HEALING OF SMALL MECHANICALLY INDUCED BRAIN INJURIES IN RABBITS DURING EARLY ONTOGENY

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Previous investigations [1, 2] have shown that the mammalian myocardium in the late intrauterine period is still capable of complete cellular regeneration, but loses this property immediately after birth. The aim of this investigation was to study the regenerative powers of another highly differentiated tissue, namely nerve tissue, in early ontogeny.

EXPERIMENTAL METHOD

Rabbits at the 20th-24th day of pregnancy were used. Under ether anesthesia, preceded by intramuscular injection of thiopental sodium in a dose of 40 mg/kg body weight, a midline laparotomy incision 7-9 cm long was made and the uterus, together with the fetuses present in it, were exteriorized into the operation wound. An incision not more than 1 cm long was made above one of the fetuses in the upper pole of the uterus, and the head of the fetus was brought up to the incision where it could be clearly examined through the unopened fetal membranes. In this position the fetal head was firmly fixed with the fingers and divided conventionally into two equal parts by a longitudinal midline. A perpendicular line was then drawn through its center, and the brain punctured on that line 2-3 mm to the right of the point of intersection of the two lines. The puncture was made through the fetal membranes, and then through the fetal skin and bone by means of a manipulation needle fitted to a syringe; a polyethylene guard was fitted over the needle at a distance of 6 mm from its lower end. In all cases the same parts of the brain, namely the right parietal region, were subjected to a stereotyped mechanical injury. In some cases a small quantity of ink (not exceeding 0.1 ml) was injected into the injured part by means of the syringe. After withdrawal of the needle from the fetal membranes, they were tied with catgut and returned into the uterus, which was then sutured with continuous catgut. Similar manipulations were carried out with all fetuses in the uterus. The temperature in the region of the operation wound was 38-40°C.

Operations by the method described above were performed on 21 animals. In three of them the dead fetuses aborted on the 2nd-3rd day after the operation. In the remaining cases some of the rabbits gave birth spontaneously, whereas in the rest, if necessary for histological examination, the fetuses were removed by laparotomy and caesarian section at different times after the operation. Altogether in the course of the experiment 129 animals were obtained between 1 and 45 days after injury.

After fixation in 10% neutral formalin solution the brain was embedded *in toto* in paraffin wax. Histotopographical sections were cut for histological examination and stained with hematoxylin and eosin, by Nissl's, Van Gieson's, and Spielmeyer's methods, and by Feulgen's method for DNA.

EXPERIMENTAL RESULTS

Extensive foci of necrosis of nerve tissue were found at the site of trauma and also hemorrhages into this region in the rabbits on the first day after the operation (Fig. 1a). Polymorphic changes, expressed as lysis of basophilic substance, as a rule segmental in character, as swelling of the cell bodies, and weakening or intensification of staining of the cytoplasm were observed in the surrounding regions of the brain. The changes described in

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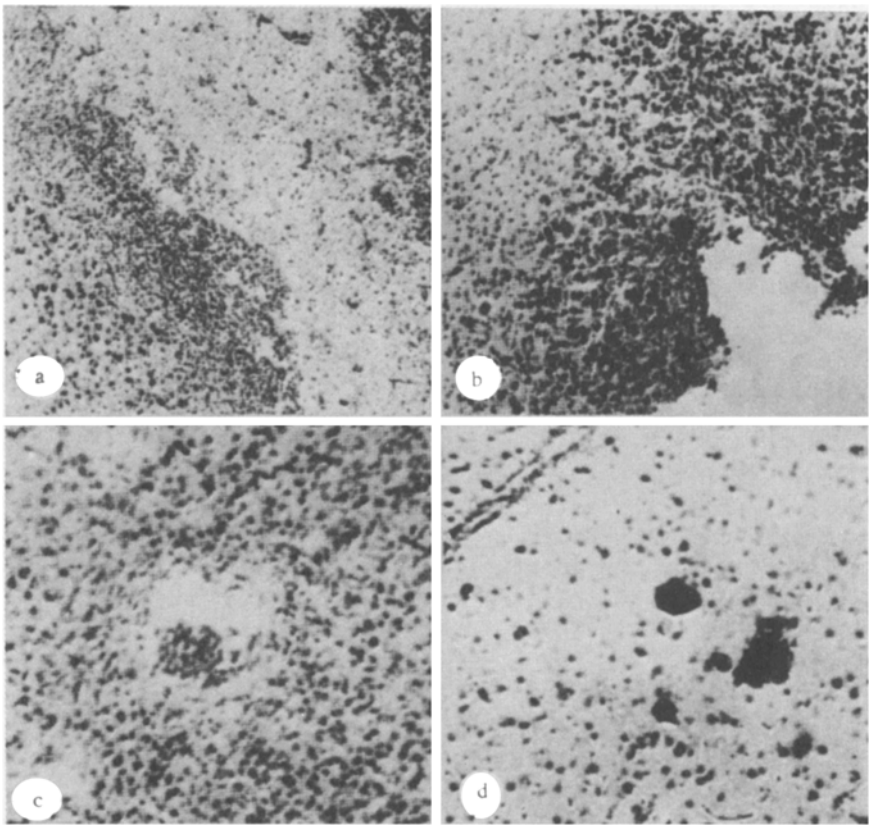

