Histological and Ultrastructural Changes in the Pineal Organ–Subcommissural Organ (PO–SCO) System of Adult *Xenopus* Induced by Various Light Conditions. I. Pineal Organ

By

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(With 2 Text-figures and 13 Plates)

Since Holmgren's (1918) classical work on the "Parietalorgan" in the frog, *Rana temporaria*, the pineal complex in lower vertebrates has attracted much attention (for references, see the review of Wurtmann *et al.*, 1968). In the anurans, the pineal organ and related structures have been investigated by a variety of techniques; ultrastructurally (Eakin *et al.*, 1963; Oksche and Vaupel-von Harnack, 1963, 1965; Kelly and Smith, 1964; Charlton, 1968; Ueck, 1968a, b; Hendrickson and Kelly, 1971), autoradiographically (Charlton, 1966b; Hendrickson and Kelly, 1969; Bunt and Kelly, 1971), electrophysiologically (Dodt and Heerd, 1962; Dodt and Jacobson, 1963; Dodt and Morita, 1967), histo- and cytochemically (Owman *et al.*, 1970; Ueck, 1971) and neuroanatomically (Paul *et al.*, 1971; Ueck *et al.*, 1971). These studies have confirmed the photoreceptive function of the anuran pineal organ.

Bagnara (1963, 1965) demonstrated that the blanching reaction occurred in larvae of the African clawed toad, *Xenopus laevis*, when they were confined to darkness. The reaction decreased after the pineal organ was removed. Charlton (1966a) and Wakahara (1970) have investigated the body coloration of larval and adult *Xenopus* with and without the pineal organ under various light conditions. From the many studies of the pineal organ in lower vertebrates it has been postulated that the organ contains, as hormonal substances, indole derivatives such as serotonin or melatonin (for references, see Bagnara and Hadley, 1970, 1973.)

Recently, Wakahara (1972) has shown that the pineal organ-subcommissural organ (PO-SCO) system in larval *Xenopus* effects the circadian rhythm in the mitotic activity of the tail fin. However, at present, it remains to be clarified how the photic stimuli received by the PO photoreceptor cells are transduced to internal humoral signals. In this respect, it was felt that more data are necessary

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on the ultrastructural alterations of the PO-SCO system. The present study describes the morphological features of the PO in young African clawed toads, *Xenopus laevis*, under different light conditions.

Materials and Methods

Xenopus laevis, used in this investigation were obtained by induced matings and cultured in laboratory aquaria. Toads three to six months after metamorphosis (about 30–40 mm in body length and 3–5 gm in body weight) were used.

For light microscopical study, thirty-six toads were divided into six groups and kept under different light conditions as follows: group 1, natural day-night conditions (in an outdoor pond); group 2, continuous darkness; group 3, LD 12:12 (100 lux at the light phase); group 4, LD 12:12 (1,000 lux at the light phase); group 5, LD 12:12 (10,000 lux at the light phase); and group 6, continuous light (10,000 lux). Throughout the experiment, the water temperature was kept at 20–22°C for all the experimental animals except those of group 1 where the temperature changed 14–25°C under natural conditions. The intensity of light used in groups 3, 4, 5 and 6 was obtained by artificial illumination with fluorescent lamps and white bulbs. One month later they were decapitated without anesthesia and fixed in Bouin's fluid for histological examination. All the animals were killed between 10:00 and 11:00 in the morning to avoid a possible circadian rhythm in the histological features of the pineal organ. Serial sagittal sections, 10 μ thick, were prepared and stained with aldehyde fuchsin (AF) after permanganate oxidation and nuclear counter stain.

