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Histological changes in the retina in experimental alloxan-induced diabetes in rabbits

The retina is one of the organs (7) where retinopathies due to diabetes are extremely distinctly manifested. Many research papers focus on the vasculopathies in that eye structure (1, 6, 7). Others describe the changes that occur in the retina following surgery (3, 5, 9, 10).

In our research we wanted to find out the damage to particular parts of the retina depending on the duration time of the experiment.

MATERIAL AND METHODS

The experiment was carried out on white rabbits (male), New Zealand, 2,880±400 g. The animals were administered a sigle dose of alloxan (Sigma, St. Louis, USA) into the marginal vein of the ear in the form of 10% solution in 0.9% NaCl at the dose 100 mg/kg b.m.

The level of glycaemia was determined after seven days. If it was 11 mmol/l or higher, the animal was chosen to the experimental group.

The rabbits were divided into 4 experimental groups and a control one, 5 animals each. In the experimental groups I, II, III and IV the material for tests was collected after 3 weeks, 6 weeks, 3 months and 6 months respectively. Over the experimental period the rabbits had free access to water and fodder. They were killed by discontinuation of the spinal cord and exsanguinaton. Next, samples of the retina were taken from the immediate areas to the papilla of the optic nerve and macula. They were fixed in glutaraldehyde and OsO_4 and immersed in Epon 812. Half-thin sections 1μ thick were stained with Swiss blue and azure. The preparations were examined under light microscope to evaluate the changes in the particular layers of the retina. The pictures were taken by a photo-camera (Carl Zeiss, Jena).

RESULTS

Control group. In the control group all layers of the retina were clearly visible. The widest of them were: layer 2 – of rods and cones and layer 4 – outer granular containing bodies of rod and cone cells (Fig. 1).

Experimental group I. After three weeks of experimental diabetes no histological differences in the particular layers of the retina were found in comparison to the controls.

Experimental group II. After six weeks following the administration of alloxan a distinct narrowed layer of rods and cones as well as a widened external limiting membrane were found. In the inner granular layer the number of 2-polar neurones was much smaller than in the controls, however, numerous macrophages were visible among the neurones. The number of ganglionic cells was several times smaller. They were not arranged regularly but distributed far away from one another (Fig. 2).

Experimental group III. In the case of three-month diabetes no cellular nuclei were found in the pigmented epithelium.

In comparison to the experimental group II even further widening of the external limiting membrane was observed. The number of two-polar neurones was small and macrophages were found in the inner granular layer. In the ganglionic layer single shrunk ganglionic neurones were observed. In the choroid membrane just at the pigmented epithelium widened vessels were filled with a big number of erythrocytes (Fig. 3).

Experimental group IV. After six months following the administration of alloxan beneath the thinned pigmented epithelium there was a narrow layer of rods and cones, about four times narrower in comparison to the controls. The outer plexiform layer was narrower than in the experimental group III. In comparison to the controls the outer granular layer was two times narrower, too. Few 2-polar neurones and neurone macrophages with typical kidney-shaped nuclei were observed in the inner granular layer. In the ganglionic layer no ganglionic neurones were observed (Fig. 4).

DISCUSSION

In various retinopathies damage to to the neurones nearly always develops, no matter what factors are responsible. A pathophysiological marker of glaucoma is necrosis due to apoptosis of ganglionic cells (8). In the oxygen deficient retina its layers get thinner, especially the inner granular layer. There is also atrophy of ganglionic cells observed (2).

In diabetic retinopathy great impact have impermeable acellular capillaries, built of thick basilar membrane only, without pericytes or endotheliocytes (11).

In our experiment three-week diabetes (experimental group I) caused no morphological changes visible under light microscope. However, after six weeks (experimental group II) the changes were distinct. First of all, the number of ganglionic cells decreased. Undoubtedly those cells are the most sensitive in the retina.

In the inner granular layer the number of 2-polar neurones decreased, too. The absence of inflammation and the presence of macrophages suggest that both in diabetes and glaucoma (8) the decreased number of neurones is due to apoptosis. A narrowed layer of rods and cones and widened external limiting membrane also suggest damage to those cells.

