CHANGES IN RAT CONNECTIVE TISSUE ASSOCIATED WITH THE DEVELOPMENT OF TUMORS CAUSED BY IMPLANTATION OF CELLOPHANE

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It has been shown, by the works of a number of authors [3, 5, 8], that the introduction of plastics into the connective tissue of animals causes the formation of tumors. In this case, the frequency with which tumors arise varies, depending on the dimensions of the plastic introduced [5]. When the same plastics are administered in the form of fibers or powder, tumors do not develop. Studying the processes of tumor genesis from implantation of polymeric plastics carries considerable interest, both from a practical and a theoretical point of view.

Plastic prostheses are widely used in surgery, and thus, investigating the genesis of these tumors is important for medical practice. Along with this, the possibility of obtaining tumors in animals by introducing plastics opens a new pathway in studying the mechanism of cancerogenesis, since, in contrast to the cancerogenic polycyclic hydrocarbons, which are chemically active substances, the majority of plastics represent chemically inert compounds.

In a previous report [4], we described the morphological changes in the connective tissue around cellophane, over the first six months after the implantation.

In this work we describe the changes observed in the connective tissue in the period directly preceding the appearance of the tumor (7-15 months).

METHOD

The experiments were performed on 500 non-pedigreed rats and rats from the Vistar lines. A portion of the animals were sacrificed at various intervals for the morphological investigations, and the remaining ones were kept so as to obtain tumors in them.

All of the animals were divided into four groups. The rats of the first group were injected subcutaneously with ground cellophane; the diameter of each fragment was less than 0.1 cm. The rats in the second group were injected with cellophane that was 1 × 3 cm in size, the third group of rats – 2 × 3 cm, and the fourth group of rats – 7 × 2.5 cm.

To study the reactions of the connective tissue, we used the customary stains (hematoxylin and eosin, picrofuchsin according to the method of van Guison, Gomori's silver impregnation) and special histochemical methods for polysaccharides (toluidine blue stain, PAS reaction), for protein functional groups (tetrazone reaction, Barmett's reaction for SH-groups and Barmett and Zeligman's reaction for COO-groups) and for lipids (Sudan-III and Sudan Black).

RESULTS

As can be seen from the data given in the table the frequency of tumor genesis in the rats in the area around the cellophane depended on the measurements of the latter. The greater the size of the implanted plastic, the higher the percent of animals with tumors. Thus, in rats of the first group, where the ground cellophane was implanted, not a single tumor formed in the course of a year, while, for the same period, tumors arose in 3.2% of the rats in the second group and in 16.6% of the third and fourth groups.

All tumors arose at the site of implantation of the plastic, except for one rat, where the tumor appeared at a distance of 1.5 cm from the edge of the plastic. The cellophane became somewhat contorted, but retained its original form and dimensions, and was always located in the center of the tumor or displaced to its edge.

Frequency of Tumor Genesis in Rats, Depending on the Form and Dimensions of the Implanted Cellophane

Dimensions of the plastic (in cm)	No. of animals surviving 10 months	Animals with tumors arising within a year	
		absolute number	%
0.1 (ground cellophane)	144	0	0
1 × 3	184	6	3.2
2 × 3	36	6	16.6
7 × $2^1/_2$	30	5	16.6

In the course of a year, 17 tumors appeared in the three groups of rats. Among them were 9 polymorphocellular sarcomas, 4 spindle cell sarcomas, one fibrosarcoma, one giant cell sarcoma, one osteoblastic tumor, and one tumor of the mammary gland.

Around the cellophane, in the course of the first 6 months following the implantation, a dense collagen capsule formed, which was heterogeneous in its morphological structure and consisted of three layers: an internal proliferating layer, applied directly to the plastic, the capsule itself, and the pericapsular layer.

This same structure of the connective tissue capsule was basically retained in the later intervals, after 7 months, but the separate layers underwent significant changes. In the internal layer, we observed the formation of focal cellular proliferations. In this case, in certain portions of the capsule, young fibroblasts were absent from its surface, and the internal surface was lined with only one layer of mature fibroblasts. In contrast to this, we observed copious growth of young fibroblasts in other portions of the capsule.

As a result of the unequal propagation of fibroblasts on the surface of the capsule, focal cellular proliferations arose. The cells in these were atypical, the dimensions of the nuclei varied, and individual cells contained fat inclusions (Fig. 1). The cytoplasm of the cells was rich in RNA, yielded a weakly pink, diffuse, color in the PAS reaction, and a more intense reaction for SH-groups, COO-groups and the tetrazone test than was observed with the fibroblasts in the other layers. Using the silver impregnation technique, a fine network of argyrophilic fibers appeared between the cells.

