PIGMENTED TUMORS IN RATS INDUCED BY INTRODUCTION OF PLATINUM AND CELLOPHANE FILMS INTO THE CHAMBER OF THE EYE

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A piece of cellophane film or thin platinum foil was implanted into the eyeball of rats so that the lens was separated from the retina. Tumors developed in 26.9% of the animals, and 85% of the tumors were obtained in animals in which platinum foil was implanted. The tumors were detected in the eye and adjacent tissues as microscopic nodules consisting of large basophilic cells which, in some cases, contained melanin. These cells migrated along the blood vessels to organs including the liver, lungs, thymus, and lymph glands. Metastases often grew to a large size (as big as a pigeon's egg), and the tumor tissue completely replaced the tissue of the organ. Good growth of tumor tissue was obtained after transplantation in diffusion chambers.

Cellophane tumors were first obtained by Oppenheimer and co-workers [7] in 1948 by implantation of cellophane film beneath the skin in rats. In their subsequent work these writers showed that cellophane powder or shredded cellophane never induces tumors [8]. This fact suggested that the induction of tumor growth by implantation of cellophane film is due to disturbance of the interaction between the tissues in the region of the films [5].

Studitskii has shown that disturbance of the normal relations between tissues by any barrier of non-carcinogenic nature (cellophane film, platinum or gold foil) will lead to malignant degeneration of tissues. For instance, wrapping the gastrocnemius muscle of a rat is followed by the development of a rhabdomyoblastoma, and if the tibia is wrapped subperiosteally, an osteogenic sarcoma develops [2, 4]. These results have provided the basis for development of the "disturbed regulation" theory of cancer. According to this theory a disturbance of tissue regulation creates conditions under which cells deprived of their mutual exchange of information become capable of independent growth [3]. Confirmation of the disturbed regulation theory was later given by Korobko [1], who observed the development of malignant tumors in many organs, including the testis, when wrapped in cellophane.

The object of the present investigation was to obtain tumors by disturbing tissue contacts in the eye in rats. The eye is the classical example of an organ in which tissue inductions take place, and for this reason it appeared to be a particularly interesting model.

EXPERIMENTAL METHOD

Cellophane film or thin (0.1 mm) platinum foil was implanted into the posterior chamber of the eye of an adult rat. The method of implantation was as follows: with a sharp razor an incision was made in the cornea at the boundary between its transparent and opaque parts facing the upper lid. Sterile disks of platinum foil or cellophane were placed into this incision so that the lens was in front of the barrier. As a result of the operation the retina was separated from the lens. The eyelids were sutured after the operation.

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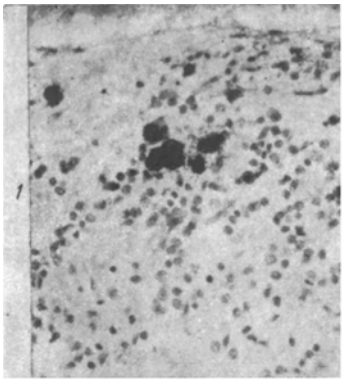

Fig. 1

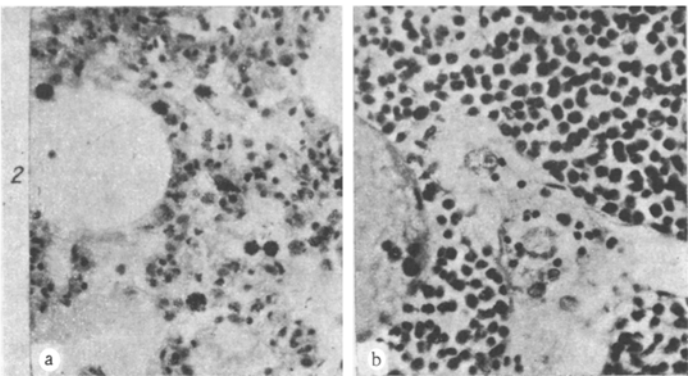

Fig. 2

Fig. 1. Retina of rat after transplantation of platinum foil; chaotically arranged pigmented cells can be seen. In this and other figures, sections stained with Carazzi's hematoxylin, 160 ×.

Fig. 2. Penetration of tumor cells into lung tissue (a) and into sinuses of a lymph gland (b), 160 ×.

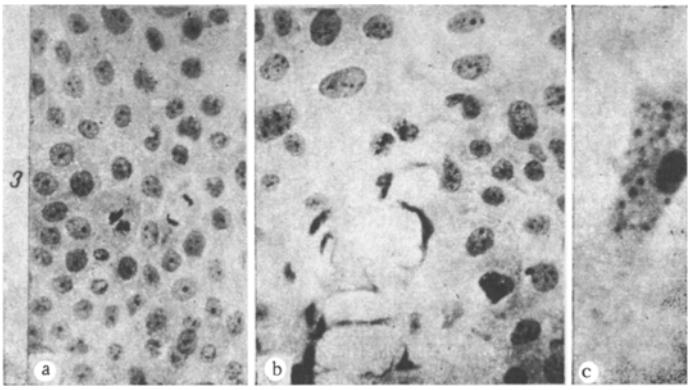

Fig. 3. Cultivation of tumor cells in diffusion chambers: a) layer of tumor cells on 7th day of cultivation (240 ×; total preparation); b) structure of transplanted tumor on 14th day of cultivation (240 ×; total preparation); c) pigmented cell on 14th day of cultivation (360 ×; total preparation).