In three-month diabetes (experimental group III) the atrophy of pigmented epithelial cells was found in the retina. Some other authors also report on the degeneration of the epithelium in diabetes

(4). The inner granular layer got narrower in comparison to the experimental group II. Similar changes were observed in others layers. Much widened blood vessels, filled with the erythrocytes found in the choroid membrane suggest changed dynamics of circulation.

In the experimental group IV (six months following the damage of the islet cells of the pancreas by alloxan) we observed great narrowing of all layers. In the inner granular layer single neurones and big number of macrophages were present, however, ganglionic cells were not found in the ganglionic layer. The number of rod and cone cells was much reduced. Thus, it may be concluded that such a great damage to the retina after six months of experimental diabetes does not allow its normal function and is irreversible.

CONCLUSIONS

1. Pathological changes in the retinal neurones develop after six months of experimental diabetes.
2. Ganglionic neurones are the most susceptible to damage.
3. The atrophy of the retinal neurones is due to the involvement of macrophages and is likely to be caused by apoptosis.
4. Six-month experimental diabetes results in distinct thinning of all layers in the retina, the changes are irreversible.

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EXPLANATION TO FIGURES

Fig. 1. Retina in rabbit, control group. Stained with Swiss blue and azure. Magn. ca 400 x.

Fig. 2. Retina in rabbit, experimental group II. Visible narrow layer of rods and cones, wider external limiting membrane, macrophages in the inner granular layer and absent ganglionic cells. Stained with Swiss blue and azure. Magn. ca 400 x.

Fig. 3. Retina in rabbit, experimental group III. Visible progressing narrowing of the inner granular membrane and absent pigmented epithelial cells. Stained with Swiss blue and azure. Magn. ca 400 x.

Fig .4. Retina in rabbit, experimental group IV. Visible great narrowing of all layers. Stained with Swiss blue and azure. Magn. ca 400 x.

SUMMARY

Mature rabbits were given a single dose of alloxan at the dose of 100 mg/kg of b.m. After 3 and 6 weeks and after 3 and 6 months the retina samples were taken from the aveas immediate to the papilla of the optic nerve. Half-thin sections were stained with Swiss blue and azure, and the changes in the particular layers were evaluated under the light microscope.

Morphological changes in the form of decreased number of neurones (especially ganglionic) and narrowed layer of rods and cones occurred after 6 weeks.

After 3 months even further increase in the number of neurones and atrophy of the pigmented epithelium cells were observed.

After 6 weeks nearly total atrophy of ganglionic neurones and distinct narrowing of all the layers were found.

Histologiczne zmiany siatkówki królika pozostającej pod wpływem doświadczalnej cukrzycy wywołanej alloksanem

Dojrzałym królikom podano jednorazowo alloksan w dawce 100 mg/kg m. c. Po 3 tyg., 6 tyg., 3 mies. i 6 mies. z okolicy tarczy nerwu wzrokowego pobierano do badań siatkówkę. Półcienkie skrawki barwiono błękitem metylenowym i azurem i oceniano w mikroskopie świetlnym zmiany w poszczególnych warstwach. Zmiany morfologiczne w postaci zmniejszenia liczby neuronów (zwłaszcza zwojowych) i zwężenia warstwy pręcików i czopków pojawiły się po 6 tygodniach. Po 3 miesiącach obserwowano zanik komórek nabłonka barwnikowego i dalsze zmniejszenie liczby neuronów. Po 6 miesiącach stwierdzono prawie całkowity zanik neuronów zwojowych i wyraźne zwężenie wszystkich warstw.