Fig. 1. Stages of healing of small mechanical injuries to the rabbit fetal brain. a) Area of necrosis of brain tissue on first day after operation. Hematoxylin—eosin, 140 ×; b) proliferation of gliocytes on boundary with focus of injury on 7th day after operation. Hematoxylin—eosin, 200 ×; c) proliferation of gliocytes in focus of injury on 11th day after operation. Nissl's stain, 200 ×; d) ink drops among glial-fibrous tissue at site of injury on 40th day after operation. Nissl's stain, 200 ×.

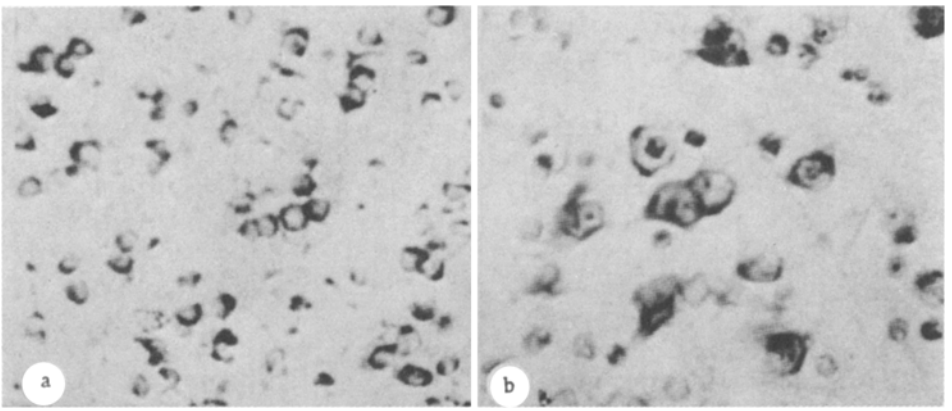

Fig. 2. Amitoses in rabbit fetal nerve tissue: a) amitotic division of gliocytes near focus of injury; b) amitotic division of neuron at a distance from focus of injury. Nissl's stain, 900 ×.

the neurons can be classed as evidence of retrograde degeneration and also as edematous or, to some extent, ischemic. They were variously expressed both close to and far from the site of trauma. Palely stained spaces visible around the cells and vessels confirmed the presence of edema of the brain tissue.

On the 3rd-7th day marked proliferation of glial cells could be seen in the brain of the traumatized fetuses (Fig. 1b). Division of gliocytes took place both by mitosis and by amitosis, as shown by the presence of many glial cells in the preparations with an incomplete transverse constriction band in the nucleus. Intensive division of gliocytes was observed on the boundary with the focus of injury. Absorption of necrotic tissue, some of it through the activity of proliferating gliocytes, took place simultaneously. The gliocytes grew in size in such cases and granules of debris appeared in their cytoplasm.

On the 10th-20th day processes of formation of a glial barrier separating the undamaged areas of the brain from the traumatic focus developed, pursued their course, and were largely terminated in the rabbit fetuses. Young connective-tissue cells also evidently took part in the formation of this barrier. A certain number of lymphoid cells, histiocytes, and also elongated cells of fibroblast type were found almost everywhere among the proliferating gliocytes. On the 20th day after the operation at the site of trauma cavities, empty or containing proliferating cell forms in their lumen, were observed most frequently (Fig. 1c). After 40-45 days, besides cystic cavities drops of ink, lying in glial-fibrous tissue, could be observed in some animals (Fig. 1d). Evidently in some cases proliferating glial-fibrous structures completely filled the small defects formed at the site of the focus of necrosis. Mechanical approximation of the edges of the cystic cavities, associated with growth of the nerve tissue of the brain, likewise cannot be ruled out. In these cases displacement of the brain tissue into the free space formed by the cysts took place.

