THE INNERVATION OF THE TRANSPLANTED BROWN-PEARCE TUMOR OF RABBITS

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The problem of the innervation of malignant tumors in man and animals has long attracted the attention of researchers. The study of this problem gives a deeper understanding of the relationship between the tumor and the host, and is thus of practical as well as theoretical importance. A point of special importance here is the question of the innervation of transplanted tumors, but little reference to it is to be found in the specialized literature. Whereas references to the innervation of experimental tumors number several tens of authors, those dealing with the innervation of transplanted tumors are to be found only very rarely.

The first research in this direction was carried out by Milone [9], who studied the innervation of the rat sarcoma. In this author's opinion, nerve fibers growing into a transplanted tumor, placed between the ends of the divided nerve, bore no relationship to the tumor. M. N. Meissel and L. F. Larionow[8], however, who carried out similar experiments, observed on the other hand that there was a close morphological connection between the nerve cells and the tumor cells, which, in their opinion, implies functional association too. Later, A. G. Chernyakhovskii [5, 6] found nerve endings in intimate contact with tumor cells in an Ehrlich's mouse carcinoma.

In recent years Ukranian workers [1, 2, 3], studying a transplanted M-1 sarcoma of rats and a mouse strain of Crocker's tumor, have described nerve cells in these tumors. At the same time these authors emphasize the presence of marked dystrophic changes in the nerves of these tumors. Ducan and Bellegie [7] described the ingrowth of nerve fibers from the posterior columns and dorsal roots of the spinal cord into a transplanted rat sarcoma No. 319 after inoculation of the tumor in the cerebrospinal canal.

Little or no work has been done on the innervation of the Brown-Pearce tumor. It is therefore necessary to dwell in greater detail on the investigations of Shapiro and Warren [10], who studied the stage of the nerves in 13 transplanted Brown-Pearce tumors in the anterior chamber of the rabbit's eye. The peripheral end of the corresponding sympathetic nerve of the eye was stimulated with the electric current, and the reaction of the vessels was observed visually: blanching of the vessels in the transplant indicated that the vasomotor effect extended to the malignant tumor. Impregnation of the tumors by Bodian's method revealed solitary nerve fibers in the tumor, or else fibers collected in bundles of 10-12 or more in each. These authors particularly stress the presence of nerves on the ninth day after transplantation of the tumor. Since the tumor was transplanted into the anterior chamber of the eye, Shapiro and Warren [10] considered these findings to be important evidence that the nerve fibers are not nerves embroiled by the tumor in the course of its invasive growth. From their investigations these authors concluded that the constriction of the blood vessels of the Brown-Pearce tumor transplants is the result of stimulation of the sympathetic nerve supplying them.

METHOD

Our experiments were carried out on chinchilla rabbits, mainly males, weighing about 2 kg, on which the operation of retroperitoneal injection of 0.05 ml of tumor emulsion into the left adrenal gland was carried out under ether anesthesia. The tumor suspension was prepared in sterile conditions by cutting the tumor finely in a Petri dish by means of Cooper's scissors. The minced tumor was diluted with physiological saline to the required concentration (25%) and filtered through a double layer of gauze. The fluid injected contained no visible tumor particles. Animals were sacrificed after 10, 14, 21, 30 and 40 days. The adrenal glands (right and left) were fixed in AFA mixture by Lavrent'ev's method for one hour, and then transferred to 20% neutral formalin. Sections were cut out to a thickness of 40-50 μm with a freezing microtome, impregnated by Kampo's method and stained with hematoxylin-eosin to determine the situation of the tumor.

RESULTS

A total of 52 left adrenal glands were investigated after primary implantation of a Brown-Pearce tumor, and nerve structures were found in 39 tumors in these glands;

the same number of right adrenal glands was examined, in nine of which metastases were detected microscopically and nerve cells were demonstrated therein.

Nerve fibers were observed among the tumor cells in the left adrenal gland on the tenth day after transplantation of the tumor. They issued from plexuses, gave off branches, curved around the tumor cells on every side and ended on them in the form of thickenings (Fig. 1).

