CORRELATION BETWEEN THE STRUCTURAL FEATURES OF INDUCED SALIVARY GLAND TUMORS AND THEIR INNERVATION

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 54, No. 10, pp. 102-106, October, 1962
Original article submitted September 12, 1961

It is now generally accepted that tumors have their own nerve supply [1-5, 9, 10]. In this connection the morphological study of the nervous apparatus of tumors and the comparison between its structure and that of the tumor are of particular interest. The object of the present research was to make a neurohistological investigation of the region of injection of a carcinogen, the site of malignant change in the tissues and tumor formation.

EXPERIMENTAL METHOD

Experiments were carried out on 40 mongrel albino rats; under ether anesthesia 5 mg of 9, 10-dimethyl-1,2-benzanthracene dissolved in 0.1 ml of vaseline oil was injected into the right submandibular salivary gland.

During the next $1^1/_2$ yr 17 of these animals developed a neoplasm in the submandibular region. Material was fixed in 12% neutral formalin. Sections were cut on a freezing microtome to a thickness of 40-60 mμ, impregnated by Kampos's method, and stained by the ordinary histological methods (hematoxylin-eosin, picrofuchsin, and impregnation by Foot's method). Histological investigation of the tumors showed them to be highly polymorphic in their structure. Both epithelial and connective-tissue tumors were present (see table). Many showed multicentric growth.

Classification of Tumors Caused by Injection of 9,10-Dimethyl-1,2-benzanthracene into the Submandibular Salivary Gland of a Rat, and Their Innervation

Type of tumor	Total no. of tumors	No. of tumors in which nervous structures were found
Tumors of connective-tissue		
origin	1	1
Histiocytoma	2	1
Lymphosarcoma	2	2
Fibrosarcoma	6	5
Spindle-cell sarcoma		
Total	11	9
Tumors of epithelial origin		
Adenoma	3	3
Epithelioma of the salivary gland	1	1
Mixed salivary gland tumor	1	1
Squamous-cell carcinoma	1	1
Total	6	6

EXPERIMENTAL RESULTS

We investigated 17 epithelial and connective-tissue tumors, and in 15 of them we found nerves. We also investigated 15 animals without visible changes in the submandibular region. In the normal submandibular salivary glands of rats the nervous apparatus is situated along the course of the capillary ducts, forming a plexus. Bundles of nerve fibers give off single fibers to the parenchymatous tissue. Some nerve fibers curve around the acini at a right angle, while others pass through them and terminate in a "bouton" on one of the secreting cells.

The submandibular salivary glands receive their nerve supply from nerve plexuses and bundles of nerve fibers penetrating the hilum in the company of blood vessels and entering the tissue of the lymph gland. Bundles of 3-5 nerve fibers penetrate the parenchyma of the lymph gland in the region of the medullary cords, where they are situated on individual fibers. Each fiber is directed towards a lymph follicle, and by dichotomous ramification forms a compound receptor of free type. It must be pointed out that the densest ramification is found in the peripheral zone of the follicle, and free nerve fibers pass only rarely to the germ centers, in the form of one of the branches of a receptor. The increased argyrophilia of the nervous structures and the fact that sensory formations can be detected in the parenchyma of the lymph glands are evidence, in our opinion, that the stimulation arising in the course of carcinogenesis is inadequate.

The zone of proliferating connective tissue forming around the oily solution of the carcinogen also developed a nerve supply. In this zone many nerve fibers were found, both in the oleogranuloma itself and in the surrounding connective tissue. Many arborization endings were seen in the latter, forming an extensive receptor field (see figure, 1). Fine ramifications of nerve fibers terminated in loops and thickening on the fibroblasts. A bundle of nerve fibers entered one part of the oleogranuloma, bent around a cavity full of oily solution of carcinogen, ramified, and terminated in the connective tissue of the oleogranuloma (see figure, 2). The nerve fibers were slightly argyrophilic, fairly straight in their course, and sometimes underwent dichotomous division.

