INTRAMITOCHONDRIAL GLYCOGEN PARTICLES IN RAT RETINAL RECEPTOR CELLS

TOYOKO ISHIKAWA and YEN FEN PEI. From the Department of Anatomy, Kyushu University, Fukuoka, Japan, and the Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, New York.

Intramitochondrial granules or bodies have been described by many investigators in various cell types. Although the advanced techniques of electron microscopy in general have clarified the existence of several types of intramitochondrial components, and in some cases even their chemical characteristics (1, 2), the chemical nature of most such substances is still unknown.

During this electron microscope study of the normal rat retina, a new type of particle was noted within the mitochondria of the receptor cells. These intramitochondrial particles, unlike any of the known types, appear to be similar to glycogen particles morphologically as well as histochemically. This note reports intramitochondrial glycogen particles in rat retinal cells, and the identification of glycogen in a tissue section at the electron microscope level.

MATERIALS AND METHODS

Two groups of male Wistar albino rats (obtained from animal care at College of Physicians and Surgeons, Columbia University, and Animal Breeding Laboratory, Kyushu University, respectively) were used in this study. Retinas from 20 normal rats, ranging in age from 1 day to 28 months, were examined. The

eyeballs were enucleated under ether anesthesia, and the retinas with choroid prepared as follows: One group of retinas was fixed directly with 1 per cent osmium tetroxide in veronal buffer for 1 hour, and the second group was prefixed with 0.6 M glutaraldehyde in phosphate buffer for 2 hours. After washing overnight with buffer, the tissues were incubated in saliva for 3 hours at room temperature, and then postfixed in 1 per cent OsO_4 for 1 hour. The control group was prepared in the same way as the second group except that saliva incubation was omitted. The fixed materials were then dehydrated in a series of ethanols, and embedded in Epon. Thin sections were cut with a Porter-Blum microtome, stained with lead salt, and examined in a Siemens Elmiskop I or Hitachi HU 11A. Thick sections obtained from all three groups of Epon-embedded tissues were stained by the periodic acid–Schiff (PAS) reaction for light microscopy.

OBSERVATION AND DISCUSSION

Fig. 1 shows longitudinally sectioned rat retinal cells at the level of outer and inner segments. In the region of the ellipsoid, a mass of dense particles enclosed by distinct membrane can be seen. From the topographical relationship, it appears that such masses of particles may correspond to PAS-positive particles in the thick sections stained by the periodic acid–Schiff reaction. The size of the masses varied from that of a single particle to as much as 3 μ in diameter in thin sections which were large enough to be detected with the light microscope. The variations found in thin sections probably represent various stages of the same process. Small clusters of similar particles were found within the cristae of mitochondria, as shown in Figs. 2 and 5 (arrows), or occasionally in the space between the outer and inner mitochondrial membranes. The particulate aggregates of medium size, shown in Figs. 6 and 7, were also found inside mitochondria within a single bounding membrane. In such mitochondria, the typical pattern of the cristae was clearly seen. On the other hand, masses of larger size, usually larger than ordinary mitochondria, were found in structures which did not resemble mitochondria. Occasionally, however, some of the cristae and the double membranes of a mitochondrion were recognizable on the limiting membranes of these particle masses (Fig. 4). This seems to suggest a process of particle accumulation occupying and expanding the space inside of mitochondrial cristae. On very rare occasions, a part of the limiting membrane of the mass disappears and no bounding membranes between the mass and surrounding cytoplasm remain in such areas (Fig. 8). The majority of the particle masses, however, are found within the mitochondria entirely separated from surrounding cytoplasm by mitochondrial membranes. This was confirmed with serial sections. Therefore, the disruption of the limiting membranes may be an artifact which occurred during the preparation. It seems improbable that the particles are located in the cytoplasm which protrudes into mitochondria. The real site of glycogen deposit is presumed to be within the cristae of mitochondria, appearing at the first within the cristae and accumulating there, followed by expansion of the internal space of the cristae.

Identification of glycogen in sections has recently been reviewed by Revel (3). The intramitochondrial particles are believed to be glycogen, for several reasons. Their staining is intensified with lead, they are *ca* 200 A, resembling beta glycogen particles. The particles were removed by enzyme digestion in glutaraldehyde-fixed tissue, which was then post-fixed in OsO_4. Sections of such tissue are shown in Figs. 3 and 4. Similar results were obtained when these techniques were applied to glycogen in chick retinal paraboloids or rat liver.

