CHANGES IN THE SUBCUTANEOUS CONNECTIVE TISSUE OF THE RABBIT EAR IN THE PROCESS OF TUMOR DEVELOPMENT INDUCED BY 9,10-DIMETHYL-1,2-BENZANTHRACENE

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Morphological and histochemical changes in the cutaneous epithelium in the process of development of cancer have been investigated rather extensively [7, 9], while study of the changes in the connective tissue has been undertaken only in isolated works [11, 13, 14]. At the same time, in various proliferation processes the epithelial changes are usually correlated with the changes in the underlying connective tissue [2, 3]. Certain investigators assign great, even leading, significance to the changes in the dermis in relation to the genesis of cancer of the skin [8, 12].

The purpose of this work was to study any morphological and histochemical changes of the dermis that develop at different stages of cancerogenesis caused by the presence of a chemical cancerogenic substance in the skin of a rabbit.

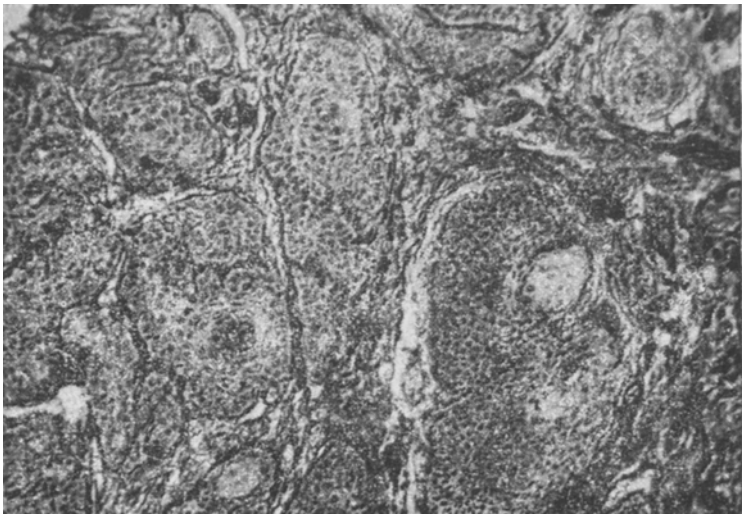
EXPERIMENTAL METHOD

The experiment was set up on 33 rabbits. We used 14 rabbits in the first group. The skin of both ears of 7 of the rabbits was smeared with one application of a 1% solution of 9,10-dimethyl-1,2-benzanthracene (DMBA) in benzene and the ears of the other 7 rabbits, with pure benzene. The animals were sacrificed 1-5 and 10 days after the application. In the second group, the skin of the ear of 17 rabbits was smeared with 1% DMBA in benzene and the skin of the 2 control rabbits with pure benzene three times a week over the course of the entire experiment. The rabbits were sacrificed at various intervals after initiation of the experiment (from 38 to 279 days).

Pieces of the ear were fixed in Carnoy's fluid and a 10% solution of formalin, and then imbedded in paraffin. Sections were stained in hematoxylin-eosin and in picrofuchsin (Van Gieson method), and were impregnated with silver according to Gomori. Histochemical methods were applied for demonstration of ribonucleic acid according to Brasha; desoxyribonucleic acid (according to Fuelgen); certain protein groups (fragments of tyrosine, tryptophan and histadine, using the reaction of tetrazone combination; sulfhydryl groups according to the method of Barnett, Tsou, and Zeligman); acid mucopolysaccharides using toluidine blue and Alcian blue stains (control preparations were treated with testicular hyaluronidase); polysaccharides containing 1,2-glycol groups, using the reaction with periodate and Schiff's reagent (control preparations were treated with amylase).

EXPERIMENTAL RESULTS

In the course of the first month of applications, we observed a diffuse hyperplasia of the epithelium; we also noted hyperplasia and cornification of the hair follicles. At this time, in the dermis under the hyperplastic epithelium, focal infiltrates appeared, consisting of polynuclear and lymphoid cells. The polynuclears almost completely disappeared from the tissue by the end of the first month, while the lymphoid infiltrates were seen throughout the course of the entire application period (up to 9 months). Following the first applications, the arrangement and histochemical characteristics of the collagen fibers and ground substance of the dermis changed very little. Later (by the end of the first month), the connective tissue under the hyperplastic epithelium gradually grew porous, and a considerable number



Retention of a basal membrane around cancerous cores after 5 months of applications. In certain areas the basal membrane is thickened, while in others it disintegrates into a network of thin, reticulin fibrils. Silvering. Magnification of 590.

of young fibroblasts appeared in it; the ground substance in this dermis, as in the dermis of normal skin, stained orthochromatically with toluidine blue. With Gomori's silvering technique, the basal membrane was clearly seen under the hyperplastic epithelium; this membrane yielded a PAS-positive reaction, as well as positive reactions for protein groups (reaction of tetrazone combination, reaction for carboxyls and sulfhydryls).

