INNERVATION OF RABBIT VIRAL PAPILLOMA

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In studying the neural mechanisms involved in tumor development, morphological analysis of the course of reflexive and trophic influences of the nervous system on this process is of importance. It may be assumed that the influence of the central nervous system is transmitted to malignant neoplasm directly via the peripheral nervous system, or indirectly via some part of the humoral system. However, the problem of which of these pathways is the most important is at present unsolved. The obvious approach is to ascertain whether or not there is a direct neural pathway connecting the tumor with the central nervous system. The majority of investigated tumors, both human and animal, have been found to have their own innervation [2,5-9, etc.]. In the available literature, we did not find any similar investigation of viral malignant papilloma in rabbits.

Considerable attention for some time has been devoted to the virus as an etiological factor in the formation of pathological tumors. Investigations of the present type, therefore, are of great interest.

METHODS

Experiments were carried out on 32 rabbits. Papillomas were produced by smearing virus material onto scarified skins. The Campos method was used for staining nerves. Papilloma innervation was studied at various post-inoculation intervals: 10 days, 1,2,5, 7, 8, 14, 16 months. Papilloma malignization usually set in at the 10-12th month. With these procedures we studied the innervation during both the benign and malignant stages.

RESULTS

Ten days after virus inoculation, microscopy revealed considerable hypertrophy of cutaneous epithelium and epithelium of hair bulbs. Neural elements at this time were found in considerable quantity. The majority of nerve fibers, with various calibres from very thick to very fine fibrils, are located in the primary connective tissues. In some preparations, fine branches can be seen to leave the principal fibers and travel out to areas between hair bulbs. Some of these fibers entwine the hair bulbs, and single fibers may penetrate the epithelial lining of the hair sac. Fine nerve fibers, devoid of myelin sheath, penetrate the cutaneous epithelium to form tightly coiled receptor structures. The morphology of these fibers appeared normal (Fig. 1).

At 1 month after virus inoculation, small individual papillomas, 0.2 × 0.2 cm in size, were present in the skin. Study of these growths also revealed nerve fibers located chiefly in the connective tissue stroma beneath the papilloma. Individual bundles of fibers travel out to the basal layer of papilloma epithelium. We were not successful, however, in finding any branches of these fibers in the overlying epithelial growth. These nerve elements appeared normal. It should be pointed out that satisfactory staining of nerve fibers at 10 days or 1 month following virus inoculation was difficult to achieve; neural elements in the tissue often could not be exposed.

Study of neoplasm at 2 months after virus inoculation revealed a distinct transition of hair bulb epithelium. The papilloma at this time shows a uniform basal layer submerged in subcutaneous tissue. Its peak is covered with a small horny mass and is elevated above the skin. Great numbers of nerve bundles, composed of fibers of various calibres, are found in the underlying connective tissue. Some of these fibers form delicate single nerve endings. Individual fibers, running in subcutaneous tissue, penetrate the papilloma epithelium a considerable distance. In some areas preserved coils of nerve fibers around hair bulbs can be noted. The very fine fibers forming these coils show reactive changes (vacuoles, axolysis, swelling) along their course.

At 5 months after virus inoculation papilloma had attained considerable size, and their surfaces were covered with an extensive horny mass. These growths

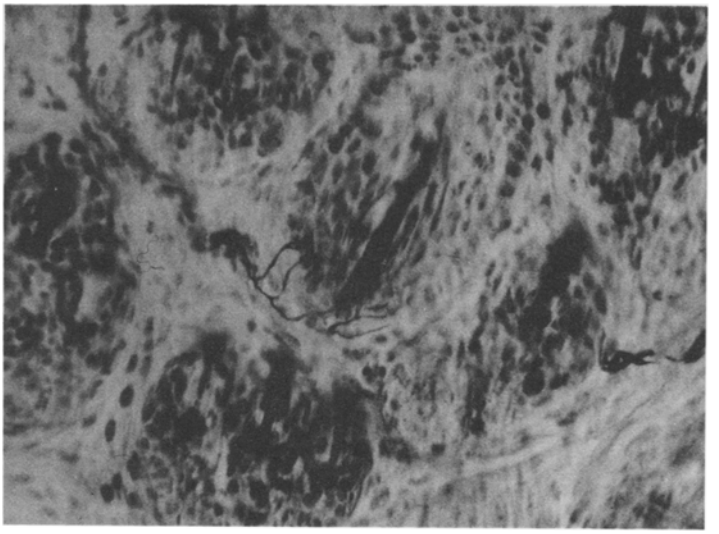

Fig. 1. Innervation of hypertrophied hair bulbs 10 days after virus inoculation. Objective, 32×; ocular, 7×; coefficient, 1.4. Campos stain.

