HISTOPATHOLOGY OF PRECANCEROUS CHANGES IN THE CEREBELLUM OF ALBINO RATS AFTER IMPLANTATION OF 9,10-DIMETHYL-1,2-BENZANTHRACENE

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The development of gliomas in the rat cerebellum under the influence of a 9,10-dimethyl-1,2-benzanthracene pellet is preceded by three distinct components: 1) the formation of a fibrous capsule around the DMBA pellet; 2) severe changes in the blood vessels of the fibrinoid necrosis type; 3) progressive hypertrophy and proliferation of the neuroglia.

Most morphological studies of chemical carcinogenesis in the central nervous system have been undertaken on the cerebral hemispheres of laboratory animals, and most have been carried out to study the final stage of the process, viz. the onset of tumors [5, 9, 10, 14-17]. The spectrum of neoplasms arising in the rat cerebellum, and the actual process of tumor formation in this part of the central nervous system have received very little study.

In an attempt to obtain new experimental data on the dynamics of development of the preglioma, the cerebellum was chosen as test object, more especially because many tumors of children arise in this part of the central nervous system.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred female albino rats weighing 60-70 g. A pellet of chemically pure 9,10-dimethyl-1,2-benzanthracene (DMBA)† weighing 1.5-2 mg was implanted in the region of the nuclei of the right half of the cerebellum. A pellet of paraffin wax of the same weight was implanted into the same region of control animals [10-12].

Altogether 214 animals were used in the experiments, 153 experimental and 61 control. The animals were under observation for 1 year. For the first 14 days the animals were sacrificed daily, and later until the 240th day they were sacrificed once a week, in batches of two rats from the experimental and one from the control group. To determine the percentage yield of tumors and the mean latent period of onset and the spectrum of the growing neoplasms, 31 animals were left alive.

The cerebrum and cerebellum were taken for histological investigation. Material was embedded in paraffin wax and gelatin. Sections were stained with hematoxylin-eosin, with azan, for fibrin by Shueninov's method, and impregnated with silver by Miyagawa's and Lashkov's methods [6] and with gold by Cajal's method. During the investigation particular attention was paid to reactive changes appearing at different stages of the experiment in all structural elements of the cerebellum.

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†Manufactured by the firm of Fluxa A. G., Switzerland.

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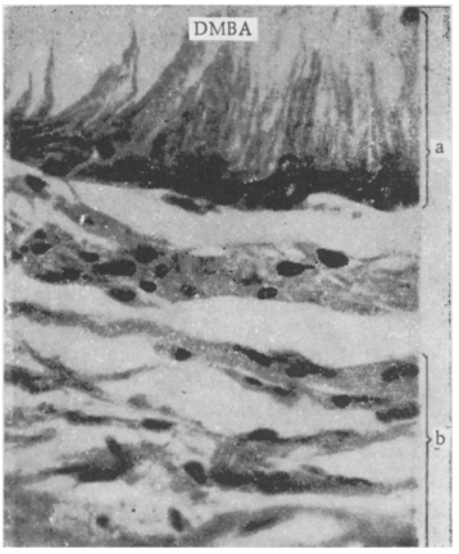

Fig. 1. Capsule around DMBA pellet: a) fringed cells; b) circular connective-tissue layer. Hematoxylin-eosin, 400×.

EXPERIMENTAL RESULTS

The results reflecting the morphology of carcinogenesis in the central nervous system differed significantly from descriptions of chemical carcinogenesis in the skin and other internal organs, in which a regular succession of phases of proliferation, ultimately leading to the growth of tumors, is found [1, 2, 4, 7, 8]. In the rat cerebellum after implantation of a DMBA pellet, three distinct components of the preglio-matous changes developed.

The first component of chemical carcinogenesis in the central nervous system is the formation of a capsule around the pellet of carcinogen. After 24 h the DMBA pellet was surrounded by small connective-tissue cells resembling polyblasts, with a dark oval nucleus and foamy cytoplasm. After 48 h, elongated cells with an oval, hyperchromic nucleus were lying perpendicularly to these cells. By the 5th day of the experiment the cytoplasm of the cells lying next to the carcinogen was considerably increased in volume, and tiny granules and vacuoles of various sizes had appeared in it. On the 8th day the apical part of these cells was split longitudinally and had become fringed (Fig. 1a). One or several round, often hyperchromic nuclei, with a clearly defined nuclear membrane and a large nucleolus could be seen at the base of the cells.

After 3-4 weeks, filamentous outgrowths of the apical part of the cells in contact with DMBA crystals had increased in length, and small vacuoles and basophilic granules had appeared in them. Next to the fringed cells was the second circular layer of long connective-tissue cells with an oval nucleus, staining blue with azan (Fig. 1b). The third layer of the capsule consisted of a curiously contorted network of small loops of dilated capillaries and larger blood vessels resembling venules. Preexisting cerebellar vessels became involved in this unusual process: they ran toward the pellet and the wound channel, and were dilated and congested with blood. This structure of the capsule around the pellet of carcinogen persisted throughout the experiment. In the brain substance surrounding the DMBA pellet, an aseptic inflammatory reaction characteristic of a focus of trauma after introduction of a foreign body into the brain [3], was observed from the 1st until the 30th day.

In no case was a capsule observed around the paraffin pellet in the control animals. After the 30th day, when the inflammatory reaction induced by the trauma and implantation of the pellet had subsided, at all stages of the experiment the paraffin pellet was surrounded by only a single layer of elongated connective tissue cells or by a band of hyalinized tissue.

