

# ONCOLOGY

## ON THE SPECIFICITY OF THE SHOPE VIRUS

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That viruses play a definite role in the development of certain animal tumors has been definitely established, but how important they are in the etiology of human tumors is not yet clear.

Tumorigenic viruses are usually considered to possess exceptional tissue specificity. This, however, is completely false. The virus causing cancer of the liver in the leopard frog is known, under certain conditions, to also cause tumors in the tissues of the triton [9]. In this case, tumors develop in the periosteum, joints and liver of the triton. In chickens, lymphomatosis causes great periosteous growths, and when leukemic material is passaged to chickens, endotheliomas, hemangiomas, osteochondromas and sarcomas develop [5].

The "milk factor" has been successfully cultivated on mouse connective tissue [2] and even on human embryonal connective tissue [4]. The skin of rat embryos processed with the Shope virus and transplanted to the brain caused typical papillomatous affections [6].

All these facts suggest that, despite the selective affinity of viruses to this or that tissue, they can adapt to new conditions of existence and multiply in other tissues of the same animal or even in the tissues of a different kind of animal.

Although it is easy to cause cutaneous papillomas in rabbits which become malignant in almost 100% of the cases by means of the Shope virus, to date, no tumors have resulted from an injection of this virus into other tissues or organs. The Shope virus has been injected by different authors intravenously, subcutaneously, intraperitoneally, intracerebrally, into the testicle, the epithelium of the oral cavity, the trachea, the rectum, the urinary tract, the external gonads, and into the mammary and salivary glands [11, 10, 7, 1]. Only with the intravenous injection did papillomas appear on the skin; no tumors were obtained anywhere else.

The negative data of these works permit the proposition that the Shope virus possesses an exceptional tissue specificity, that it is "strictly dermatropic." However, if one considers the data given above from these works, this proposition cannot be considered conclusively proved. It is possible that the Shope virus caused another reaction in the other tissues.

The purpose of this work was to make a histological study of the changes occurring in various tissues injected with the papilloma virus.

### EXPERIMENTAL METHODS

Tissue from a Shope papilloma preserved in 50% glycerin was washed free of the glycerin, ground with a glass pestle and diluted with an equal volume of a physiological solution. The resulting suspension was injected into the rabbits (in a dose of 1 ml per rabbit) through a hypodermic needle into certain organs or rubbed into a scarified mucous membrane.

The submaxillary salivary glands of ten rabbits were injected with the papilloma suspension (the skin was cut open and then sutured); the material was injected into the testicle of five male rabbits, into the bladder wall (after the celiac cavity had been opened) of twelve rabbits, and rubbed into the mucous membrane of the external gonads of 11 animals (7 males and 4 females).

A suspension made from normal rabbit skin prepared in a similar way was injected into the corresponding organs of the control group of rabbits.

The rabbits were observed. The animals were killed and dissected after intervals of from one month to one year; all changes were recorded, and the experimental organs were removed, fixed and histologically processed.

### EXPERIMENTAL RESULTS

No tumors were obtained at the place where the papilloma suspension had been injected into the salivary glands of any of the ten rabbits thus injected. Only in one rabbit after 6½ months did two small papillomas appear on the skin in the thoracic region. Their growth was not active, and they disappeared after a short while. The salivary glands of the experimental group grew somewhat larger than those of the control group. Histological examination showed, both in the early and late stages (over 7 months) of the experiments, epithelial growth in the ducts: thickenings and, in places, formations of massive accumulations. No mitoses could be found in them (Fig. 1). No abnormal changes were observed at these same stages in the control experiments.

A papilloma appeared the 4th month on the skin of the right flank in one of the five rabbits injected with the papilloma suspension through the testicle. The papilloma grew actively. No tumors appeared at the place where the material had been injected. Histological examination disclosed no changes in the testicle tissue as compared with the control.

Nor were tumors obtained in the rabbits injected with the papilloma suspension through the thickness of the bladder wall. However, many multinuclear cells appeared in the epithelial layer of the bladder wall, which were different from the ordinary multinuclear cells of a normal transitional epithelium. Normally, they are only found in the sheath layer, but, in the experimental material, they were found in the middle cellular layers as well.

In one field of vision with a small microscopic magnification (objective 8, ocular 7), one could count up to 17 multinuclear cells, whereas only 2-3 are visible normally. The small cells contained 5-8 nuclei, larger cells, about 20, and the giant cells contained up to 40 nuclei. The nuclei were arranged either in a bunch in the center of the cell, or in a chain, if the cell was elongated, and in separate groups in the giant cells. In some multinuclear cells, the nuclei were arranged like a wreath around the periphery. The cytoplasm here was lumpy and coarse, and one could sometimes see vacuoles in the cells (Fig. 2).