For electron microscopical study, the toads were confined to the following light conditions for three weeks: (1) eight toads were placed under continuous light (10,000 lux); (2) eight were placed in continuous darkness; (3) thirty-six toads were kept in the natural day-night conditions of the laboratory or outdoor pond. The toads were killed by decapitation between 10:00 and 11:00 in the morning and the dorsal halves of the brains were dissected out and fixed with the following fixatives at 0–5°C: a) 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 (Millonig, 1961), b) glutaraldehyde plus formaldehyde buffered to pH 7.5 with 0.1 M phosphate buffer, and c) a three fold mixture containing glutaraldehyde, formaldehyde and acrolein buffered to pH 7.5 with 0.1 M phosphate buffer (Rodríguez, 1969). The brains, including the pineal organ, were trimmed in the fixative. The dissected pieces of tissues were immersed in fresh fixative for 2 to 4 hours, and after the thorough washing with the same buffer, were postfixed in 1% osmium tetroxide buffered at pH 7.5 with 0.1 M phosphate buffer for 2 hours. The tissues were then rinsed in distilled water to remove phosphate, dehydrated through the ascendant ethanol or acetone and embedded in Epon 812 (Luft, 1961). Ultrathin sections were cut on a Porter-Blum ultramicrotome and stained with lead citrate or doubly stained with uranyl acetate and lead citrate (Reynolds, 1963). Observations were made with a Hitachi HS-7 electron microscope.

Observations

1. Light Microscopical Study

Histological features of the pineal organ in the larval and adult *Xenopus* have previously been described by the present author (Wakahara, 1968). Under light microscopy, four cell types are distinguished: the photoreceptor cell, two types of supportive cells and the free cell. There is little histological variation in the pineal

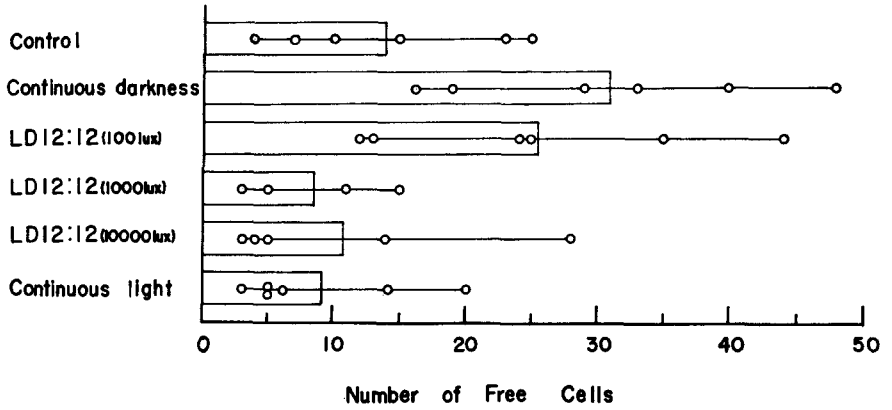

Fig. 1. Number of free cells counted in the pineal lumen of animals kept in various light conditions for a month. Each circle represents the value for each toad; columns, average values.

organ of toads reared in different light conditions, except for the number of free cells in the pineal lumen (Fig. 1).

The animals kept in continuous darkness had the largest number of free cells ranging from 16 to 48, (or an average of 30.8 cells per animal), and about twice as many as the controls, which possessed 7–25 cells (an average of 14.0 cells per animal). The toads kept under LD 12:12 conditions with a dim light (100 lux) contained 12 to 44 cells (or an average of 25.5 cells per animal). This amount is slightly larger than that in toads kept in the natural day-night conditions. In two other groups of toads reared in LD 12:12 but treated with greater intensities of light (1,000 and 10,000 lux), the number of free cells was very small, ranging from 3–15 (an average of 8.5 cells per toad) and 3–38 (an average of 10.8 cells per toad), respectively. These values are comparable to that in the continuous-light-adapted animals which possessed 3–20 cells (or an average of 8.8 cells per toad).

2. Electron Microscopical Study

All the three fixatives employed in the present study yielded similar results with respect to the form and size of the cells and the preservation of the membrane system and matrix. In the pineal wall, the ganglion cells were detected, which are characterized by cored vesicles near the Golgi-complexes within the relatively electron lucent cytoplasm. The two types of supportive cells, classified at the light microscopical level by the presence or absence of the AF positive cytoplasmic inclusions, were not recognized in the ultrathin sections.

In the following a description of the photoreceptor cell, supportive cell, ganglion cell and free cell is given, which constitute the pineal organs of the toads reared in continuous light (light-adapted) and continuous darkness (dark-adapted).

A. Photoreceptor cell

The photoreceptor cell, the principal constituent of the pineal organ, occurs much more frequently in the pineal floor than in the roof. It is composed of an outer segment, an inner segment and a nucleated basal part with a basal process extending into the neuropil zone (Fig. 2).