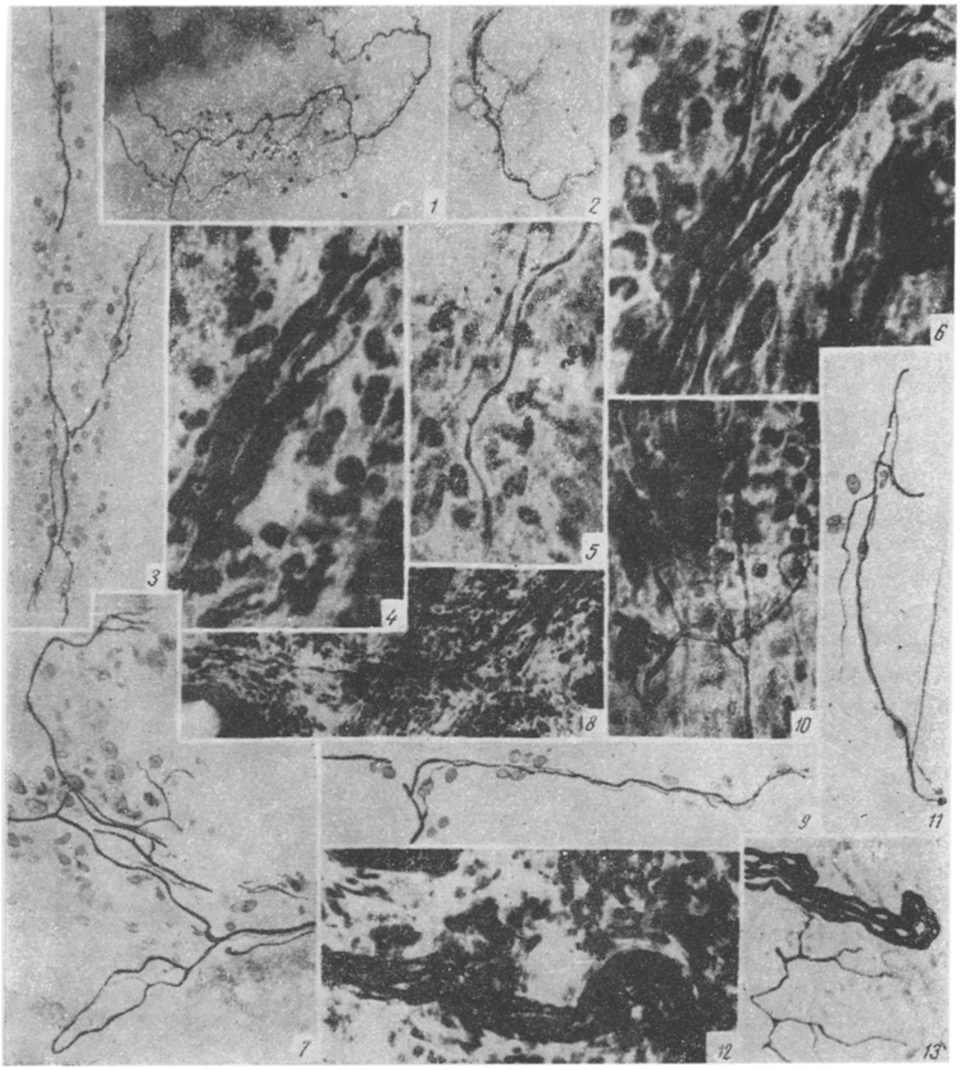
Proliferation of the connective-tissue cells led to the formation of fibrosarcomas, sometimes only detectable microscopically in the connective-tissue septa. A nerve trunk entered one part of the thin connective-tissue capsule and passed into the tumor. Its fibers were irregularly swollen, thickened, and hyperargentophilic. Some distance from its point of entry it gave off thin nerve fibers which divided dichotomously and formed an arborization receptor of a simple type (see figure, 12, 13).

It should be pointed out that all the tumors of connective-tissue origin which were small in size (2.0 × 3 cm) had a rich nerve supply. Nerve elements could not be found in only one spindle-cell sarcoma, which reached a size of 4.3 × 4.7 cm on the 130th day after injection of the carcinogen, on account of its rapid growth accompanied by necrosis. In the remaining tumors, extremely distinctive in their structure as shown by their names: histiocytoma, mastocytoma, the principle of innervation was as described above. Nerve trunks entered the tumor at one point in the capsule in company with vessels. In the tumor tissue these trunks appeared as loose bundles of thin nerve fibers. Very thin nerve fibers branching from these bundles terminated in boutons on the tumor cells. Other very thin nerve fibers extended along the vessels into the depth of the tumor, mainraining a uniform caliber throughout their course. The spindle-cell sarcoma of mastocytoma type contained numerous mast cells. In the tumor of the histiocytoma type, made up of uniform cells with a regular, round nucleus and a thin rim of cytoplasm, the character of the innervation was slightly different. The nerve elements showed more marked reactive changes and were uneven in caliber. Their straining properties were sometimes more, sometimes less marked. The thin fibers became even thinner in the region of the terminals, and varicosities could be seen along their course (see figure, 3, 4).

Hence the connective-tissue tumors in nearly all cases had a nerve supply. However, the structural features of the innervation depended on the structure and degree of maturity of the tumors. The last two qualities may to some extent also be characterized by the rapidity of onset and rate of development of the tumors. In the morphologically more mature tumors (mastocytoma, fibrosarcoma) the nervous structures were changed only slightly or not at all. In the histiocytoma (reticulosarcoma) and in the sarcomas the nerve elements were in a state of irritation (hyperargentophilia, changes in the caliber and varicosities of the nerve fibers). The pattern of innvervation of the neoplasms of epithelial origin was also largely dependent on their structural features.