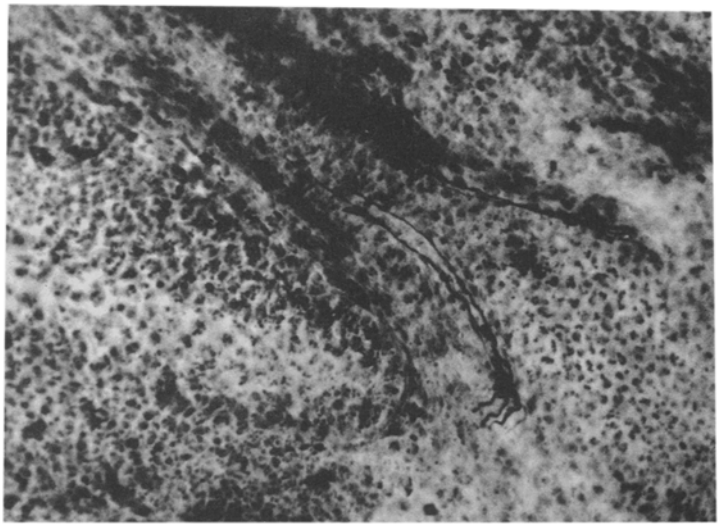

Fig. 2. Penetration of nerve fibers into papilloma at 5 months after virus inoculation. Objective, 32×; ocular, 7×; coefficient, 1.4. Campos stain.

were circumscribed by primary connective tissue, into which the epithelial protuberance was deeply wedged. Considerable evidence of mitosis was observed, particularly at the level of the 4-5th layers. The primary mass of nerve fibers is located in the underlying connective tissue, and bundles of nerve fibers travel into the connective tissue stroma of the epithelial protuberance.

The nerve fibers, penetrating the stratified squamous papilloma epithelium with equal density, are fine, straight, and unarborized, but at the same time irregularly thickened (Fig. 2). Many of these dichotomize and form more intensively argentophilic swellings. Some of the more delicate coiled fibers form club-shaped endings similar to those of receptor terminals. Many of the fibers accompany vessels and branch into their walls. In some places itis possible to observe individual fibers sending one branch to vessel walls and another to epithelial cells.

At 7 months after virus inoculation the morphological structure of the papilloma agreed with the usual description. The large epithelial growth is deeply wedged in the surrounding connective tissue, and the latter clearly circumscribes the basal layer of epithelium. The epithelial protuberance has expanded to a form of papillo-acanthosis. Evidence of extensive mitosis is visible. Microscopy of these papillomata reveals that nerve bundles containing

3-4 fibers or more leave the connective tissue and penetrate a considerable distance into the stratified squamous papilloma epithelium. These fibers are uniform, smooth, and devoid of any pathological changes. Neural structures similar to receptors are found both in the connective tissue stroma of the epithelial growth and in the surrounding connective tissue. In the latter a great number of vessels are seen. In some areas the nerve fibers are vacuolized and irregularly thickened due to irritation.

The neural elements present approximately the same morphological picture at 8 months after virus inoculation. In this case also the nerve fibers penetrate the stratified squamous papilloma epithelium. These are fine, delicately stained fibers, whose characteristics indicate that they grew into the papilloma and were not located there previously. Some of these fibers give off fine branches with bead-shaped varicose enlargements typical of growing axons. In the surrounding connective tissue the nerve fibers form fine branched endings similar to recepters. Club development can be noted on individual fiber terminals. In this case, therefore, there is the appearance of stimulation and development of the nerve fibers of papillomatous tissue.

These characteristics differed from those observed during the benign period of papilloma growth. Studies of even later stages of the tumor process, i.e., at the beginning of malignization or after the complete transformation of the papilloma into a malignant tumor, revealed more conspicuous changes in nerve fiber structure. Such a picture was observed at 14 months after virus inoculation, i.e., at the initial stage of the malignant process. Randomly located islands of stratified squamous epithelium occur in the loose connective tissue. Their centers contain a mass of keratosic epithelium. Together with these changes occurs a region of preserved normal papilloma structure. Nerve fibers of various diameters can be noted among the stroma collagen fibers, and between stroma epithelial cells. The majority of nerve fibers however, are fine, irregularly thickened, and hyperargentophilic. In some cases, thickened nerve fibers containing vacuoles can be seen.

