

TRANSPLANTABLE STRAIN OF SHOPE'S RABBIT CARCINOMA

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In experimental oncology it is of the greatest importance to establish a model cancer, both in order to study pathogenesis, as well as to seek for effective treatments of malignant growths. Because recently a widespread study of the part played by viruses in the etiology of tumors has been made, the establishment of models of tumors with a well-known virus etiology acquires particular interest. Shope's virus papilloma belongs to this category. In the benign stages of development, the virus is readily detected in the tissue of the papilloma, but after malignancy has developed it is not easy to distinguish it. The phenomenon of "disappearance" of the virus is characteristic not only of Shope's papilloma, but is found also in the development of the Rous sarcoma, and in poliomas and other tumors.

Characteristics of the Primary Strain of Shope's Rabbit Carcinoma

| Number of generation | Time of grafting the tumor (in days) | Age of animals (in days) | Total animals | Date of appearance of tumor (in days) | Number of animals | | | | | Size of tumor (in cm) |
|----------------------|--------------------------------------|--------------------------|---------------|---------------------------------------|-------------------|----------------|---------------------------------|---------------------|------------------|-----------------------|
| | | | | | with tumors | without tumors | with tumors which were absorbed | died without tumors | died with tumors | |
| 1 | 90 | 7 | 10 | 90 | 1 | 9 | — | — | 1 | 7×8 |
| 2 | 43 | 6—30 | 30 | 6,23 | 7 | 23 | — | 4 | 3 | 1×1 0,8×0,7 |
| 3 | 40 | 6 | 5 | 28—34 | 2 | 3 | — | — | 2 | 7×8 |
| 4 | 56 | 6—12 | 35 | 41—48 | 2 | 33 | — | 10 | 2 | 5,5×6 |
| 5 | 50 | 7 | 23 | 6—8 | 8 | 15 | 1 | 9 | 7 | 6,5×6 |
| 6 | 32 | 4—30 | 21 | 7—9 | 10 | 11 | 1 | 6 | 9 | 4×4,5 |
| 7 | 20 | 6 | 23 | 7—17 | 12 | 11 | 5 | 1 | 7 | 3,9×5,5 |
| 8 | 23 | 5 | 15 | 4 | 14 | 1 | 8 | — | 6 | 6×5,5 |
| 9 | 24 | 6 | 21 | 4 | 21 | — | 12 | — | 9 | 4,5×5,8 |
| 10 | 22 | 6 | 18 | 4 | 18 | — | 3 | — | 15 | 3,5×5,3 |
| 11 | 19 | 6 | 20 | 5 | 20 | — | 2 | — | 18 | 3×4×6 |
| 12 | 11 | 6 | 8 | 3 | 8 | — | — | — | 8 | 4,5×5 |
| 13 | 17 | 6 | 6 | 3 | 6 | — | 1 | — | 5 | 3,7×5 |
| 14 | 17 | 6 | 8 | 6 | 8 | — | — | — | 8 | 3,6×4 |
| 15 | 30 | 7 | 17 | 4 | 17 | — | 6 | — | 11 | 3,3×4,5 |
| 16 | 20 | 6 | 19 | 4 | 19 | — | 11 | 4 | 8 | 4×4,5 |
| 17 | 17 | 1—2 | 10 | 4 | 10 | — | 2 | 2 | 8 | 3,8×4,6 |
| 18 | 17 | 1—2 | 12 | 6 | 12 | — | 6 | — | 6 | 2,8×3,2 |
| 19 | 15 | 2 | 8 | 7 | 8 | — | 3 | 2 | 5 | 3,2×4,1 |
| 20 | 16 | 1—2 | 14 | 3 | 14 | — | 5 | 1 | 9 | 4×4,2 |
| 21 | 26 | 1—4 | 23 | 3 | 23 | — | 13 | 4 | 10 | 2,8×3,9 |
| 22 | 21 | 1 | 16 | 3 | 16 | — | 11 | 5 | 5 | 4×4,5 |
| 23 | 30 | 1—2 | 15 | 4 | 15 | — | 9 | 4 | 6 | 3,7×2,9 |
| 24 | 24 | 1—2 | 22 | 4 | 22 | — | 13 | 6 | 9 | 3,9×4 |
| 25 | 24 | 1—2 | 27 | 3 | 27 | — | 14 | 9 | 13 | 4×2,7 |
| 26 | 24 | 1—3 | 17 | 3 | 17 | — | 7 | 3 | 10 | 5×3,2 |
| 27 | 23 | 1—2 | 16 | 4 | 16 | — | 8 | 3 | 8 | 4,2×4,3 |
| 28 | 27 | 2 | 16 | 4 | 11 | — | 2 | 1 | 9 | 3,9×4 |
| 29 | 21 | 1—2 | 11 | 3 | 11 | — | 3 | 1 | 8 | 4×4 |
| 30 | 29 | 2—3 | 19 | 3 | 19 | — | 7 | — | 4 | 3×3,5 |