Fig. 1. Fine nerve fibers among tumor cells on the tenth day after inoculation of the tumor in the left adrenal gland. Rabbit No. 1964. Silver impregnation by Kampos' method.

On the 14th day and also on the 21st day after transplantation of the tumor the following picture of innervation of the tumor was observed. At first the nerves usually passed through the cortical layer and then entered the tumor, where breaking up of the bundles took place, the individual fibers being separated from each other by tumor cells. Some fibers in the bundle penetrated into the tumor for a considerable distance, taking a course in the direction from the periphery to the center. Occasionally nerve fibers were also observed in necrotic areas of the tumor. Growth bulbs could be seen very often at the ends of the nerve fibers, together with dichotomous division of the fibers (Fig. 2).

In every case signs of irritation of the nerve fibers were seen, in the form of increased argentophilia, varicose swellings along the course of the nerve fibers and bublar thickenings at their ends. In one case (rabbit No. 603) considerable changes were observed among the fibers situated in the substance of the cortex. Special mention must be made of a case (rabbit No. 514) in which the tumor nodule, situated outside the adrenal gland, partially engulfed the gland and infiltrated into its cortical layer, although nowhere did it reach the substance of the medulla (the tumor was separated from the medulla by a strip of cortex). Nevertheless, here too, nerve fibers were found. Some of them were greatly thickened and argentophilic, others very thin—intermingled on all sides with the tumor cells. There were many such fibers and they could be followed for a considerable distance. On the 30th day, while the distribution of the nerve fibers bore the same character, the development of endings was observed. For instance, some fibers, after undergoing dichotomous division, terminated among the

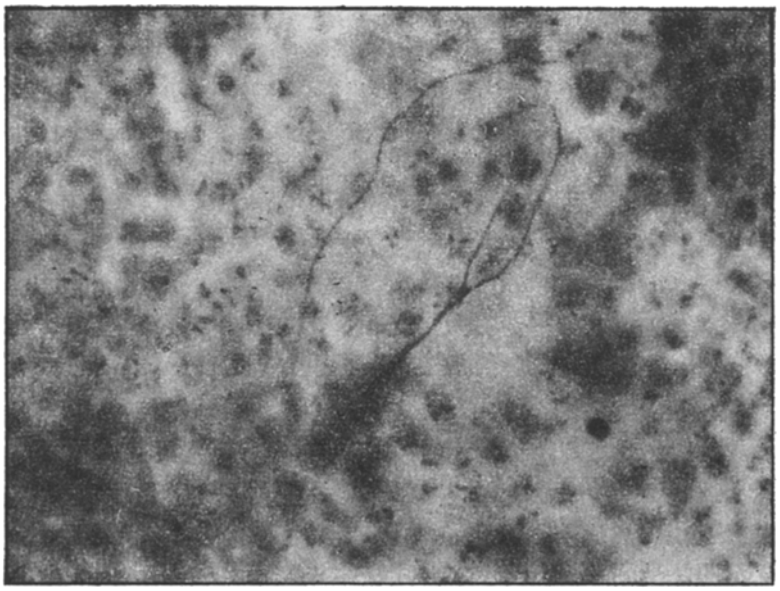

Fig. 2. Ramification of thin nerve fibers in a tumor on the 21st day after inoculation of the tumor in the left adrenal gland. Rabbit No. 603. Microphotograph. Silver impregnation by Kampos' method. Ocular 10 X, objective 32 X.