Beginning with the 2nd month of the experiment, clearly defined papillomas appeared on the skin, consisting of multilayered, cornifying epithelia which were separated from the surrounding connective tissue by an argyrophilic basal membrane; in certain areas, the latter was thickened, while in others, conversely, it disintegrated into a net of thin reticulin fibrils. This membrane yielded a weaker protein reaction than the basal membrane on the epidermis of normal skin, but the intensity of the PAS-reaction remain unchanged. The connective tissue of the papilloma contained numerous thin collagen fibrils, with fibroblasts and lymphoid cells distributed between them. When stained with toluidine blue, the ground substance of this connective tissue was orthochromatic, or contained only small amounts of chromotropic mucopolysaccharide.

By the third month of the experiment, in certain areas of the treated skin, along with papillomas, there appeared malignant proliferations of epithelium, showing infiltrative growth; they began from the base of the papilloma or from the hyperplastic epithelium. In addition to large, cancerous tumors penetrating the muscle and cartilage, microscopic investigation often disclosed small foci of invasively growing epithelium. Around the cancer nucleus and extensions we usually observed a basal membrane with a varying width. These membranes were argyrophilic, yielding positive PAS-reaction, and also reactions for various protein groupings. In certain areas around the cores of epithelium, instead of a compact membrane we observed a thin network of argyrophilic fibrils (see figure). Around the invasively growing epithelial cells there was always an intense proliferation of connective tissue; the zone of this proliferation was usually considerably wider than the zone of invasive growth of the epithelium. The proliferating connective tissue was rich in young fibroblasts. It contained a large number of reticulin fibrils, and the ground substance between the fibroblasts and the epithelial cores, as a rule, was very rich in mucopolysaccharides, which stained metachromatically with toluidine blue and was disrupted by testicular hyaluronidase.

The facts described show that the processes of cancerogenesis in the cutaneous epithelium of the rabbit ear are accompanied by profound changes in the underlying connective tissue. One of the very early, and stably retained, reactions in this tissue is the focal lymphoid infiltration. The mechanism of its development is unclear, but a similar reaction also arises with the injection of cancerogenic hydrocarbons under the skin of rats [1]. Possibly, it is a unique, local, immune reaction to changes in the antigenic properties of proteins that are caused by cancerogens.

It should be noted that in the early stages of cancerogenesis we did not observe marked changes in the histochemical characteristics of the dermis ground substance. In particular there were no accumulations in the dermis of

chromotropic mucopolysaccharide, which accumulates in the dermis within several days after the first application of the cancerogen [14].

Proliferation of fibroblasts in the connective tissue was seen in the first months of the experiment, but was manifested weakly. On the other hand, in the connective tissue surrounding the invasively growing extensions of malignant epithelium we noted very marked proliferative processes. It may be postulated that during malignant change the cutaneous epithelium acquired "stromatogenic properties", with the capacity to cause marked proliferation of fibroblasts. A characteristic property of these young fibroblasts was their ability to produce large amounts of chromotropic, acid mucopolysaccharide. It is interesting to note that, as was shown by our previous experiments [5], with inflammatory proliferations the invasive growth of normal rabbit cutaneous epithelium ceased immediately after accumulation of chromotropic mucopolysaccharide began in the surrounding connective tissue. On the other hand, proliferation of cancerous epithelium is apparently not inhibited within an environment containing this mucopolysaccharide. Obviously, a similar "local environment" differed in its effect on the invasive growth of normal and tumor epithelial cells. The accumulation of chromotropic mucopolysaccharide in the invasive growth zone of cancerous tumors has also been noted by certain other authors [4, 15].

Disruption of the basal membrane of the epithelium is usually regarded as a necessary condition for invasive growth [10]. As was shown in our experiments, in the early stages of cancerogenesis the integrity of the basal membrane of the epidermis is basically retained, and only in certain areas does one observe its attenuation. Invasive growth of the malignant epithelium is accompanied by new formation of a membrane around the epithelial cores. Changes in the membrane during invasive growth of cancerous epithelium are basically similar to those which are observed with invasive growth of non-tumor epithelium associated with inflammatory proliferations in the skin [5] or in the mammary gland of the pregnant mouse [6]. This fact confirms the common features of the basic mechanisms behind invasive growth of tumor and normal cells.

SUMMARY

33 rabbits were used in the experiment, and the carcinogenic treatment employed was painting the ears of rabbits with 1% 9,10-dimethyl-1,2-benzanthracene in benzene 3 times a week. After such treatment, a high incidence of skin tumor was observed. The processes of carcinogenesis in the epithelium of rabbit ear were accompanied by marked alteration of the dermal connective tissue. Lymphocytic infiltration in the derma appeared at an early stage and persisted up to the end of the experiment.

Invasion of dermis by the cancerous epithelium was accompanied by marked proliferation of the surrounding connective tissue, in which a great amount of chromotropic mucopolysacchride was found. In the early stage of carcinogenesis the basal membrane of the epithelium was preserved; at a later stage around the invasively growing epithelial zones newly developed basal membranes were observed.

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