According to most authors [1-5], the virus does not disappear from the tumor, but remains in it in a masked form. Smith and others (1952) succeeded in unmasking the virus of the Shope papilloma by transplanting the tumor intramuscularly into newborn rabbits. Under these conditions the tumor grew well, and the authors succeeded in establishing a transplantable strain which they called Vx_3 - Vx_4 - Vx_6 .

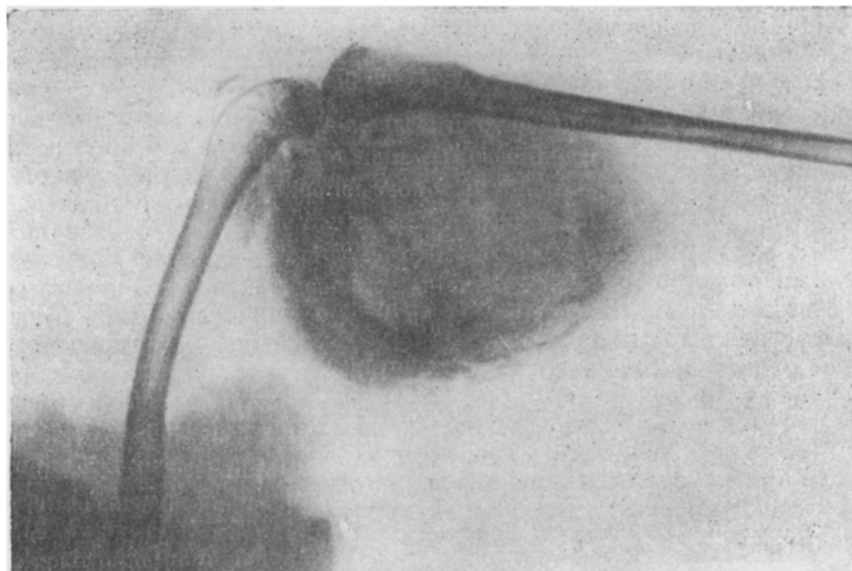


Fig. 1. Tumor developed in a rabbit three months after transplantation of a skin cancer into the thigh.

Recently several such strains have been obtained. However, the virus could be separated only from strain Vx_3 .

In the present work we describe a transplantable strain of Shope tumor, obtained in our laboratory in 1959*.

The material for establishing the transplantable strain was a skin cancer formed through the development of malignancy in a papilloma. Excised fragments of the tumor were treated in physiological saline with 25-30 thousand units of penicillin. After they had been carefully washed, the fragments of tumor were placed in a mortar, and cut up finely with scissors to form a porridge-like mass. The mass was then diluted 3:1 with physiological saline. One ml of the mixture prepared in this way was then injected into the thigh muscles of newborn rats. In certain cases fragments were grafted. For this purpose we used Sukhumi rabbits (not pure bred) aged from 2-3 days to 1-2 months. Histological preparations were stained in hematoxylin-eosin.

