EARLY MORPHOLOGICAL CHANGES IN THE CONNECTIVE TISSUE OF RATS AROUND AN IMPLANTED CELLOPHANE DISK

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In recent years information has appeared in the literature concerning the development of malignant tumors around plastic disks implanted in the connective tissue of animals [2, 6-10]. The development of tumors is dependent on the shape and size of the implanted disk [4, 8].

There is hardly any information in the literature on the morphological changes in the connective tissue preceding the development of sarcoma after implantation of plastics. The only paper on this subject is that of Oppenheimer and collaborators [8], who in the course of the first six months after implantation failed to find any characteristic morphological changes which could be regarded as the initial manifestations of the process of carcinogenesis. According to Oppenheimer and co-workers, at this period a dense collagen capsule is formed around the disk, in which only a few fibrocytes are present.

The object of our investigation was to make a detailed study of the morphological and of certain histochemical reactions of the connective tissue around an intact plastic disk and one which had been cut up into small pieces.

EXPERIMENTAL METHOD

Sterilized cellophane was implanted beneath the skin of 100 rats, weighing 30 and 100 g, either in the form of a disk measuring 1 × 3 cm or as pieces cut up with scissors and measuring less than 0.1 cm. The rats were sacrificed 1, 2, 3 and 4 weeks and 2, 3, 4, 5, 6 and 7 months after implantation of the cellophane.

In order to study the reactions of the connective tissue we used the ordinary stains (hematoxylin and eosin, Van Gieson, Gomori) and histochemical methods for detection of polysaccharides (periodic acid-Schiff reaction, staining with toluidine blue at different pH values, alcyan blue) and also Hale's method with colloidal iron in combination with treatment with enzymes (hyaluronidase, collagenase). For the detection of RNA we used the method of staining with methyl green-pyronine with ribonuclease.

EXPERIMENTAL RESULTS

The whole early period of development of connective tissue reactions (until seven months) around the cellophane disks may be conventionally divided into three phases: the development of an inflammatory reaction, the formation of a collagen capsule and the further changes in this capsule.

One week after implantation an extensive inflammatory cell infiltration can be seen around the disk, consisting of leukocytes, lymphocytes and young fibroblasts. Two weeks after implantation collagen fibers appear

at a short distance from the disk. Next to the disk at this stage is a layer rich in young fibroblasts, in which no collagen fibers are to be seen. Subsequently there is a gradual increase in the number of newly formed collagen fibers. At the end of the first month a loose collagen capsule is formed around the disk, between the fine collagen fibers of which are areas of focal round-cell infiltration and proliferation of fibroblasts. During the second month the individual collagen fibers unite to form bundles, and the round-cell infiltration in the collagen capsule appreciably diminishes. The inner layer of the capsule, in contact with the disk, is rich in young fibroblasts. In this layer binuclear and multinuclear cells may also be observed.

In the 3rd-4th month there is usually a connective tissue capsule around the disk in which three layers can be distinguished (Fig. 1). The outer layer, furthest from the disk, i.e., the pericapsular layer consists of loosely arranged collagen fibers and numerous small blood vessels. The middle layer, or true capsule, consists of dense collagen bundles, between which are dilated capillaries and areas of focal proliferation of fibroblasts. The third layer, in direct contact with the disk, consists of fibroblasts orientated along the disk, and of young fibroblasts with a round nucleus (Fig. 2). Among the latter may be seen cells with larger nuclei than the young fibroblasts and with a well defined nucleolus. The cytoplasm and nucleolus of the young fibroblasts given an intensive reaction for RNA. Staining by Gomori's method shows a network of small loops of argyrophilic fibers in this layer. The presence of young fibroblasts and argyrophilic fibers indicates that collagen formation is taking place in this layer.

Fig. 1. Connective tissue capsule around a cellophane disk 4 months after subcutaneous implantation. Three layers are distinguished in the capsule: pericapsular, true capsule, and inner proliferating layer. Stained with hematoxylin-eosin. Magnification 270×.

The morphological changes in the connective tissue in the experiments with the small pieces of cellophane in their early stages follow the same course as those described above around the intact cellophane disks. The inflammatory reaction around the small pieces of cellophane, however, follows a slower course, and the encapsulation of these particles takes place 1-2 weeks later than the encapsulation of the complete disk. The connective tissue capsule formed around the cellophane particles is thinner than that around the intact disks, and the proliferation of fibroblasts in the inner zone of this capsule is uniform and more conspicuous.

For detection of glycoproteins we used the periodic acid-Schiff reaction. At early periods (1-4 months) the PAS-positive component can be seen in the collagen fibers, and diffusely stains the gound substance between the collagen fibers of the true capsule and in the inner proliferating layer. At later periods (5-7 months) when a dense collagen capsule has been formed, the PAS-positive component is seen in the collagen fibers predominantely. The diffuse staining between the fibers disappears, but that in the inner proliferating layer remains.

In sections treated with hyaluronidase the PAS-positive component is unchanged, but after treatment with collagenase it disappears.

Investigation of the distribution of acid mucopolysaccharides, giving a metachromatic staining with toluidine blue, showed that in the first two months after the experiment weak metachromasia is observed in the granulation tissue surrounding the disk. This metachromasia is diffuse in character and is unevenly distributed in the middle layer of the capsule.