The most conspicuous and densest network of nerve fibers was found in the walls of the epidermoids, where these fibers formed two types of plexuses depending on the depth at which they lay: some were superficial, others near the epithelial cover. They gave off nerve fibers, which divided in the dense connective-tissue membrane of the cyst to form triangles of neuroplasm, and terminated in sharply pointed endings on numerous connective-tissue cells and near the epidermal cover. The innervation of the adenomatous salivary glands (see figure, 8) in no way differed in its character from the innervation of the connective-tissue formations.

Its pattern was largely identical with the pattern of innervation of the epitheliomas of the human salivary gland [6]. The mixed salivary gland tumor induced in one rat 442 days after injection of the carcinogen had a complex structure and a rich innervation. This tumor consisted to a large extent of cells of fibroblast type, in places giving way to deformed giant cells. In one place masses of hyalin were situated among the cells, imitating bony trabeculae constructed in the manner of a pathological osteoid. At another place, this tumor had a structure resembling tubes with walls consisting of 3-4 layers of epithelial cells. In places the tubes changed into continuous sheets of epithelial cells. The latter were interlocked and connected to each other by prickle-like processes. The innervation of the tumor was heterogeneous. Nerves penetrated the tumor through the capsule as bundles in the company of blood vessels (see figure, 6). Each bundle consisted of nerve fibers of one type with a uniform caliber. The nerve fibers divided dichotomously and terminated among the tumor cells (see figure, 5, 10). Numerous and varied



Nerve endings in salivary gland tumors in rats. 1) Arborization receptor in the connective tissue surrounding the oleogranuloma. Rat No. 26 on the 302nd day after injection of carcinogen. ×400. 2) Bundle of nerve fibers terminating in the connective tissue of the oleogranuloma. ×400. Rat No. 19 on the 302nd day after injection of carcinogen. 3) varicosity of nerve fibers along the course of the terminals in a histiocytoma. Rat No. 5 on the 327th day after injection of carcinogen. ×400. 4) Bundle of thickened nerve fibers in a histiocytoma. The same case. ×400. 6) Bundle of nerve fibers penetrating a mixed salivary gland tumor. Rat No. 12 on the 442nd day after injection of carcinogen. ×400. 5, 10) Ramifications of nerve fibers in the same tumor. ×400. 7) Polyvalent receptor. 9, 11) simple receptors in the same tumor. ×400. 8) Bundle of nerve fibers ramifying in an adenoma of the salivary gland. Rat No. 14 on the 488th day after injection of carcinogen. ×400. 12) Bundle of nerve fibers in fibrosarcoma. Rat No. 20 on the 302nd day after injection of carcinogen. ×400. 13) Dichotomously dividing nerve fibers in the same tumor. ×400.

receptors were present. Some of these, as a result of the great length and numerous branches of the nerve fibers along their course formed an extensive receptor field embracing vessels, tumor cells, and stroma and could be regarded as polyvalent receptors (see figure, 7). Others, consisting of very thin dichotomous branches of a nerve fiber, terminated on the tumor cells by contact thickenings, forming receptors of a simple type (see figure, 9, 11). Finally, receptors of a third group were found among the parts of the tumor with the structure of a tubular adenoma.

Hence, the presence and character of the innervation were largely dependent on the time of appearance, the rapidity of growth, and the structural features of the induced tumors. Tumors of a more benign structure with a slow onset and rate of growth were characterized by a clearly defined pattern of innervation and by the presence of complex endings of sensory type. In the case of malignant change, this pattern of innervation was accompanied by irritation of the nerve fibers and simplification of the receptor apparatus as a result of reduction in the size and number of branches of the nerve fiber. In necrotic tumors the nerve elements could not be seen, and in the surrounding tissues they showed signs of irritation.

These findings are in full agreement with the results of our investigations of human salivary gland tumors, the transplantable Brown-Pearce tumor of rabbits, and transplantable sarcoma M-1 of rats [6, 7, 8]. The presence of sensory endings in human tumors and the appearance of very similar formations in experimental tumors demonstrate that these apparatuses are a means whereby the tumor exerts an influence on the organism.

SUMMARY

This work was devoted to the comparison of the structural peculiarities of induced salivary gland tumors with their innervation. A total of 17 epithelial and connective-tissue tumors of rats were studied. The structure and the functional properties of the innervation apparatus, present in almost all the neoplasms, depended on the degree of malignization. Benign neoplasms (mixed tumor of the salivary gland) had the most differentiated receptors with numerous arborization, as well as polyvalent receptors. More simple terminals of malignant tumors had irritation phenomena.

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