The distribution of mitochondria containing glycogen particles is restricted in visual receptor cells to the level of the inner segments in which longitudinally arranged, slender mitochondria accumulate, and to the level of synaptic spherules in which mitochondria are usually spherical in shape. In these studies, no intramitochondrial glycogen was found in other retina cells; *e.g.*, bipolar, ganglion, or glia cells.

The appearance of intramitochondrial glycogen in the rat retina may be related to age. Accurate measurement of the number of mitochondria containing glycogen particles is difficult in thin sections; however, the presence of intramitochondrial glycogen can be detected with ease in the retina of rats 1 year or older. On the contrary, we cannot find any glycogen in the retina of new-born rats or those a few weeks of age. In retinas of 3-month-old animals, glycogen was visible for the first time as a small cluster of particles within the mitochondrial cristae. We assume, therefore, that glycogen particles in receptor mitochondria first appear within mitochondrial cristae and may have some relationship with age.

Explanation of Figures

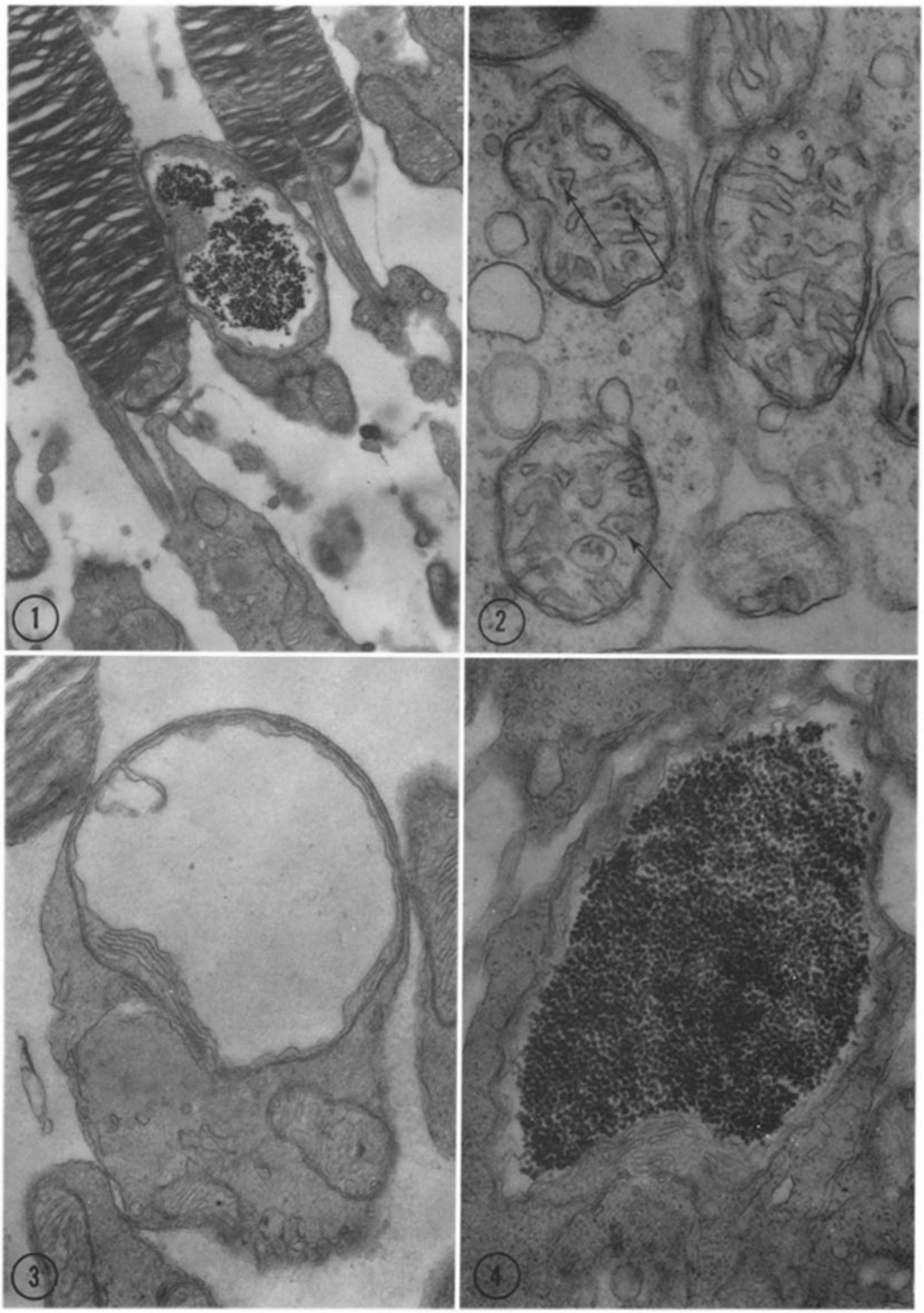
All figures are electron micrographs of thin sections stained with lead salt.