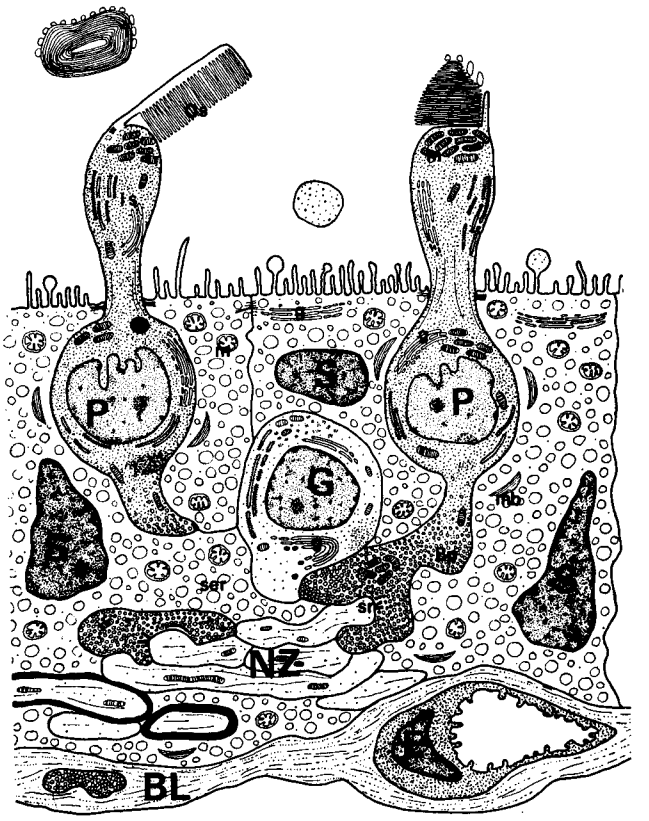

Fig. 2. Semi-diagrammatic illustration of the pineal wall of adult *Xenopus*. For explanation, see text. BL, basal lamina; Bp, basal process of photoreceptor cell; E, endothelial cell; G, ganglion cell; Is, inner segment of photoreceptor cell; NZ, neuropil zone; Os, outer segment of photoreceptor cell; P, photoreceptor cell; S, supportive cell; g, Golgi-complex; m, mitochondrion; mb, myeloid body; ser, smooth surfaced endoplasmic reticulum.

Outer segment

The outer segment of the photoreceptor cell is a stack of flattened sacs of the plasma membrane of ciliary derivatives. It is connected to the inner segment

by a connecting piece in which the ciliary fibrils arise from the centriole located in the inner segment (Figs. 7 and 8), and by finger-like cytoplasmic processes which also arise from the inner segment (Figs. 11–15). The outer segment varies in form and size, from those evidently fully developed (Figs. 3 and 4) to the one with a thin stack of sacs (Fig. 10). The outer segment with a thinner stack of sacs has a longer plasma membrane. The outer segment composed of about 100 flattened sacs is of 1–3 μ in width, while the one with a stack of 30–50 sacs measures 10 μ or over. Concentrically arranged plasma membranes sometimes accompanying whorls of myelinated membrane in the center are observed in the lumen (Fig. 11).

In general the outer segments are less developed in the two experimental groups than in the control group (Figs. 5 and 6). The segment with a stack of 100 or more flattened sacs of the plasma membrane, are rare in the light- and dark-adapted animals. Instead, only an apparent remnant, membranous structure is present (Fig. 6, arrows). The outer segment in the light- and dark-adapted animals, thus shows a wide diversity in morphology, presumably due to a lesser degree of development. The transfiguration of the plasma membrane into vesicles and tubules, a phenomenon sometimes encountered within the osmium tetroxide-fixed materials, was not observed in this study.

Inner segment

The inner segment of photoreceptor cells is distinguished from the nucleated basal part by a constriction, or neck, where the cell is connected with neighboring supportive cells by desmosomal junctions (Fig. 18). The inner segment contains mitochondria, Golgi-complexes, rough and smooth surfaced endoplasmic reticula, centrioles, free ribosomes and presumed glycogen particles. Occasionally, in all groups of toads, microtubules are observed running from the basal part to the inner segment through the neck region (Fig. 18). In contrast to the relatively underdeveloped outer segment in the light- and dark-adapted animals, the inner segment of the experimental groups usually contains a more developed rough surfaced endoplasmic reticula and Golgi-complexes than the control group (Figs. 16 and 17). The rough endoplasmic reticula, mostly with parallel cisternae but in some cases vesicular in form, are observed in the apical half of this segment. The Golgi-complexes are distributed from the neck region to the subapical region, an association with the vesicles and tubules. They usually contain medially electron dense matrix.

