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Department and Institute of Histology and Embryology with the Laboratory
for Experimental Cytology, Medical University of Lublin
Department and Institute of Human Physiological Anatomy,
Medical University of Lublin

ALICJA ZARĘBSKA, MIROSŁAW ŁAŃCUT,
KATARZYNA BAKIERA, EULALIA MATEJKO,
KRYSTYNA CZERNY, GRAŻYNA KIŚ, ZBIGNIEW WÓJTOWICZ

Ultrastructural changes in the receptor parts of retinal rods in experimental alloxan-induced diabetes in rabbits

The majority of research papers on the pathology of the retina describe that process in connection with eye surgery performed in diabetes (5, 7, 11, 12). Other observations of the changes in the retina that occur in diabetes focus mainly on the damage to the small blood vessels and describe that eye structure in clinical aspects (3, 4, 8).

The aim of our investigation was to find out the degree of damage to the receptive areas of retinal rods depending on duration time of diabetes induced by alloxan.

MATERIAL AND METHODS

The research was done on white male rabbits (New Zealand, body mass 2880 ± 400 g). The animals were administered a single dose of alloxan (Sigma, St. Louis, USA) into the marginal vein of the ear in the form of 10% solution in 0.5% NaCl at the dose 100 mg/kg b.m. The level of glycaemia was determined after 7 days. If it was 11 mmol/l or higher the animal was chosen to the experimental group.

The rabbits were divided into four 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.

The animals were allowed free access to water and fodder. They were killed by discontinuation of the spinal cord and exsanquination. Next, the samples of the retina were taken from the areas immediate to the papilla of the optic nerve and retinal macula. The samples were fixed in glutaraldehyde and OsO_4 and immersed in Epon 812. Ultrathin sections were dyed with uranyl acetate and lead citrate according to Reynold's method. The receptive parts of the rods were examined under electrone microscope BS-500 (Tesla).

RESULTS

Control group. The receptive parts of the rods presented a pattern of parallel membranes, closed at the periphery (at the cell membrane). Those membranes formed so-called discs. Inside discs there were numerous electronically darker grains (Fig. 1).

Experimental group I. After three weeks following alloxan administration the membranes forming discs and interspaces between them were hardly visible. The whole inside of the receptive rod parts was scattered with small and bigger grains. The cellular membrane covering the rod was folded in many places and expressed "diffusion reaction" (Fig. 2).

Experimental group II. After six weeks following alloxan administration distinct morphological changes were observed in comparison to the controls. The pattern of focal membranous discs did not show regular distribution. At such places, electronically light widened interspaces were observed. They resulted from the enlargement of certain discs. At places, where no enlarged discs were found the structures presented higher electronic density in comparison to the controls. The cellular membrane, electronically light, was discontinued at places (Fig. 3).

Experimental group III. After three months of diabetes duration the damage to the dendrite part of the rod was great. It mostly presented as deformations and atrophy of many discs. The cellular membrane close to the damaged area of the rod was mostly fragmented (Fig. 4).

Experimental group IV. After six months of experimental diabetes single, well visible rods were found, some of those were narrow in comparison to the controls.

DISCUSSION

Diabetic retinopathy is due to the impaired function of blood–retina barrier. It results from generalised microangiopathy, which is an early physiological manifestation of diabetes (1, 2, 10).

Damage to the retinal neurones mainly results from oxygen deficiency. Impermeable acellular capillaries built of thickened basilar membrane without pericytes and endotheliocytes have great impact (9, 14).

Considering the fact that nervous stimulus is brought about in the dendrite part of the retinal neurone I, we decided to investigate the ultrastructural changes that occur in that part of the rod cell in the course of experimental diabetes.

After three weeks following the administration of alloxan we found no morphological damage to the dendrite rod area. Hardly visible discs membranes and much granulated structure of that cell part pints to so-called "metabolic shock", which is a manifestation of impaired physiology, i.e. biochemical change of rodopsine stimulated by light. Bigger and electronically denser grains are likely to be Ca++ ion clusters, which together with Na+ have a significant impact in light stimulated hyperpolarisation of the rod cell membrane. However, the increased number of small grains and their presence in the interspaces between discs might result from liabilisation and release of protein molecules.

After six weeks of the experiment we found subtle morphological changes manifested as enlarged discs. Those were typical of the initial stage of damage to the cell due to the impaired function of sodium-potassium pomp, changed permeability of the membranes and the inflow of water into the cell (15).