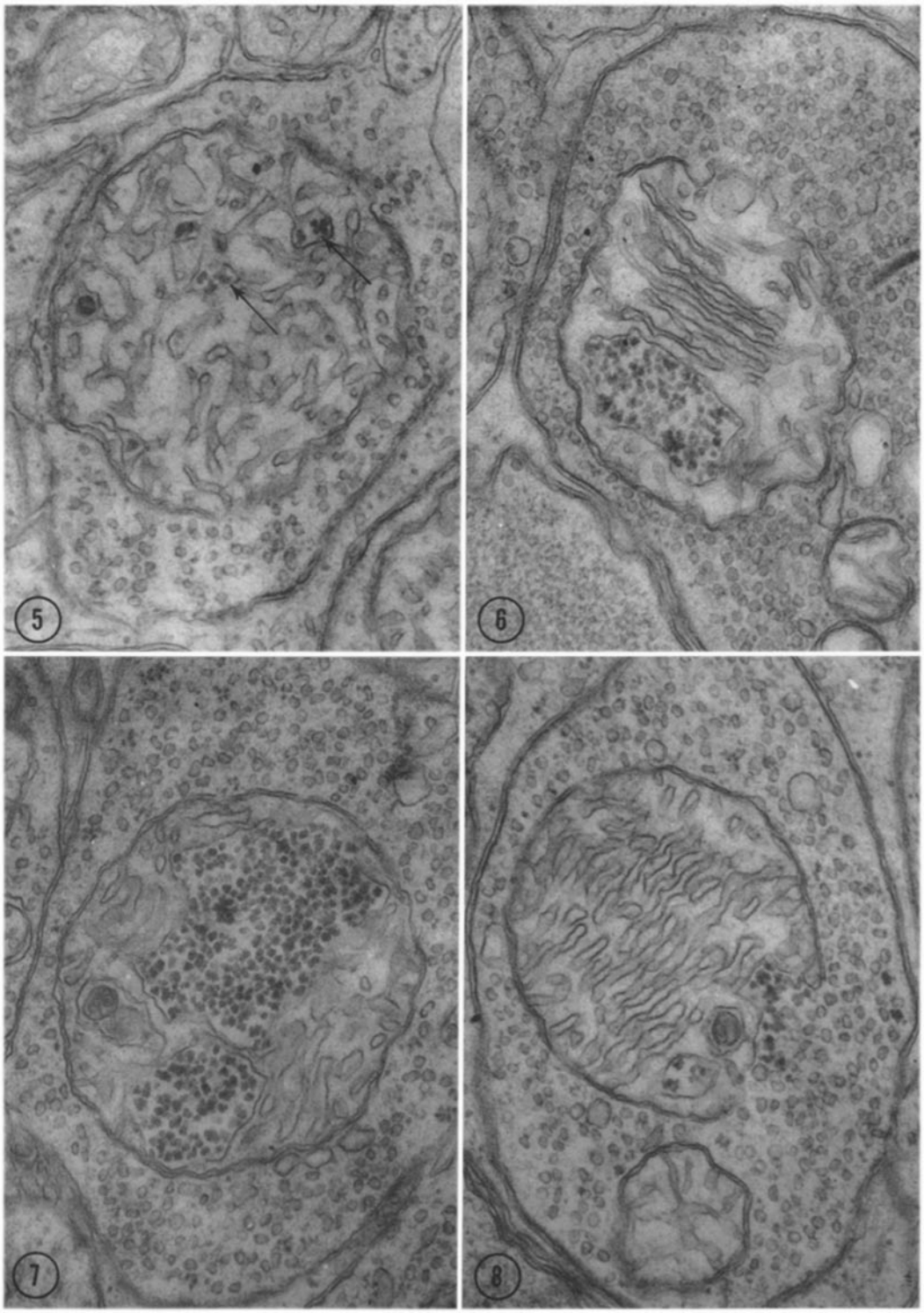
FIGURE 1 Part of longitudinally sectioned receptor cells of rat retina, showing characteristic lamellar structure of outer segments and the region of ellipsoid. At the tip of the inner segment, a mass of dense particles with distinct limiting membranes is noted. Glutaraldehyde-OsO_4 dual fixation. × 12,000.