Basal part

The photoreceptor cell has in its basal part an indented nucleus usually with a prominent nucleolus and disseminated chromatin. The perinuclear cytoplasm contains mitochondria, Golgi-complexes, rough and smooth surfaced endoplasmic reticula, lysosome-like bodies, free ribosomes and presumed glycogen particles. No apparent difference was found among the three groups of animals on the ultrastructure of the nucleus and cytoplasm except for the lysosome-like bodies, which are usually larger in number in the light- and dark-adapted animals than

the control group.

The basal process extending into the neuropil zone contains numerous small vesicles about 400–600 Å in diameter and a few mitochondria within a relatively electron dense cytoplasm. Frequently, the basal process of the photoreceptor cell containing numerous small vesicles penetrates the basal lamina which separates the pineal organ from the habenular commissure fiber (Fig. 21). In one specimen of the light-adapted group, numerous, presumably glycogen, particles are present in the basal part of the photoreceptor cell (Fig. 19). They are electron dense, about 500–700 Å in diameter, without the external limiting membrane, and therefore can be distinguished from the vesicles in the basal processes of other photoreceptor cells.

B. Supportive cell

This type of cells characteristically contains large, spherical mitochondria, a considerable amount of vesicular smooth surfaced endoplasmic reticula and a peculiar membrane structure, or "myeloid body". In the dark-adapted animals, the myeloid body is larger than those both in the normal control and light-adapted animals (Figs. 22–27). The length of the myeloid body in the dark-adapted animals (an average of 2.14 μ in 42 myeloid bodies) and is more than those of the control and light-adapted animals (an average of 1.48 and 1.45 μ in 48 and 22 myeloid bodies, respectively). Also there are larger numbers of myeloid bodies in the dark-adapted animals than in the other two groups, although no conclusive evidence is yet available because of a relatively small number of specimens examined.

In constrast to the supportive cells in the pineal floor, those located in the pineal roof frequently have peculiar whirled membranous structures of remarkable morphological diversity (Fig. 29). Some are morphologically similar to the membranous structures in the free cell. In the light- and dark-adapted animals, peculiar whirled membranous structures were frequently encountered within the supportive cells of the pineal roof and sometimes even in those of the pineal floor. Further, many lysosome-like bodies, of various size and forms, are present in the supportive cells of the light-adapted animals, and their contents indicate that the membranous structure is in different stages of disintegration. In one case, several such lysosome-like bodies were observed occupying a large part of the cytoplasm (Fig. 30). In one specimen of the light-adapted group, a supportive cell was found to attach closely to the outer segment of the photoreceptor cell (Fig. 31).

Differences in the smooth endoplasmic reticula, mitochondria, Golgi-complexes, microtubules and fine filaments (Fig. 28) are hardly noticeable ultrastructurally.

C. Ganglion cell and neuropil zone

The ganglion cell is characterized by its relatively round nucleus and electron lucent cytoplasm. In normal animal, the nucleus contains a prominent nucleolus

and uniformly distributed chromatin granules (Fig. 32). The cell is also characterized by abundant ribosomes that are aggregated to form rosettes and by cored vesicles (about 800–1,500 Å in diameter) located near elaborate Golgi-complexes (Fig. 33). The lamellar and vesicular rough surfaced endoplasmic reticula are well developed in the perikaryon. A number of electron dense particles without external limiting membrane, presumably glycogen, are also present. Mitochondria are generally small in size, but sometimes an extremely large mitochondrion is found with the packed cristae running parallel to the long axis. Also present in a normal animal was a large electron dense body (about 0.8–1.2 μ in diameter) consisted characteristically of membranous whirled structures (Fig. 32). Ultrastructurally the ganglion cell in the two experimental groups is almost the same as that depicted in the control group except in the number of cored vesicles. In the light-adapted animals, the cored vesicles are more abundant in the perikaryon than in the dark-adapted and control animals. However, no difference was found in the size and form of the cored vesicles among the three groups.