No tumors were obtained in the animals rubbed with the papilloma suspension in the mucous membrane of the external gonads at the place where the material was administered. The mucous membrane was histologically normal. Only in one male, a month after the beginning of the experiment, was a node 2 x 8 mm in size discovered where the papilloma suspension had been administered, under the mucous membrane of the preputial sac. This node persisted for 2 weeks, and then disappeared; at that time, an enlarged lymph node in the left groin began to be apparent. By the third month of the experiment, thick, rather inactive nodes could be felt in the celiac cavity.

The rabbit was killed on the 96th day of the experiment. The mucous membrane at the place where the papilloma suspension had been rubbed in was unchanged. An enlarged lymph node, 2.5 cm in diameter was found under the skin of the left groin; two large groups of fused lymph nodes 9 and 4 cm in diameter were found transperitoneally at the base of the mesentery and on the aorta. There were two small, round nodules 2 and 1.6 cm in diameter in the spleen. On cross-sections of the nodes, one could see macroscopically foci of necrosis and small hemorrhages. Live tissue remained mostly along the peripheries of the nodes. In this case, histological examination showed that a tumor had formed.

In all the nodes, the tumor was made up of more or less similar cells, poor in cytoplasm, which were larger than the lymphocytes and distinguished from them by the character of the nuclei. The latter were oval or round and stained rather lightly. Both normal and abnormal mitoses were found in abundance. There was practically no stroma in the tumor.

In other portions of the tumor, along with the small cells with darkly stained nuclei, large cells, which were both uninuclear and binuclear, were found. There were many giant cells containing up to 10 nuclei (Fig. 3).

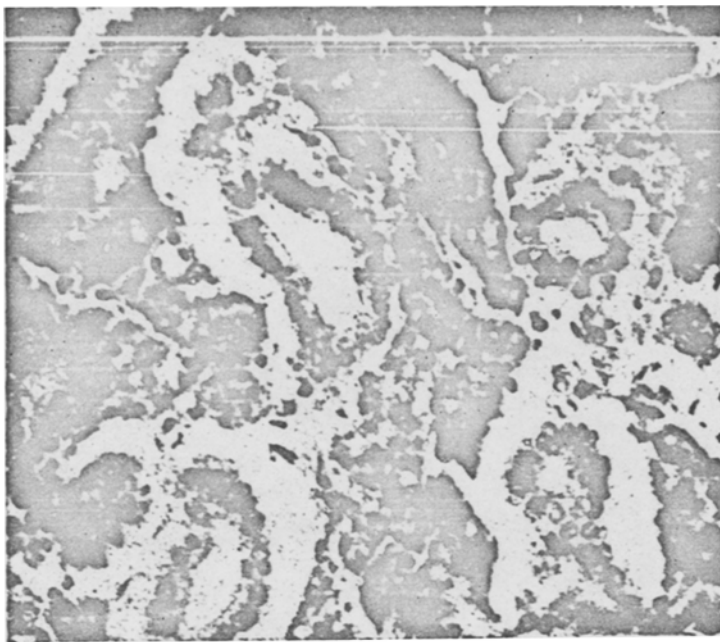


Fig. 1. Section through a salivary gland, 200th day of the experiment. Epithelial growth in ducts of salivary gland. Photomicrograph. Stained with hematoxylin-eosin. Magnified 300 times.

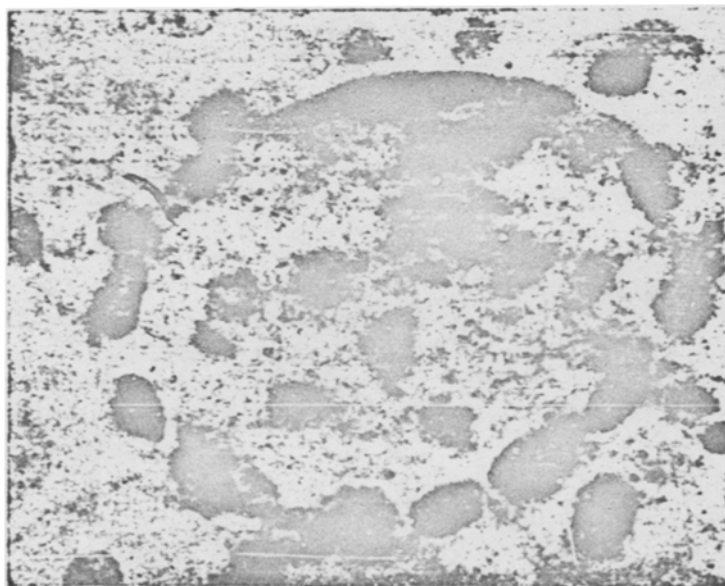


Fig. 2. Total preparation of bladder epithelial sheath. 240th day of the experiment. Giant, multinuclear cell, 14 nuclei of which are arranged around the cell periphery. Photomicrograph. Stained with iron hematoxylin. Magnified 300 times.