During this study of healing of mechanical brain injuries in fetuses great attention was paid to regenerative changes affecting nerve tissue cells in different parts of the brain. Division of neurons in mammals was the most interesting question of all.

Multiplication of neurons by mitosis is accepted by some workers not only in early ontogeny, but also in adult animals [3, 7]. In the present experiments mitoses could not be observed among neurons in the rabbit fetuses.

The question of direct division of neurons also is no less interesting. In the literature evidence is given of the active formation of new neurons by amitosis in both young and adult animals [3, 4].

Amitoses, which were observed, can be conventionally divided into two groups. In the first group many cells were distinguished with an incomplete transverse constriction band in their nuclei, and without any doubt these were glial cells. In particular, this was shown by their relatively small size, the absence of tigroid in their cytoplasm, and so on (Fig. 2a). Amitotically dividing neurons were classed in the second group (Fig. 2b). They were found extremely rarely in the histological sections. In the writer's view, there were certain single neurons which can undoubtedly be considered to be amitotically dividing nerve cells.

Neurons lying in the immediate vicinity of each other were observed fairly often in the nerve tissue of the brain. The impression was gained that such neurons could arise through amitosis. However, the frequent presence of such figures in the sections combined with the virtually complete absence of neurons with a partial transverse constriction band in their nuclei make it more likely, in the writer's view, that these were simply neurons which happened to be close together. This conclusion also was confirmed by careful visual study of these cells.

Examination of morphological changes arising in the brain of the rabbit fetuses after mild mechanical injury showed an increase in the number of binucleolar neurons. Counting showed a statistically significant and marked increase in the number of these cell forms in the experimental animals compared with the normal; the increase was most marked in the injured cerebral hemisphere close to the site of trauma. The number of binucleolar neurons was increased in the earliest stage after the operation, and their number remained high throughout the experiment.

During examination of processes of regeneration in the CNS particular attention has been paid in recent years to hypertrophy of nerve cells. This is because, according to data in

the literature [5, 8], the basic regenerative processes in the CNS of man and animals take place at the intracellular level. This is manifested morphologically by hypertrophy of the cells. In the material in the present investigation no hypertrophy of neurons could be detected.

Cellular regeneration of the injured tissue thus does not take place in rabbit fetuses at the 20th-24th day of intrauterine life after mild mechanical brain trauma. Manifestations of a regenerative character affecting the brain neurons include a marked increase in the number of binucleolar neurons developing after brain trauma. Some workers [6, 9] consider that this is a compensatory and adaptive phenomenon.

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EFFECT OF T AND B LYMPHOCYTES ON PHAGOCYTIC ACTIVITY OF HUMAN PERIPHERAL BLOOD POLYMORPHS

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Immune lymphocytes can stimulate phagocytes, which then digest infectious agents more actively [4, 6, 8, 9]. Stimulating properties are ascribed predominantly to T cells [4]. Isolation of different subpopulations of T and B lymphocytes makes possible a more detailed study of this problem, and this is currently important because during infection definite correlation exists between the number of individual lymphocyte subpopulations and the form and course of the pathological process [1, 2]. The investigation described below was carried out for this purpose.

MATERIALS AND METHODS

Lymphocytes and neutrophils were isolated from the heparinized venous blood of 28 patients with suppurative surgical infections in a 1.077 Ficoll—Verografin density gradient. The rosette formation test was carried out with the lymphocytes, using papainized [14] sheep, rabbit, and mouse erythrocytes or an EAC diagnostic kit with bovine erythrocytes, after which the cells were separated into subpopulations by repeated centrifugation on the same gradient. If necessary, the cells were freed from erythrocytes by hypotonic shock. As a result a total lymphocyte population (T + B + "null" cells), T lymphocytes, active (activated) T lymphocytes (T_{act} — a combination of helpers, depressors, and killers) [11], T lymphocytes with receptors

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