More delicate membrane structure compared to the controls suggests lower protein content. It may be concluded their renewal is not normal at that stage.

Diabetes lasting three months caused strong damage to the receptor areas in the majority of rods. Great enlargement and atrophy of many discs and fragmented cellular membranes are the evidence

that at that stage of damage rod cells cannot perform their function or it is much defective.

After six months of diabetes duration time only single rods were found to be present in the retina. They were quite well preserved. Atrophy of the majority of them is a consequence of the changes in the neuroglia as well as in the capillaries. The distribution of changes is not uniform (6, 13). The rods located near less damaged capillaries are likely to be preserved or less damaged.

CONCLUSIONS

1. Three-week diabetes results in functional changes in the rod cells of the retina in rabbits.

2. After six weeks of diabetes duration first morphological changes develop, which is connected with impaired function of sodium-potassium pomp.

3. Three-month diabetes results in great damage to the rod cells.

4. After six months of experimental diabetes duration only single rods are present in the retina; they are probably located in the adjacent areas of the less damaged vessels.

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EXPLANATION TO FIGURES

Fig. 1. Control group. Regular pattern of disc membranes in the dendrite parts of two neighbouring rods. Magn. approx. 32,000 x.

Fig. 2. Experimental group I. Oblique section of two receptor areas of the rod. Hardly visible disc membranes, elecronically dense grains and folded cell membrane. Magn. approx. 32,000 x.

Fig. 3. Experimental group II. Many discs enlarged. Magn. approx. 32,000 x.

Fig. 4. Experimental group III. Visible greatly changed receptor areas of the rod. Magn. approx. 32,000 x.

SUMMARY

Mature rabbits were administered a single dose of alloxan at the dose 100 mg/kg b.m. After 3 and 6 weeks and after 3 and 6 months, the samples of the retina were taken from the areas immediate to the papilla of the optic nerve. Ultrathin sections were dyed according to the Reynold's method, and the receptive parts of the rods were examined under electrone microscope BS-500 Tesla.

After 6 weeks following alloxan administration, distinct morphological changes in the form of enlargement of certain discs in the receptive parts of rod cells were observed.

After 3 months the majority of the discs was damaged, and after 6 months only single, quite well preserved rod cells were found to be present in the retina.

Ultrastrukturalne zmiany części receptorowej pręcików siatkówki królika w 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. Ultracienkie skrawki barwiono wg metody Reynoldsa i badano części receptorowe pręcików w mikroskopie elektronowym PS-500 firmy Tesla. Po 6 tygodniach stwierdzono zmiany morfologiczne w postaci obrzmienia niektórych dysków w częściach receptorowych komórek pręcikowych. Po 3 miesiącach większość dysków była silnie uszkodzona, a po 6 miesiącach pozostały w siatkówce pojedyncze, w miarę dobrze zachowane komórki pręcikowe.