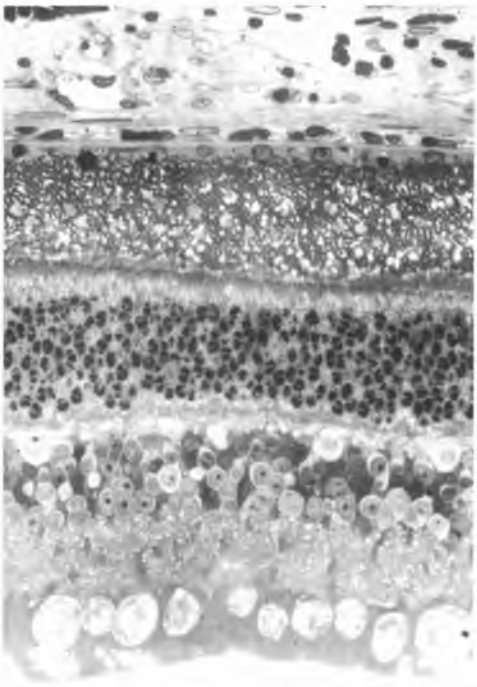

Fig. 1

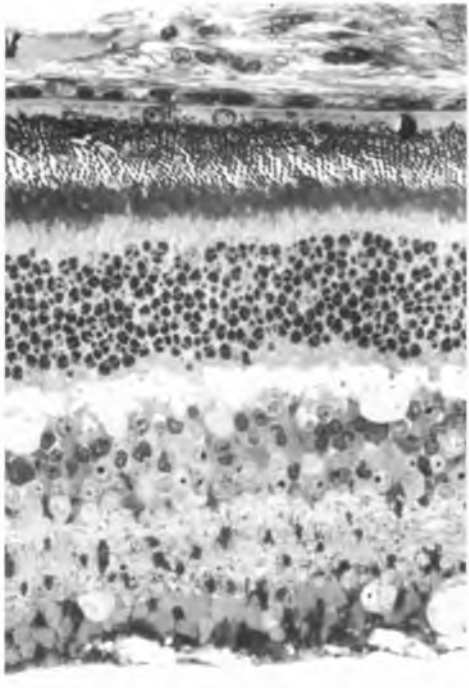

Fig. 2

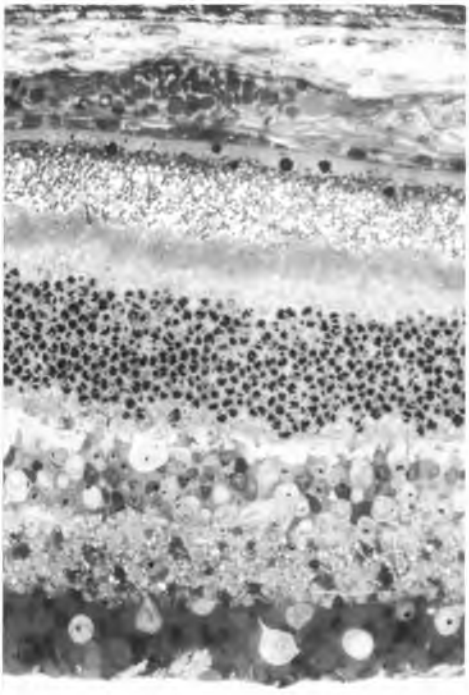

Fig. 3

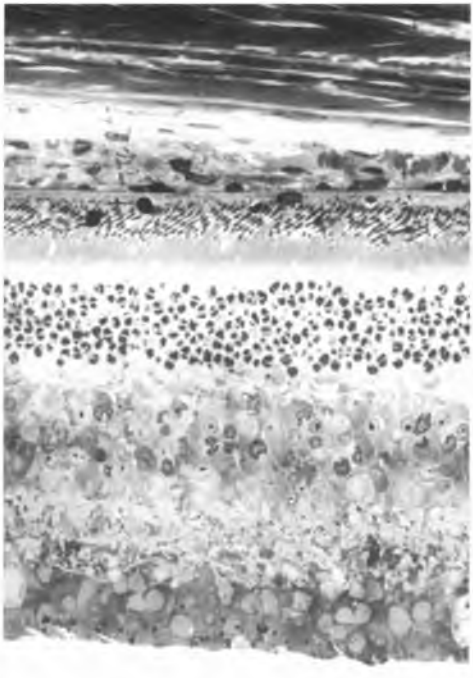

Fig. 4