FIGURE 2 Transverse section of inner segments. Small clusters of particles can be seen within the expanded cristae (arrows) of mitochondria. OsO_4 fixation. × 38,000.

FIGURE 3 "Empty" structure found in the inner segment of rat retina incubated in saliva. Mitochondrial double membranes and cristae are clearly seen at the periphery. Dense particles were completely removed. Glutaraldehyde-OsO_4 dual fixation with saliva digestion. × 30,000.

FIGURE 4 Glutaraldehyde-OsO_4 dual fixation without saliva digestion. Large, expanded mitochondrion is filled with dense glycogen particles. × 35,000.


1
2
3
4



FIGURES 5 TO 7 Intramitochondrial glycogen particles (arrows) within spherical mitochondria in the synaptic spherules. Note membrane-bounded particle mass and typical mitochondrial cristae in a single mitochondrion. OsO_4 fixation. × 38,000.

FIGURE 8 Disruption of mitochondrial double membranes can be seen. There is no separation between particles and the surrounding cytoplasm, in which numerous synaptic vesicles are distributed. OsO_4 fixation. × 38,000.

Descriptions of intramitochondrial glycogen or even of the relationship between mitochondria and glycogen formation have not been frequent. Beaulaton (4) has reported intramitochondrial accumulation of glycogen in the silkworm. From available evidence in the present study, we believe that the substance which appeared and accumulated within the mitochondria of rat retina cells is glycogen. However, this would not necessarily imply that the mitochondrion itself can synthesize glycogen, for it has not been shown whether the visible glycogen particles found were built up of smaller, and therefore invisible, glycogen units which had diffused into the mitochondria, or were synthesized there.

The role of intramitochondrial glycogen in the rat retina is not understood at present. However, its consistent appearance in normal rat retinas seems to indicate that it plays some role in retina tissue. The co-existence of different types of mitochondria suggests that possibly they have different functions.

The authors express their thanks to Dr. G. K. Smelser of the Department of Anatomy and Ophthalmology, Columbia University, and to Dr. Eichi Yamada of the Department of Anatomy, Kyushu University, for their valuable advice and support during the course of this study. We also are indebted to Miss V. Ozanics of the Department of Ophthalmology, Columbia University, for reading the manuscript.

Part of this work was done while one of the authors (T. Ishikawa) was a Visiting Fellow, Fight for Sight Research Fellowship F 157 (C2), at the Department of Ophthalmology, Columbia University.

This research was supported in part by United States Public Health Service research grants NB 03614-02, NB 1202-09, and a Postdoctoral Training Grant from the National Institute of Neurological Diseases and Blindness, 5 TI NB 5324-04.

Received for publication, October 13, 1964.

REFERENCES

1. PEACHEY, L., Electron microscopic observations on the accumulation of divalent cations in intramitochondrial granules, *J. Cell Biol.*, 1964, **20**, 95.
2. NASS, M. M. K., and NASS, S., Intramitochondrial fibers with DNA characteristics. I. Fixation and electron staining reactions. II. Enzymatic and other hydrolytic treatment, *J. Cell Biol.*, 1964, **19**, 593.
3. REVEL, J. P., Electron microscopy of glycogen, *J. Histochem. and Cytochem.*, 1964, **12**, 104.
4. BEAULATON, J. M., Sur l'accumulation intramitochondriale de glycogéne dans la glande prothoracique du Ver à soie dur Chêne Antheraea pernyi (Guér) pendant les quatrième et cinquième stades larvaires, *Compt. rend. Acad. sc. Paris*, 1964, **258**, 4139.