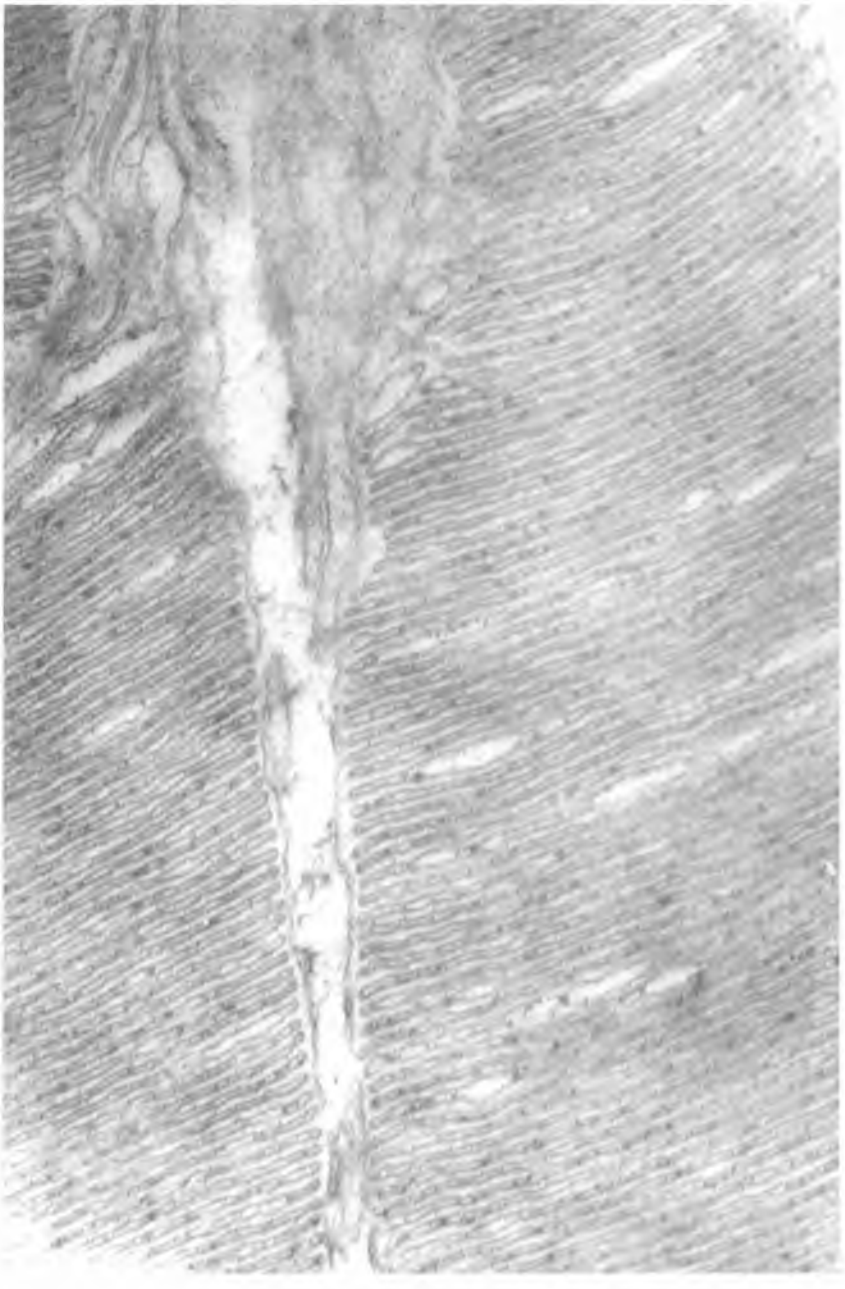

Fig. 1