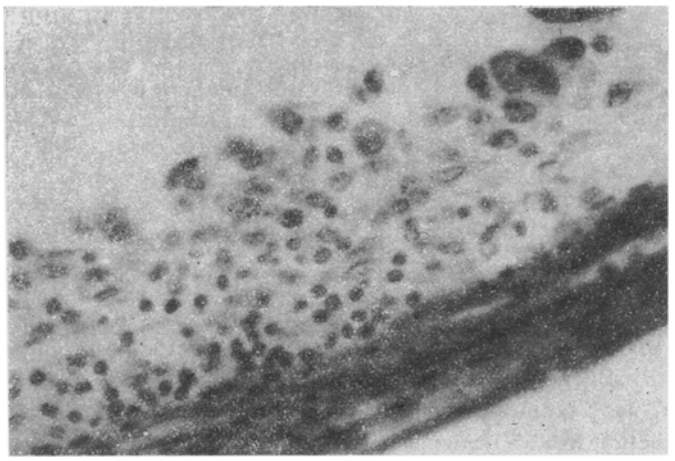

Fig. 1. Growth of atypical fibroblasts in the internal layer of the capsule, around an intact fragment of cellophane measuring 7 × 2.5 cm, after 10 months. Stained with hematoxylin-eosin. Magnification 500. Photomicrograph.

Changes in the form of the collagen fibers in the middle layer of the capsule were observed at the same time as the heterogeneous proliferation of the fibroblasts. Staining with picrofuchsin, the collagen did not take up the color uniformly. In some portions of the capsule, the fibers lost their clear outlines, as though fusing into a single "homogeneous" mass (Fig. 2); in others, we observed an increase in the number of fibroblasts for the middle layer.

The intensity of the PAS reaction in the capsule varied markedly. The swollen collagen fibers, and the portions that appeared like a homogeneous mass, yielded a weak PAS reaction. An intense PAS reaction was observed in the ground substance around the focal cellular proliferations, and in the areas where the collagen retained its normal morphological structure.

Acid mucopolysaccharides, yielding a metachromatic stain with toluidine blue, were observed in the areas where the collagen appeared as a dense homogeneous mass, and in the ground substance around the cellular proliferations.

In investigations for the distribution of functional protein groups in the connective tissue capsule, the most intense reaction was observed in the cells (nucleolus, nuclear chromatin, cytoplasm) and in the ground substance of the internal layer; a less intense reaction was seen in the collagen fibers of the middle layer.

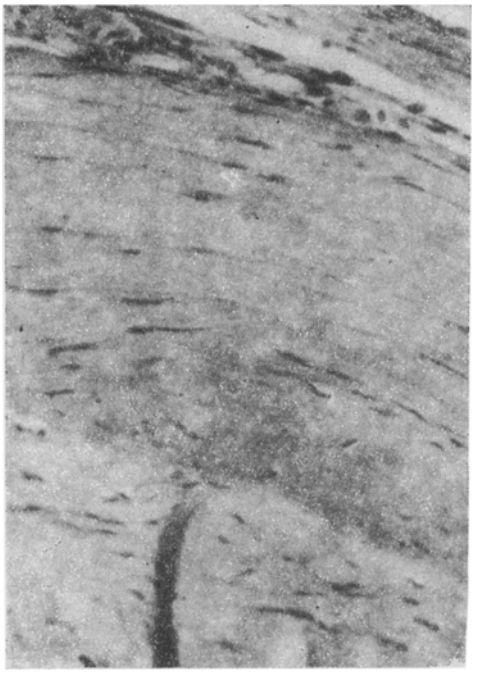

Fig. 2. Disruption of the observable fibrous structure in the collagen of the capsule around a cellophane fragment measuring 1 x 3 cm, after 12 months. Stained by the method of van Guison. Magnification 500. Photomicrograph.

In the trials with the ground cellophane, the connective tissue capsule surrounding the individual fragments of cellophane remained just as thin as in the early period, even 7-14 months after the beginning of the experiment. The morphological structure of the collagen also remained unchanged. Proliferation of the fibroblasts on the surface of the capsule and in the middle layer was widespread and homogeneous, and we did not observe atypical cells or the formation of focal cellular proliferations in isolated portions of the capsule.