By the time of appearance of the first tumors 52 animals were still alive. Altogether 12 tumors were obtained in the rats into which platinum foil was implanted but only 2 tumors in the rats receiving cellophane. The difference in the incidence of the tumors was evidently due to the fact that the cellophane was easily crumpled during the operation, so that it could not completely isolate the retina from the lens. The first tumor appeared 1 year 7 months and the last 2 years 1 month after the operation.

The material was fixed in Bouin's fluid or in Zenker's fluid with the addition of acetic acid, and also in neutral formalin. Paraffin sections, 4-5 μ in thickness, were stained with Carazzi's hematoxylin and counterstained with eosin, stained with Heidenhain's iron hematoxylin and counterstained by Mallory's method, or stained with azocarmine and counterstained by Mallory's method; for determination of RNA the Brachet's reaction was used.

To study the histogenesis of the tumors, the tumor tissue was cultivated in diffusion chambers [6]. The diffusion chambers were implanted intraperitoneally in rats. The chambers were removed 3, 7, and 14 days after transplantation and the filters were fixed in alcohol-formol and total preparations were stained with Carazzi's hematoxylin.

EXPERIMENTAL RESULTS

As was stated above the incidence of the tumors after introduction of cellophane films and platinum foil into the rats' eye was fairly high, namely 26.9%. Microscopic study of the specimens showed that the operation disturbed the layers of the retina. The retinal cells lost their regular arrangement, although something like the layers continued to be observed throughout the experiment (Fig. 1). Around the platinum or cellophane a connective-tissue capsule was formed, and it was most highly developed at the corners of the eye. On the side of the lens the capsule consisted of several layers of collagen fibers with a few fibroblasts. On the side of the retina as a rule there was no capsule, for the retinal cells were either in direct contact with the platinum or they were separated from it by a single layer of fibroblast-like cells.

None of the tumors attained a large size. However, individual nodules of tumor tissue could always be found in the region of the retina and also in the tissues surrounding the eyes. The cells of these nodules stained strongly by Brachet's method. They measured 15-20 μ in diameter and contained large nuclei and large pyroninophilic nucleoli. Many of these cells contained pigment, so that the nodules formed by them could be clearly seen in the specimens. Many of the cells, both pigmented and nonpigmented, could be seen in the blood vessels of the eye and in the adjacent tissues.

Large tumors, the size of a pigeon's egg, were found in the lungs and liver. Macroscopic tumor nodules (0.5 cm^3) were present in nearly every case in the thymus and in mediastinal lymph glands.

The character of invation of the various organs by tumor cells was very similar: tumor cells migrated along the blood vessels and penetrated from the capillaries into the parenchyma of the organ (Fig. 2). The tumor cells found in the blood vessels hardly ever contained pigment, but as soon as they penetrated into the tissue of an organ (lung, liver, lymph gland, or thymus) melanin appeared in them (Fig. 2). The tumor cells were always larger than the cells of the affected organ, and very often they contained two nuclei. In the blood vessels the shape of the tumor cells was highly variable, many of them having outgrowths of different types, but on entering the parenchyma of the organ the tumor cells acquired their usual round shape. Most of the organ was gradually replaced by tumor tissue, and only its stroma retained its original structure.

During cultivation in diffusion chambers, the tumor cells grew well regardless of the original material: whether the tumor nodules were taken from the eye itself or from the metastases. In the early stages of cultivation (3 days) the cells migrated actively from the original graft and spread over the filter. In the later stages of cultivation (7 and 14 days) nearly the whole of the filter was penetrated by tumor tissue. The central part of the filter was usually invaded by a sheet of cells of approximately uniform shape and size. In this zone the cells divided intensively (Fig. 3a). In peripheral parts of the transplanted tumor some degree of differentiation of the cell and the beginning of formation of definite structures could be observed (Fig 3b). In this zone the cells showed considerable polymorphism. In those parts of the filter which were not yet completely covered by tumor tissue, cells containing pigment were frequently found in the late stages of cultivation (Fig. 3c). Pigment granules were usually situated in the central part of the cell, and the outgrowths did not contain pigment granules.

Disturbance of the contacts structures in the eyeball thus led to the formation of pigmented tumors, which metastasized actively in various organs, in which they attained a large size. This type of growth is characteristic of melanomas and it has been described for melanomas of carcinogenic origin [9].

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