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EFFECT OF EARLY MONOCULAR DEPRIVATION BY LEUKOMA OR CATARACT ON RETINAL DEVELOPMENT

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Key Words: retina, ganglion cells, monocular deprivation, cataract, corneal leukoma.

The cellular mechanisms of plasticity of the brain in visual deprivation have been studied in detail in structures of the visual cortex and basal ganglia [3, 6]. The receptor section (the retina) has received far less study, and information on the character of the changes taking place is extremely contradictory. Some workers [1, 5] have described marked changes of a degenerative character, whereas others deny the existence of any significant morphological disturbances in occlusive amblyopia [4]. The study of the retina in unilateral deafferentation is particularly interesting because changes at the cortical level in this case are maximal [2].

The aim of this investigation was to study the action of early unilateral deprivation on maturation of the retina. Occlusion resulting from induced leukoma or cataract was used as a completely unstudied model.

EXPERIMENTAL METHOD

Experiments were carried out on male kittens (*Felis domestica*) reared in the laboratory under ordinary conditions of lighting and with monocular deprivation at the age of 2-3 days. Cataract was induced by division of the capsule of the lens, and leukoma of the cornea was formed after a burn inflicted with a 25% aqueous solution of ammonia. The retina of the kittens was studied at the age of 1, 4, 7, 14, 28, 56, 84, and 120 days of postnatal development. Material was fixed in Carnoy's fluid and embedded 5-6 μm thick were stained with fast cresyl violet by Nissl's method in a modification. Total protein was determined by staining with Naphthol yellow S, followed by photometry on an SMP-01 scanning microscope ("Opton"). The results were analyzed by "Wang-720C" microcomputer, by the "Areascan Print" program. The area of cross section of the cell bodies was measured with the "Microvideomed-11" attachment. The intensity of protein metabolism was assessed with the aid of ^{3}H-lysine labeling. Labeled lysine ("Amersham," specific radioactivity 10 MBq/mmole), was injected into the vitreous body in vivo in a dose of 12×10^5 Bq, and enucleation was carried out 20 min later. In a series of experiments in vitro, the enucleated eye was opened and placed in incubation medium containing 3.7×10^{-5} Bq/ml of ^{3}H-lysine at 37°C for 15 min. Autoradiographs were obtained by the usual method.

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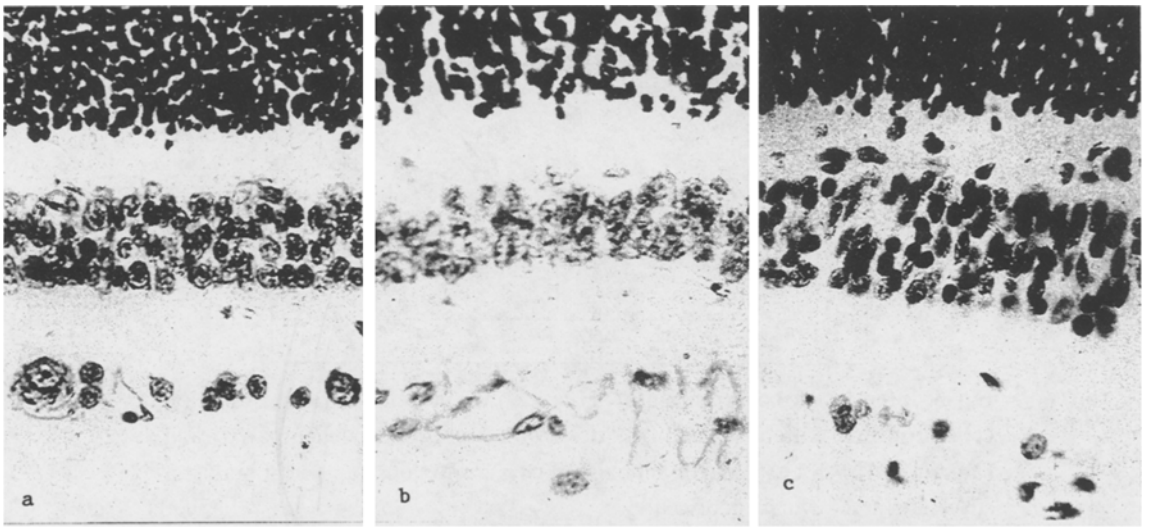

Fig. 1. Retina of normal adult cat (a) and of cat with deprivation-induced leukoma (b) and cataract (c). Fast cresyl violet. 400×.