Sometimes nerve fibers, running in primary epithelial tissue, reach almost up to the surface of the tumor. It was our impression that the apex of the nerve bundle occurs in the peripheral zone of the tumor and is exposed to destruction. The nerve fibers in this region undergo degenerative changes. The large myelinated fibers are severely thickened and have become tightly coiled. They form a rough curve, and turn to travel back towards the base of the tumor, gradually thinning out. From the thickened parts of fibers emerge very fine collaterals with club-shaped endings, and these collaterals sometimes show a retrograde course into the base of the tumor. Parts of the thick fibers are exposed to complete destruction into clumps. In the main bundle, where nerve fibers are loosely arrayed, secondary protuberances are also prominent, emerging both from fine and thick nerve trunks which exhibit signs of pathological excessive growth of neural elements. In some cases, nerve fibers adjacent to epithelial cells are abruptly twisted, with considerable irregularity in diameter and thorniness of contours. The endings of very fine nerve fibers are conspicuously thickened. In the depth of the tumor, where there is a mass of connective tissue, the fibers are ordinary, with uniform contours and small swellings on the endings.

Study of tumors at 16 months after virus inoculation, i.e., when the papillomata had made a complete transition to the malignant stage, revealed more conspicuous degeneration of neural elements. At this stage the tumor is deeply ingrown into the mass of muscle. It can be seen that some nerve fibers found in the connective tissue stroma are irregularly swollen and highly argentophilic. Single nerve fibers which leave the main bundle to form structures very similar to receptors can occasionally be seen. These structures occur in the connective tissue layers between epithelial cells. The nerve fibers have conspicuous swellings which produce secondary protuberances, and these undergo degenerative destruction. Axolysis of axis cylinders is observed in the thickened regions. Fine terminal rings lie scattered in the tissue. In the loose connective tissue in the depth of the tumor are found bundles of nerve fibers in various stages of degeneration (Fig. 3).

A great many nerve fibers running in bundles occur in the connective tissue stroma among epithelial cells. Some of these fibers also show signs of destruction and excessive growth (conspicuous swelling along the length of the fiber, degenerating secondary growths). It can be seen how the fibers, running in bundles, vary their character when in contact with tumorous tissue. Here the nerve fibers abruptly thin out. Individual fibers, emerging from bundles, travel towards the epithelial cells.

These experiments showed that nerve fibers occur in both the benign and malignant developmental stages of viral papilloma. On the 10th day after virus inoculation, when only negligible proliferation of cutaneous and hair bulb epithelium had occurred, normal neural elements could be observed in the latter. A similar picture was also noted at 1-2 months after virus inoculation, i.e., after the pathological process had already commenced.

The first reactive change of nerve fibers was noted at 2 months after virus injection. With development of the pathological process, this change was increased. Even if these changes were reversible during the benign stage of papilloma formation, with transition to the malignant stage profound degeneration of neural elements was noted.

Along with nerve fiber degeneration, excessive growth was observed, and this, undoubtedly, is evidence of a reaction of nervous tissue to pathological stimulation, leading to nerve growth, in the later period of tumor development

Fig. 3. Large nerve trunk located deep in tumoral tissue. Degenerative and reactive changes in nerve fibers are visible. Microphoto of tumor at 14 months after virus injection. Objective, 32×; ocular, 6×; coefficient, 1.9. Campos stain.

(5-8 months, etc.), into various sections of the neoplasm. Innervation consisting of nerve fiber growth into the connective tissue capsule of the tumor is particularly evident.

SUMMARY

Innervation of viral papilloma was studied in rabbits. The nerves were stained by the Campos method. The viral papilloma of rabbits was found to be innervated. The nerve elements were revealed in 10 days, 1, 2, 5, 7, 14, and 16 months after the inoculation of papilloma virus. The nervous formations observed at the early dates after the virus inoculation could be considered as normal; later, an active growth of the nerve elements occured, manifested both in the changed character of the nerve fibers themselves, and in the appearance of growth bulbs at their endings.

Reactive and degenerative changes were also seen in these nerve elements, the extent of which increased with the development of the viral papilloma, especially after its malignization.

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