RESULTS

The results describing the strains are shown in the table.

Three months after transplantation of the skin cancer into the thigh muscles of ten newborn rabbits, in one of them a tumor of considerable size, measuring 7×8 cm was found (Fig. 1). The tumor was very dense and lumpy, and was fused to the surrounding tissue. The section showed almost entirely boney tissue, and only in the center was some epithelial tissue present. There were no metastases. A histological study showed a typical picture of flat cornifying cancer cells. There were a number of epithelial cells surrounded by connective tissue fibers. The polymorphism of these cells was striking. There were numerous mitoses. There were also accumulations of cornified epithelium (Fig. 2).

Transplantation of the second generation of this tumor was made 53 days after it had originated into 14 five-day-old rabbits and into 16 rabbits one-month-old. Growth of the tumor occurred in four of the adult rabbits after six days, and in two of the five-day-old rabbits and in one adult after 24 days. In 23 rabbits there were no tumors. The new tumors were small in size, compact, lumpy, and closely fused to the surrounding tissue. Their maximum size was 1×1 cm.

*D. N. Bochkova took part in the transplantation in the first three generations, and T. V. Lastovetskaya from the 22nd generation onwards.

The third generation was transplanted 40 days after the development of the tumors of the second generation. Transplantations were made into five rabbits one-week-old, and in two of them a tumor growth was observed after 28-34 days. No tumors were found in the other three. At first these tumors were dense, lumpy, and of small size. After 3-4 months they had enlarged considerably to attain a size of 7 X 8 cm. In section, the main mass of these

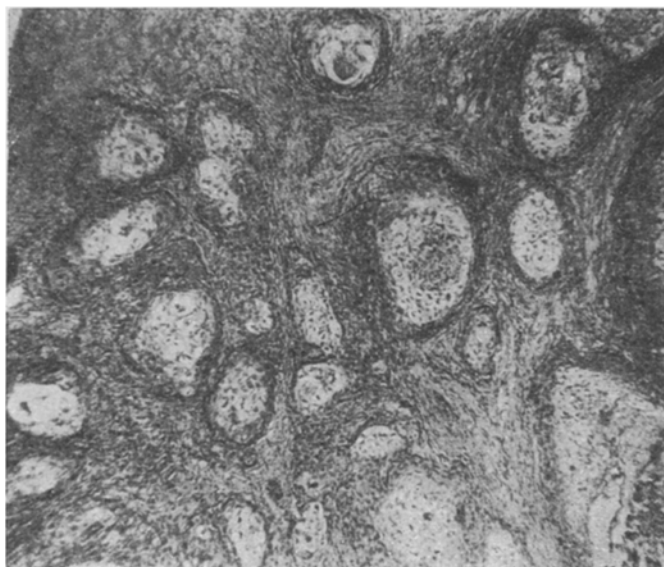


Fig. 2. The same tumor seen under the microscope.

tumors consisted of a pale pink epithelial tissue. At the same time, a considerable proportion of them were occupied by a necrotic mass consisting mainly of cornified epithelium. Histological study showed a typical picture of flat cornified cancer cells. No deposit of calcium cells was found.