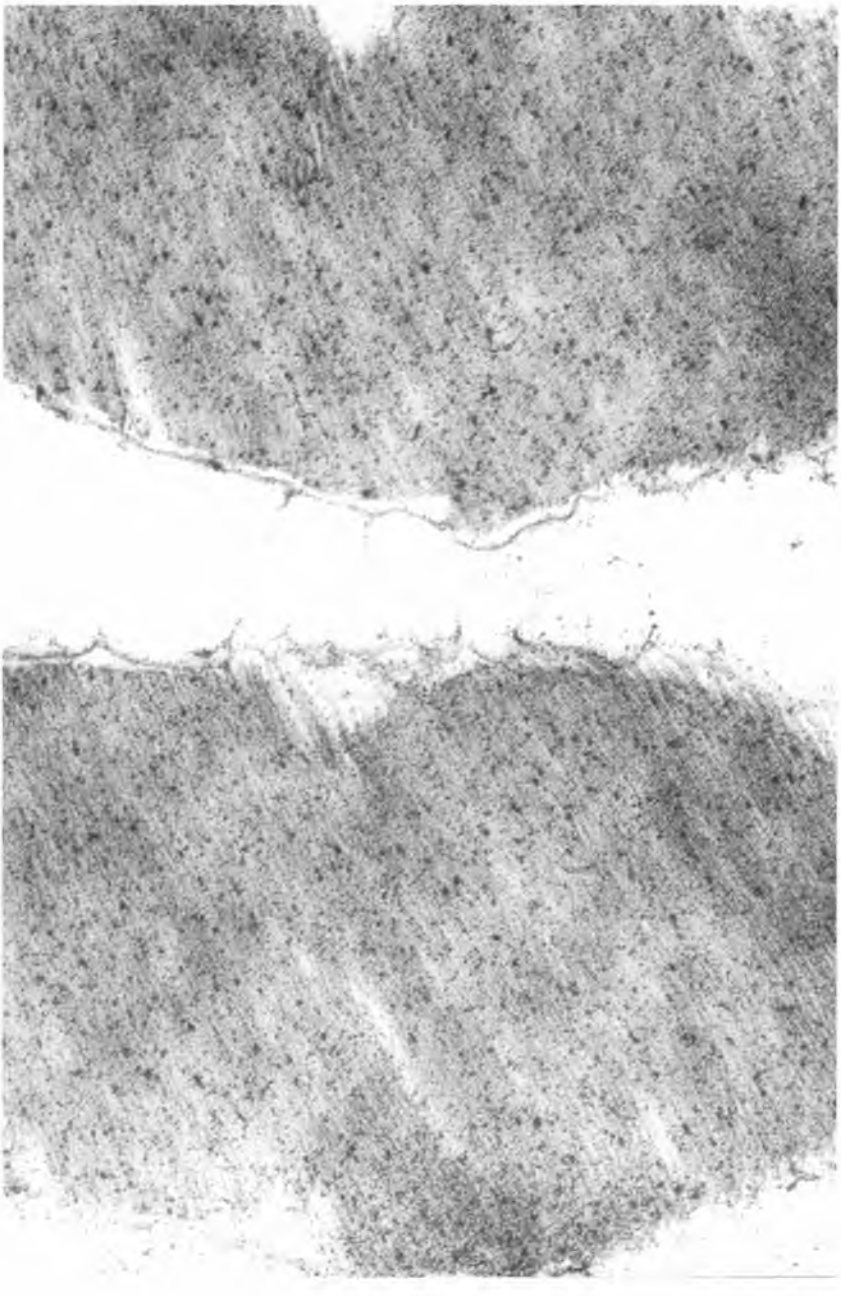

Fig. 2

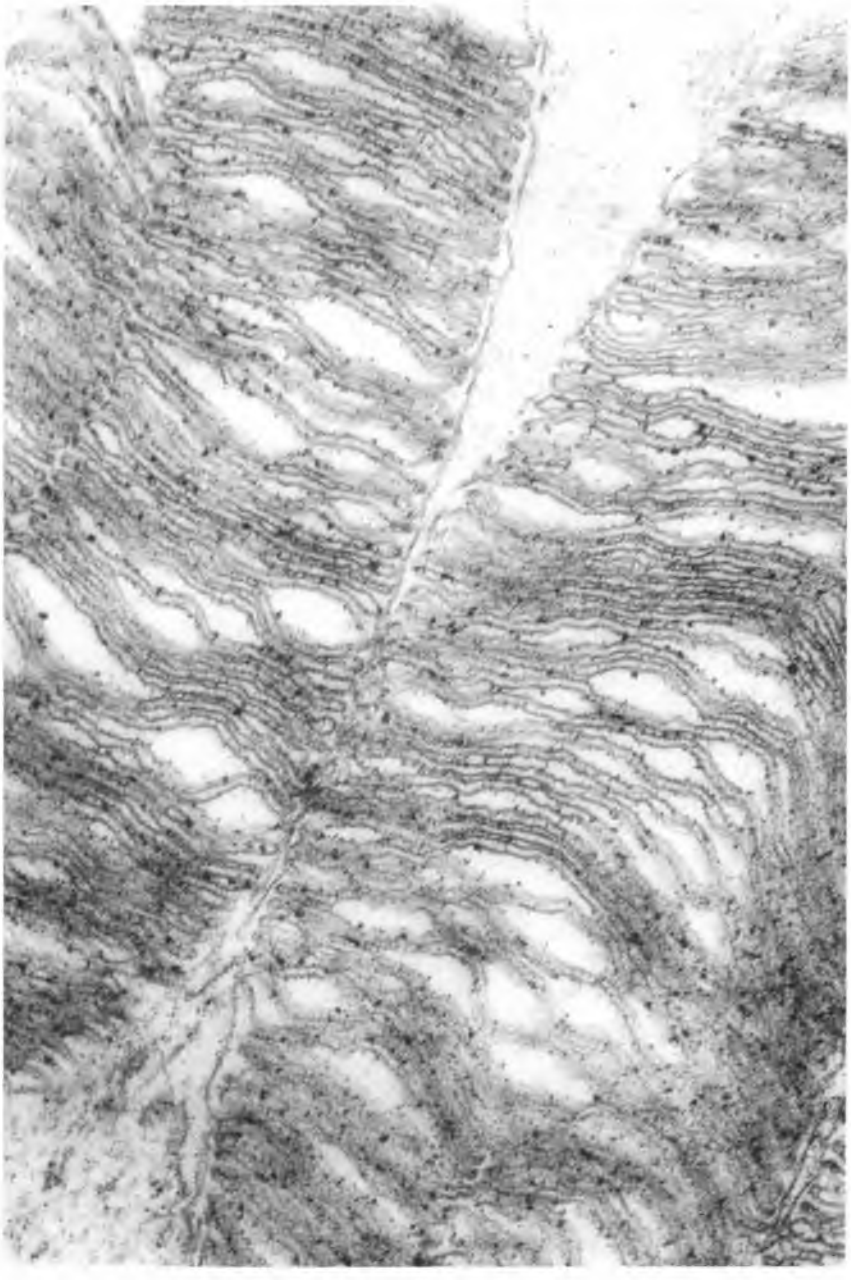