The second component of the pretumor changes in the cerebellum consisted of changes in the blood vessels. They appeared essentially after the 14th day and persisted until the end of the experiment (365 days), gradually increasing in intensity. The distinguishing feature of this process was the severe damage to the vessel wall, which became permeable to plasma proteins, with the consequent development of marked plasma transudation and deposition of fibrin in the vessel walls and its leakage into the surrounding tissue spaces, where the fibrin could be detected by Shueninov's method (Fig. 2a). The form and degree of this process varied: most commonly fibrinoid swelling of the arteries in the vascular layer of the capsule or in the subarachnoid spaces of the cerebellum was observed near the DMBA pellet. In other cases the fibrin escaped outside the walls of the large veins adjacent to the carcinogen. The principal element of this group in the thickened wall was a fibrinous mass which, together with the vessel wall, developed hyalinosis or, most commonly, necrosis. In these cases the whole mass became permeated with tubules in which recent or lysed erythrocytes and a few eosinophils could be seen. In other cases, a loose fibrin clot was formed and at the same time, marked destruction of the vessel wall took place. Such a clot could undergo recanalization with proliferation of the endothelium at the periphery of the fibrinous masses.

No pathological changes were observed in the blood vessels in the cerebellum of the control animals.

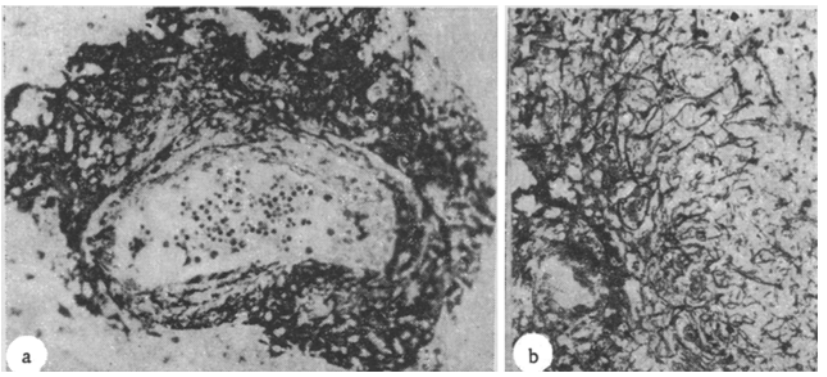

Fig. 2. Lesion of blood vessel wall, transudation of plasma, and deposition of fibrin (a). Shueninov's stain, 200×. Network of hypertrophied astrocytes surrounding DMBA pellet (b). Cajal's orthosublimate method, 400×.

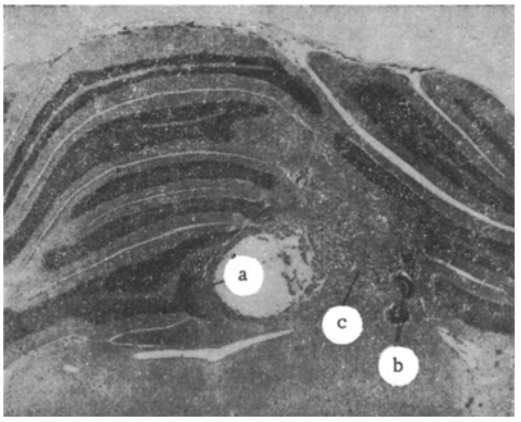

Fig. 3. Final stage of carcinogenesis in the rat cerebellum: a) capsule around DMBA pellet; b) altered vessels; c) focus of newly formed tissue. Hematoxylin-eosin, 10×.

The third constant element of pregliomatous change in the central nervous system was hyperplasia of the astrocytes. By the 21st day astrocytes in the brain substance surrounding the capsule of the carcinogen showed marked hypertrophy. Their thick vascular pedicles stretched out towards the pericapsular vascular plexus. On the 28th day a thick network of astrocytes with hypertrophied bodies formed a strong barrier around the area of the DMBA pellet (Fig. 2b). Hypertrophy of the astrocytes was observed until the 70th-80th day, after which this reaction changed qualitatively, and foci of astrocyte proliferation appeared. Intensive proliferation of the astrocytes, which occurred increasingly after the 100th day of the experiment, terminated with the formation of gliomas. In the initial stages of development of the gliomas, in most cases three components of carcinogenesis could be distinguished (Fig. 3).

In the surviving group of animals, tumors of the cerebellum developed in 27 of the 31 rats. The percentage of induced tumors in normal rats was thus 87.1. The mean latent period of development of the tumors in these animals was 246.7 ± 37.5 days. Histological study of the 27 primary tumors of the cerebellum showed that they were mainly of the glial series: three astrocytomas, four protoplasmic astrocytomas, 8 dedifferentiated astrocytomas, 1 angioastrocytoma, 2 oligodendroglioblastomas, 4 glioblastoma multiforme, and 5 mixed glioblastomas.

In the course of chemical carcinogenesis induced by implantation of a DMBA pellet into the nuclei of the right cerebellar hemisphere in albino rats, the pregliomatous changes are characterized morphologically by three distinct features, which are never observed in the control group, namely: 1) the formation of an unusual fringed capsule; 2) marked changes in the blood vessels of the fibrinoid necrosis type, frequently with perivascular seepage of fibrin; 3) progressive hypertrophy and proliferation of the neuroglia. These processes, as the writers' previous observations showed, also occur after implantation of pellets of a carcinogen into other parts of the central nervous system, but they are particularly severe in the cerebellum. The pathogenetic role of these changes is receiving further study.

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