Therefore, the tumorous process affected the lymph nodes and was also found in the spleen. The actual tissue of the tumor consisted of rather undifferentiated cells. It was similar to the so-called reticulocytoma.

This tumor was transplanted to 9 rabbits. The results of the transplantations were negative.

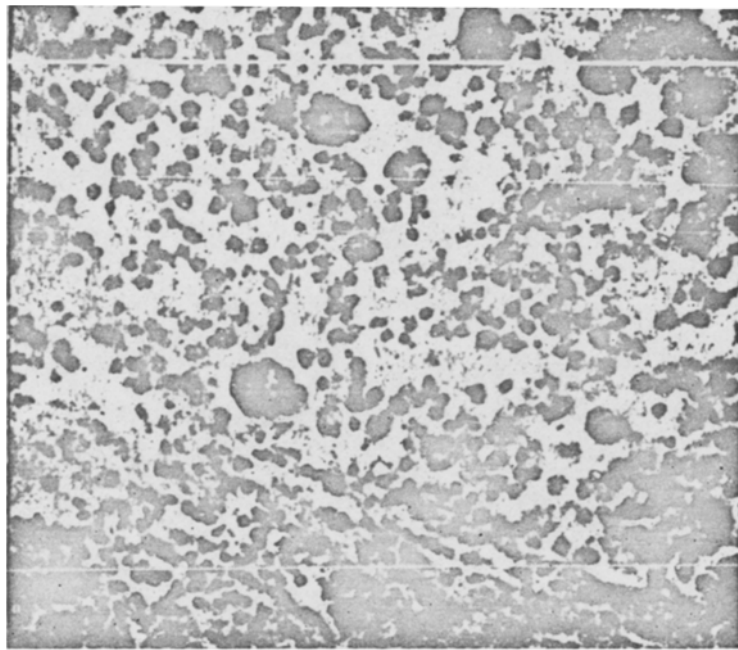


Fig. 3. Tumor section. Large multinuclear cells among small and larger tumorous cells. Photomicrograph. Stained with hematoxylin-eosin. Magnified 170 times.

The data presented deserves attention. The changes observed in the bladder epithelium and in the epithelium of the salivary gland ducts could be considered a peculiar reaction in response to the virus injection. There were no such changes observed in the control experiments.

The Shope virus causes the acceleration and intensification of the growth of rabbit skin explants, disturbs the processes of cell division and causes the appearance of atypical cellular forms [3]. But cell proliferation is not only caused by tumorigenic viruses. It is also observed in other virus infections (chicken pox, neurovaccine, etc.) as one of the stages of the disease [8].

The appearance of giant and multinuclear cells is connected with this "pathologic" proliferation.

The growth of the salivary gland duct epithelium and the appearance of giant, multinuclear cells in the epithelial sheath of the bladder can be considered as a manifestation of "pathologic" proliferation.

The mucous membrane of the external gonads of the rabbit belongs to the cutaneous type of membrane. Its sheath is a multilayer, flat epithelium, which produces mucus. One would expect that here would be the first place at which a papilloma would develop, but such was not the case. A reticulocytoma type of tumor was only observed in one rabbit.

Why did this one develop? In the first place, the tumor could have developed spontaneously, quite apart from our experiments. However, spontaneous tumors are extremely rare in rabbits. For the purposes of this article, one can suggest the following arguments. The tumor originated in the regional lymph node nearest the place of implantation, and then, the process spread to the lymph nodes located along the aorta. Its progress was traced.

Entering the lymph node through the lymph circulation, the virus found a favorable environment in the node. Adapting itself to the new conditions, it began to multiply energetically and was thus able to cause those changes in the tissues which are usually defined as malignant tumorous growths.

However, all these reasons are propositional, since we had only one case to observe. The experiment must be repeated with a similar result in order to conclusively prove that this tumor was caused by the Shope virus.

## SUMMARY

Introduction of the Shope virus into different organs of rabbits resulted in histological changes. Profuse epithelial growth was noted in the salivary gland ducts. Many giant, multinuclear cells appeared in the epithelial layer of the urinary bladder. A reticulocytoma type tumor developed in the extraperitoneal nodes of one of the rabbits. The causation of the changes elicited by the Shope virus is discussed.

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