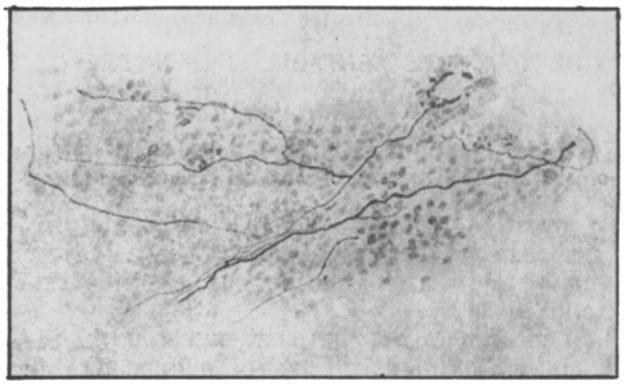

Fig. 3. Bundle of nerve fibers and individual nerve fibers undergoing dichotomous division, terminating amid tumor cells (on the 30th day after inoculation of the tumor in the left adrenal gland). Rabbit No. 192. Silver impregnation by Kampos' method.

tumor cells in the form of pointed endings (Fig. 3), and in isolated cases of pin-head structures.

It must be pointed out, however, that with an increase in the length of time after transplantation of the tumor, the number of nerve fibers in the tumor decreased, especially on the 40th day, evidently on account of necrosis of the tumor and the death of its nervous apparatus. At this stage of development of the tumor, irritation of the nerve fibers was particularly pronounced in the capsule.

In nine cases metastases were present in the right adrenal gland. In two cases metastases were only found histologically. The area affected by the metastasis was different in each case. It may be assumed that the dimensions of the metastasis were dependent on the time of its appearance.

In all cases many nerve fibers were found in the metastases, being arranged haphazardly in the tumors, sometimes in the form of a network. Some fibers were thickened and highly argentophilic. Along with these there were many thin nerve fibers, the latter undergoing dichotomous division. Along the course of the thin fiber varicose swellings could be observed.

When the findings obtained from the study of the nerve formations in the transplanted Brown-Pearce tumor in the rabbit are examined, it is above all essential to note that nerve structures were found in the tumor at different times (10, 14, 21, 30 and 40 days) after transplantation. Under these circumstances the number of nerve cells, and their character, in the tumor transplanted into the afrenal gland differed from the corresponding features of the nervous apparatus in the adrenal medulla. The same findings were observed in the metastases of this tumor in the opposite adrenal gland, into which nerve fibers grew from neighboring nerve trunks.

Our findings do not agree with the statements in the literature [9] that nerve cells, if they are present in tumors, are not in contact with the tumor cells. The microphotograph and the figures illustrate the ingrowth of very thin nerve fibers between the tumor cells. In isolated cases, at the stage of 21-30 days after transplantation of the tumor, when the nerve formations in the tumor were most numerous, nerve endings were also found.

These nerve fibers which grew into the tumor, to form growth bulbs and nerve endings, must therefore be regarded as belonging to the tumor itself.

Particularly noteworthy were the changes affecting the nerve fibers both in the tumor and in the surrounding tissues. Besides the growth bulbs, connected with the growth processes of the nerve fibers, it was possible to see varicosities of the nerve fibers, spherical thickenings and accumulations of neuroplasm, spiral coiling and hyperargentophilia. These changes were manifestations of irritations, and were present to a varying degree depending on the time after transplantation of the tumor. These signs of irritation were most pronounced on the 21st-30th day, when the tumor itself had reached its peak of growth and development, and in many cases had resulted in a fatal outcome. On the 40th day the number of nerve fibers was relatively small on account of the development of necrosis of the tumor.

The results obtained are in full agreement with those of investigations which we carried out [4] on tumors of the salivary glands in man. Although in neither case have exhaustive details been obtained on the functional significance of the nerve formations found in the tumors, the presence of endings of sensory type in human tumors and the appearance of endings very similar to sensory endings in the experimental tumors (see Fig. 3) demonstrate that these structures are indeed the medium by which nervous influences from the tumor are conveyed to the rest of the body.

SUMMARY

The author investigated the innervation of an experimental Brown-Pearce tumor transplanted to the adrenal gland in rabbits. Nerve elements were revealed in the tumor at various times after its transplantation—in 10, 14, 21, 30, and 40 days.

The tumor nerve fibers became most numerous 21-30 days after transplantation and at that time nerve endings were also revealed. Many nerve fibers were found in tumor metastases in the other adrenal gland. Thus, the nerve endings growing in the tumor should be regarded as nerves belonging to the tumor itself.

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