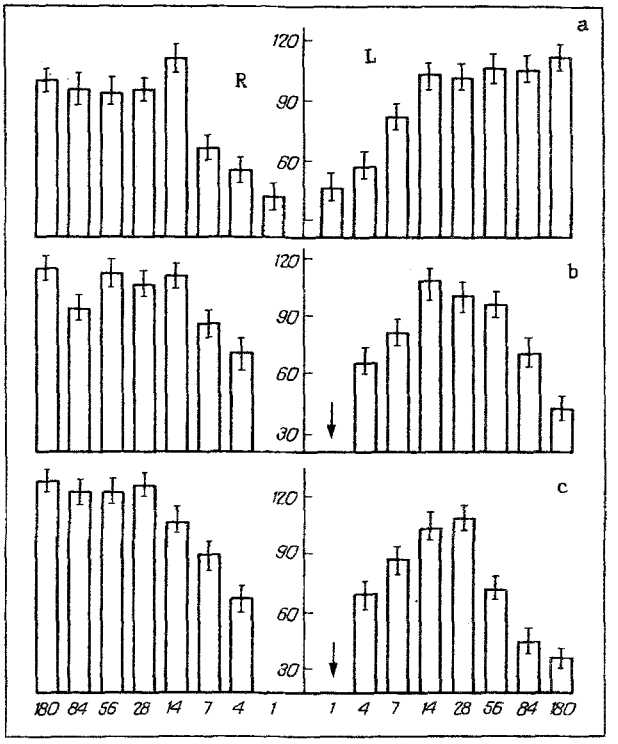

Fig. 2. Absolute protein content in cytoplasm of ganglion cells. Abscissa, age of kittens (in days); ordinate, protein content (in c.u.); a) control group, b) deprivation with leukoma, c) with cataract. R) Right retina, L) left retina; arrow indicates time of deprivation.

EXPERIMENTAL RESULTS

In animals of the control group aged 1 day the ganglion cells were large and the nucleus surrounded by a relatively thin border of cytoplasm. The protein concentration in the cytoplasm was minimal at 48-59 conventional units (c.u.), but on the 4th day after birth this amount increased. According to quantitative microscopy, the increase was due both to an increase in the area

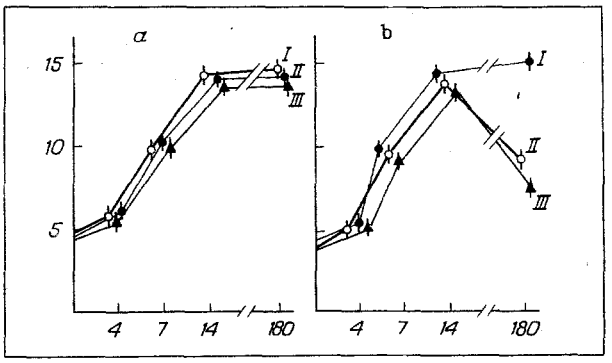

Fig. 3. Intensity of incorporation of ^{3}H-lysine into proteins of retinal ganglion cells of normal kittens (I) and of kittens with occlusion by leukoma (II) and cataract (III). Abscissa, age of animals (in days); ordinate, number of grains of silver. a) Intact eye, b) deprived eye.

of cross section of the neuron body and also to an increase in the concentration of bound dye. The ganglion cells of kittens aged 7 days could now be classified into the principal subtypes. The cells were unequal in size and hardly any further increase in the protein concentration took place, any increase in its content being produced mainly as a result of growth of the cross section of the neurons. The ganglion cells of kittens aged 14 days no longer differed from those in adult animals (Fig. 1a). Their cytoplasm contained chromatophilic substance in the form of clumps. The absolute protein content was increased to 100-119 c.u., and thereafter it did not change significantly (Fig. 2a); the right and left retinas, moreover, did not differ in this respect.

The protein concentration in the retinal ganglion cells of the deprived kittens also increased with age and reached the control values at the 14th day. In older animals a decrease in the total protein content was observed in ganglion cells of the ipsilateral retina (30-45 c.u.), whereas in neurons of the contralateral eye its content remained the same as before (Fig. 3b, c). A considerable decrease took place mainly due to a decrease in area of the cytoplasm, and the change in the protein concentration was not significant.