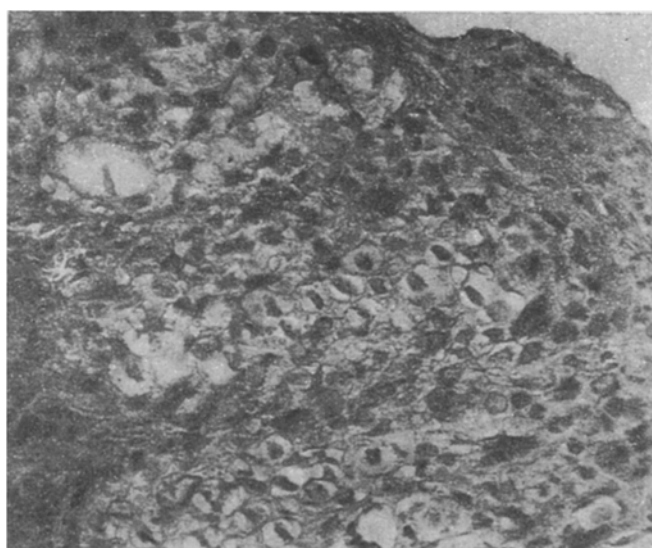


Fig. 3. Morphological picture of a tumor developing as a result of a graft of a skin cancer into a weanling rabbit.

After 56 days from the time at which the third generation tumors had originated, transplantation of the fourth generation was made into 35 rabbits one-week-old. Development of the tumors occurred in two rabbits by the 41-48th day. In 33 rabbits no tumors developed. No absorption of the tumors was observed. The lumps which developed attained a considerable size, and resembled the tumors of the third generation.

From the fifth generation onwards, the number of tumors increased considerably, and they developed much sooner. Thus, of the 23 one-week-old rabbits, a tumor developed in eight 6-8 days after transplantation. A difference from the preceding generations was that in one rabbit absorption of the tumor was observed. Subsequently the number of cases of absorption of tumors increased somewhat. The greatest number absorbed was observed in the 21-24th generations. The retrograde development of the tumor occurred 15-20 days after transplantation.

As can be seen from the table, from the 9th generation onwards, transplantation succeeded in every case. The time for the tumor to develop varied from three to six days.

From the 17th generation onwards, transplantation was made into rabbits 2-3 days after birth. In the adults the tumors failed to grow. Transplantation of tumors into rabbits at the age just mentioned was found to be more favorable for their development. There was some change in the morphological appearance, consisting mainly of a reduction in the amount of connective tissue and an increase of epithelial tissue (Fig. 3). The survival time of rabbits with a tumor was shortened. In the first tumor generations the rabbits lived 4-6 months, but from the 17th generation onwards, their survival time was reduced to 1-2 months.

However, as was pointed out above, the percentage of tumors absorbed after transplantation into newborn rabbits was not reduced. Transplantation of parts of one single tumor into weanling rabbits from different litters showed that in some rabbits the tumor took in 100% of the cases (of the 12th and 14th generations) and there was no absorption. However, in other cases, despite the fact that the tumors appeared in all the rabbits from the fourth generation onwards, absorption did occur, if not in all, at any rate in most of the animals. Evidently, absorption of tumors is associated with immunological features.

In order to have more uniform material for the experiments we discarded some of the offspring, eliminating animals in whose offspring a tumor was absorbed.

At the present time we have transplanted the 31st generation of Shope's tumor. In no single case were any metastases observed. A study of this strain for the presence in it of a virus showed that treatment of skin with an extract of these tumors in many cases causes the development of a papilloma. The developing papillomas both in outward appearance and in histological structure differ in no way from those induced by the normal virus preserved in glycerine. Thus intramuscular transplantation of a skin cancer unmasks the virus of Shope's papilloma. A more detailed account of these experiments will be presented in another communication.

SUMMARY

Transplantation of skin cancer into the muscles of the thigh of newborn rabbits led to tumor development. Further transplantations have made it possible to obtain a transplantable strain of Shope's cancer. By now 31 generations have already been transplanted. The tumors attained a considerable size (7 x 8 cm); they were tuberous and adhered closely to the surrounding tissue. A typical picture of squamous-cell cornifying carcinoma was observed histologically. There were no metastases. Papillomas were induced in a number of cases by inoculation of the skin by an extract of these tumors.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. Some or all of this periodical literature may well be available in English translation. A complete list of the cover-to-cover English translations appears at the back of this issue.