In the experiment with cellophane particles metachromasia was usually observed in the inner layers of the collagen capsule, immediately around the disk. No change in the intensity of the metachromasia in the course of six months could be observed in the experiments with either the cellophane particles or the intact disk. Metachromasia was found throughout the whole period of study.

When the sections were treated with testicular hyaluronidase, which acts on hyaluronic and chondroitin sulfuric acids, the metachromatic staining disappeared. Metachromatic staining could be detected by the use of toluidine blue solutions with pH values not less than 4. These findings are in agreement with the hypothesis that hyaluronic acid is present in the tissue, for chondroitin sulfuric acid gives a metachromatic staining at pH values below 4.

In order to detect acid mucopolysaccharides we also used Hale's method with colloidal iron and staining with alcyan blue. These reactions gave a diffuse staining which coincided with the distribution of the PAS-positive component, and intensively stained thin fibrils were discovered, the distribution of which coincided with the areas of metachromasia.

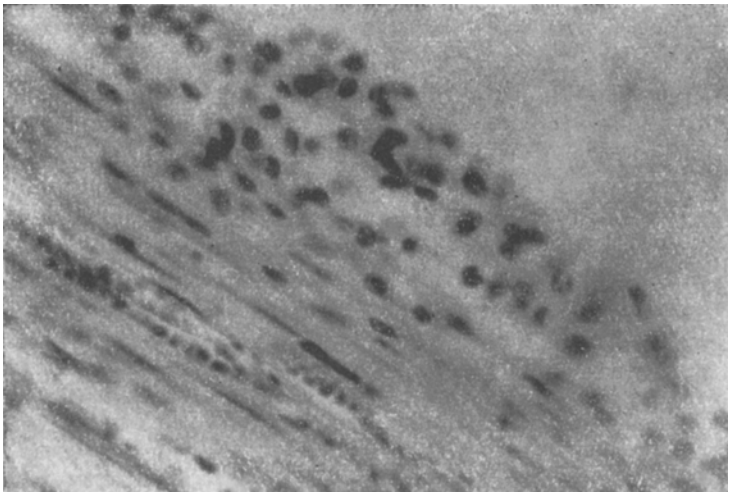

Fig. 2. Proliferation of cells of the inner proliferating layer. Stained with hematoxylin-eosin. Magnification 500×.

The results of our investigations thus showed that after cessation of the inflammatory reaction around the disk in the connective tissue the constant proliferation of cells remains. This cell proliferation takes place in the collagen capsule itself and in its inner border, adjacent to the disk. The cells of this inner zone are rich in cytoplasmic and nuclear RNA, indicating their functional activity. We cannot therefore agree with the statement of Oppenheimer, who considers that, starting after three months, the connective tissue capsule around the disk is in a "resting state," and that all active processes in it become quiescent. If the whole process were so, it would be difficult to understand why and from whence tumors would arise after a long period of quiescence.

It is evident that, in spite of the chemical inertia of the cellophane disk, it constantly influences the tissues, as shown morphologically by the constant proliferation of the cells and by the growth of a collagen capsule. Metachromatically staining acid mucopolysaccharides are known to appear [3] in the tissues in the early stages of collagen formation. The constant metachromasia in the collagen capsule around the disk is therefore evidence that the process of collagen formation is taking place all the time in the capsule. In recently published work by Oppenheimer and co-workers [5] an increase in the velocity of incorporation of S^{32} in the polysaccharides of the collagen capsule around polystyrene was noted, by comparison with the polysaccharides of normal connective tissue.

These results of biochemical investigations are in good agreement with the morphological and histochemical findings described above, demonstrating that the connective tissue capsule around polymerized plastics is not a "resting," inactive tissue. Such a capsule constantly contains cells which are proliferating and forming collagen. Focal collections of young fibroblasts during prolonged collagen formation were described by Vasil'ev [1] in experiments with pellets containing carcinogenic hydrocarbons. In respect of the experiments with cellophane disks it may therefore be postulated that the growth and activity of the connective tissue cells when the conditions of their existence are severely modified (the presence of a dense collagen capsule) are among the factors playing a role in the development of tumors. Further research is needed to give the conclusive solution of this problem.

SUMMARY

Cellophane films (1 x 3 cm in size) and small pieces of cellophane were implanted subcutaneously to rats. These animals were sacrificed at different intervals ranging from 7 days to 7 months. Connective tissue changes occurring around the films after the implantation were studied with the aid of various morphological and histochemical methods. In 3-4 months 3 zones could be distinguished in the capsule around the films: 1) pericapsular layer, 2) capsula proper, and 3) internal layer containing proliferating cells.

Chromotrophic acid mucopolysaccharides were present in the collagenous capsule up to the end of 7 months; the presence of such mucopolysaccharides may be regarded as an indication of an incomplete process of collagen formation. Foci of cellular proliferation were present in the "capsula proper" and in the internal proliferative layer. The author briefly discusses the role of prolonged collagen formation and of the focal cell proliferation in the mechanism of plastic cancerogenesis.

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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.