The label could be detected autoradiographically in animals on the 1st day of development above all the layers, but the intensity of incorporation was relatively low and the number of grains of silver was 6.0 ± 0.6. With age the intensity of metabolism increased and incorporation of the label on the 4th day of life was 6.8 ± 0.6, rising to 9.0 ± 0.8 on the 7th day and to 13.4 ± 0.9 on the 14th day, respectively. The level of incorporation of the labeled amino acid thereafter remained unchanged.

Variation in the incorporation of ^{3}H-lysine into the retinal ganglion cells of kittens of the experimental group correlated with the results of cytophotometry. The number of grains of silver in the intact eye reached a maximum by the 14th day, when it was actually a little higher than in the control, and thereafter it remained unchanged. A sharp decrease in incorporation of the label took place in the deprived eye after 14 days. By the 180th day this level was 7.2 ± 0.7 in the case of occlusion by leukoma and 6.8 ± 0.6 in the case of occlusion by cataract, respectively, evidence of a sharp decline in protein metabolism (Fig. 3a, b). Profiles of histograms of levels of incorporation in vivo and in vitro coincided.

Besides data of quantitative cytochemistry, definite morphological changes also were found in the retina of the deprived eye. A considerable decrease in the number of ganglion cells was observed: the large ganglion cells completely disappeared. Chromatolysis of basophilic substance and pycnolysis were detected in the cytoplasm. Ghost cells, with sharply reduced area of cross section of their bodies, were seen (Fig. 1b, c).

As a result of this investigation it can be concluded that normally maturation of the retina is largely complete by the 14th day of postnatal development, which coincides with the period of functional activity (when the kitten acquires vision). Development under conditions of deprivation also ends by this time. Later, degenerative changes begin to increase in the ganglion cells of the "closed" eye, and reach a maximum by the 180th day. It can be concluded that early monocular deprivation has an inhibitory action on the developing retina.

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ELECTRON-MICROSCOPIC AND MORPHOMETRIC INVESTIGATION OF THE ACTION OF ENKAD ON RETINAL PHOTORECEPTOR CELLS OF CAMPBELL RATS WITH HEREDITARY RETINAL DEGENERATION

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Retinitis pigmentosa (RP) is a heterogeneous group of hereditary diseases leading to blindness. On average this disease is found in one of every 3500 persons in the world. The features of RP are distinguished by their great variability, but nocturnal blindness, a circular scotoma, and narrowing and loss of peripheral vision with longer preservation of central vision are always observed [14]. As the disease progresses, reduction of amplitude and disappearance of the electroretinogram (ERG) are observed. Blindness arises on account of degradation of the outer layers of the retina, and in particular, of the photoreceptor layer [14]. The hereditary character of RP was established in the middle of last century. Since then the view has been held that this disease is incurable. In 1971, however, the use of the preparation enkad [8], which is a mixture of ribonucleotides obtained by enzymic hydrolysis of yeast RNA, was suggested in the Soviet Union. Clinical trials of this preparation have revealed a group of patients with a form of retinitis pigmentosa in whom it was possible to obtain temporary restoration or improvement of vision or to delay progression of the disease, sometimes with a prolonged effect, if repeated courses of injections of enkad were given [1-4, 6-9]. As early as 5-8 weeks after the beginning of treatment the eyesight of these patients began to improve, and the improvement reached a maximum after 2-3 weeks. It could include widening of the peripheral field of vision, reduction of the circular scotoma, increased dark adaptation, and enhanced visual acuity. In some cases the ERG was restored. The improvement of dark adaptation was found to be the least stable feature and reverted to its initial level during the few months immediately after the end of treatment [6-8]. As a rule repeated treatment gave more lasting results, and nowadays a second course is given 6-8 months after the first [4]. These studies served as a prototype for a successful attempt to treat RP in the USA, with similar results. As therapeutic preparation there, a mixture of 5′-nucleotides was used, followed by oral administration of their precursors, namely inositol and orotic acid [13]. The positive results of clinical treatment with enkad shed some light on a hitherto unknown aspect of the metabolic disturbance in patients with hereditary RP, namely a disturbance of nucleotide metabolism, which the name of dysnucleotidosis has been given [1].

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