Fig. 3

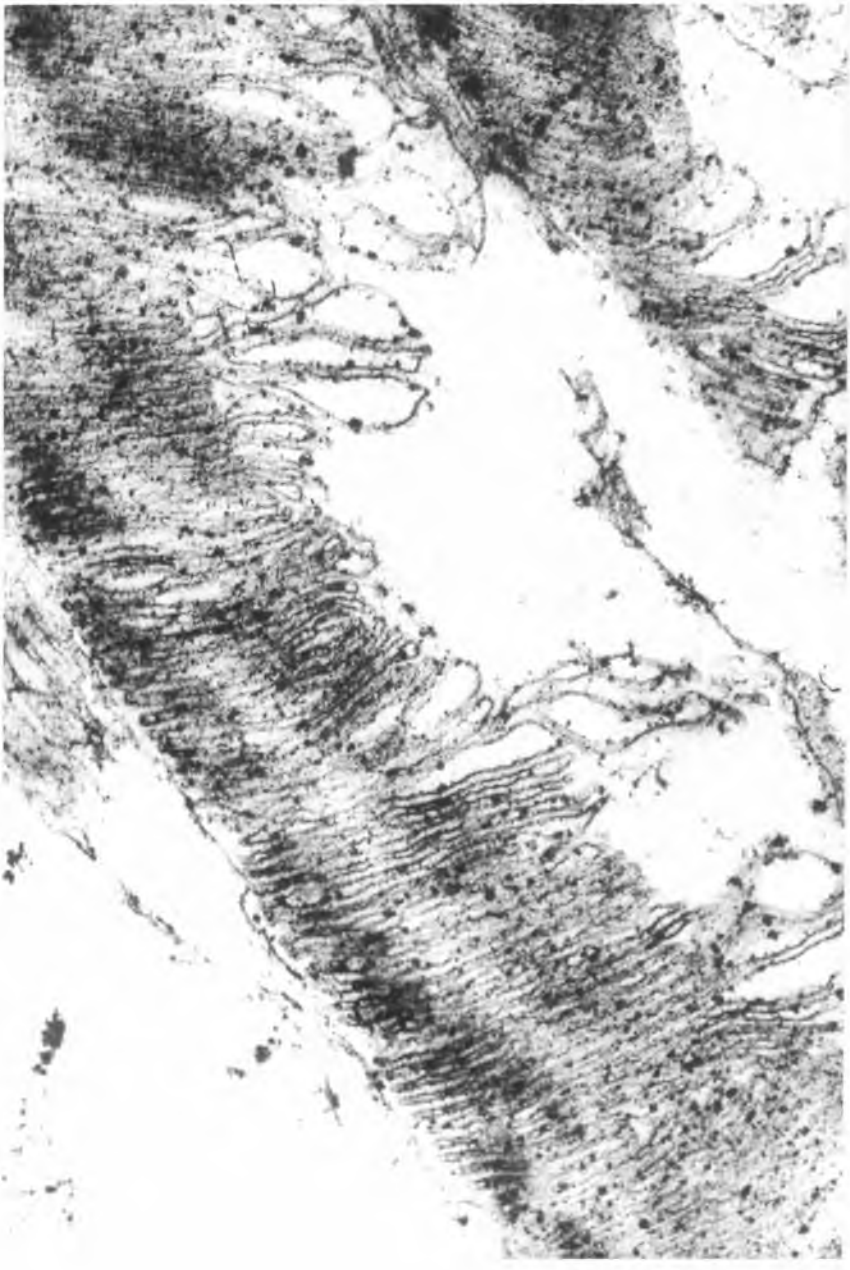

Fig. 4