The number of individual cells containing fat inclusions was somewhat greater in this case than in the capsules around the whole plastic. In the pericapsular layer, there were many groups of polygonal, round or spindle-shaped cells around the individual cellophane fragments, whose cytoplasm contained large numbers of granules yielding an intense PAS reaction (Fig. 3). When sections were treated with amylase, the intensity of the PAS reaction did not change. Using toluidine blue, these cells stained orthochromatically, or manifested weak metachromasia.

Our experiments succeeded in confirming the data in the literature on the development of tumors in animals subsequent to the introduction of cellophane; we also confirmed the dependence of the frequency with which the tumors develop on the dimensions of the implanted plastic fragment [5]. With increments in the diameter of the implanted plastic (0.1, 1 x 3, 2 x 3, 7 x 2.5 cm), the percent of tumor genesis increased correspondingly (0, 3.2, 16.6).

In the connective tissue capsule around the cellophane fragment, at a late stage (directly preceding the development of tumors), we observed morphological and histochemical changes in the collagen (unequal staining with picrofuchsin, the formation of homogeneous masses of collagen, heterogeneous distribution of PAS-positive material). Similar, but significantly more manifest, changes, around a pellet containing cancerogenic polycyclic hydrocarbons, were described by Yu. M. Vasil'ev [1].

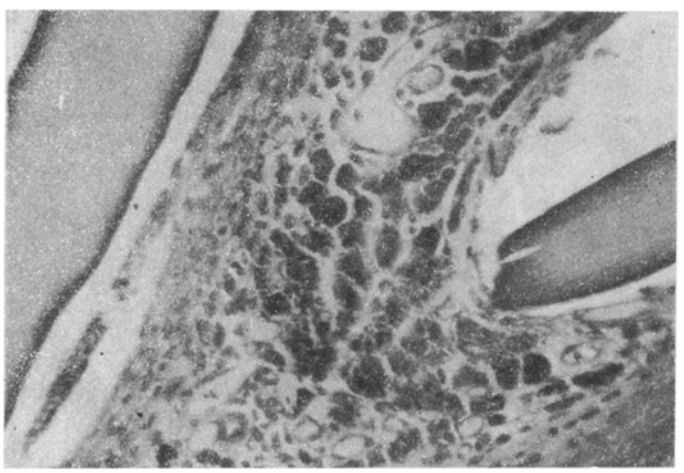

Fig. 3. Cells whose cytoplasm is rich in PAS-positive components, in the capsule around the ground cellophane after 9½ months. PAS reaction. Magn. 270. Photomicrograph.

The connective tissue capsule around the ground cellophane, which did not cause development of tumors, remained thin during the entire period of observation. An interesting characteristic of these capsules was the appearance of large accumulations of cells in the pericapsular layer, rich in PAS-positive granules. The nature and significance of these cells is not clear. It is possible that some of the newly formed collagen around the particles of ground cellophane undergoes resorption and that the appearance of cells with PAS-positive granules is related to this collagen resorption. This question is subject to further study.

Comparison of the results of trials with the intact and ground plastic shows that tumors arise only in those cases where prolonged proliferation of the fibroblasts is combined with abnormally

extensive collagen formation. On the basis of our data, it may be postulated that prolonged proliferation of fibroblasts, under conditions of altered collagen formation, leads to the development of cellular variants, which subsequently yields malignant cells.

The question of the initial localization of foci of malignant growth is in dispute at the present time. Some authors [8] believe that the growth of tumors begins at the surface of the collagen capsule, while others [7] maintain that tumors arise outside the capsule. In our investigations, the presarcomatous foci more often arose at the surface of the capsule, or sometimes, in its middle layer, which is probably related to extension of the cells from the internal layer of the capsule.

The morphology of the focal cellular proliferations, with atypical cells and argyrophilic stroma, coincides with the morphology of presarcoma foci, described by Z. V. Gol'bert and L. M. Shabad [2]. This fact also indicates the similarity of the histogenic processes of tumors associated with the action of cancerogenic hydrocarbons and with implantation of cellophane.

SUMMARY

Small pieces of cellophane and whole cellophane films were introduced subcutaneously in rats. Not a single tumor appeared in rats in which ground cellophane was introduced. In rats in whom whole cellophane films(1 × 3 cm, 2 × 3 cm and 7 × 2.5 cm in size) were implanted the tumors appeared correspondingly in 3.2, 16.6, and 16.6% of the animals.

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