The neuropil zone has a complicated structure, intermingled with the basal processes of the photoreceptor cells, presumably dendritic processes of the ganglion cells and myelinated and unmyelinated nerve fibers of unknown origin, located deeply in the pineal wall (Fig. 34). The amount of minute vesicles contained in the basal process of the photoreceptor cell is much greater at the distal end than in the proximal part. Large granules (about 800–1,000 Å in diameter) probably with the external limiting membrane are observed among numerous uniformly elaborated minute vesicles (Fig. 20). The mitochondria in the distal part of the basal process is sometimes aggregated to form a cluster. The presynaptic part of the synaptic apparatus is composed of synaptic rods or ribbons and numerous synaptic vesicles which surround them (Fig. 34). No difference was found in the number and structure of these organelles between the two experimental groups and the normal control group.

D. Free cell and other structures in the lumen

A greater number of free cells are present in the pineal organ of the dark-adapted animals. The cells are extremely variable in shape and size and usually contain mitochondria, smooth and rough surfaced endoplasmic reticula, lysosome-like bodies and a large number of membranous inclusions (Figs. 36 and 37). The membranous inclusions show various degrees of disintegration of the plasma membrane, from being almost intact concentrically arranged to the whirls of the myelinated membrane, or to the membranous debris within the lysosome-like bodies of various electron densities.

In general, the free cell has numerous endoplasmic reticula and is devoid of the Golgi-complexes. However, a peculiar type of free cell was sometimes observed which, like the photoreceptor cell, had an ellipsoid-like cluster of mitochondria, well developed Golgi-complexes with associated vesicles and even the finger-like

cytoplasmic processes closely surrounding the outer margin of the outer segment (Fig. 37). Such "photoreceptor-like" free cells are found not only in the dark-adapted animals but also in the light-adapted and control animals (Figs. 38 and 39). Further, the cytoplasmic processes containing numerous microtubules and elongated mitochondria are present in the pineal lumen of the dark-adapted animal (Fig. 40). It was not confirmed, however, where these cytoplasmic processes had originated from.

Extraordinarily large vesicles (up to 45 μ in diameter) appeared in the pineal lumen of the light-adapted animals. These vesicles contain minute particles of different electron densities. Having no external limiting membrane, these particles might be similar to the presumed glycogen particles that are observed in the pineal parenchymal cell.

Discussion

While Charlton (1968) could only distinguish two types of cells, the "include cell" and "support cell" in the pineal organ of *Xenopus laevis*, in this study at least three cell types have been detected electron microscopically: the photoreceptor cell, ganglion cell and supportive cell. The glia cell, which has been distinguished from the supportive cell by its localization and cytoplasmic properties in *Rana* by Oksche and Vaupel-von Harnack (1963), could not be distinguished in this study. In addition, the free cell in the pineal lumen, which may correspond to "Freie Zelle" (Oksche and Vaupel-von Harnack, 1963) or "macrophage" (Kelly and Smith, 1964; Ueck, 1968a), is a common constituent of the pineal organ in spite of its morphological diversity.

It has been shown light microscopically that the free cells in the pineal lumen increase in number as the animals stay in continuous darkness. Although some alterations in the morphology of the pineal organ or frontal organ of the lower vertebrates have been reported to occur under various light conditions (Grunewald-Lowenstein, 1956; Eakin *et al.*, 1963), the increase in the number of free cells in the pineal lumen of the dark-adapted *Xenopus* was reported for the first time in this paper. This increase is also confirmed by electron microscopy. It should be noted the toads treated with a 24-hour cycle of LD 12:12, with a dim illumination of 100 lux at the light phase, have a similar number of free cells to those of dark-adapted animals.

Kelly and Smith (1964) postulated that the free cell in the anuran pineal lumen originated from the macrophage. The present investigation, however, clearly demonstrates the presence of "photoreceptor-like" free cells. Because the "photoreceptor-like" free cells in the pineal lumen were observed in the two experimental animals as well as in the control animals, and as these cells and their neighboring tissues were well preserved electron microscopically, the possibility of these cells being fixation artifacts is excluded. Further, the supportive cells contain a membranous structure similar to the ones observed in the free cells

and the presumed dendritic or axonal processes of the ganglion cell are present in the pineal lumen. These features indicate that the free cells in the pineal lumen originated from the pineal parenchymal cells.

The diverse morphology of the outer segment of photoreceptor cell in lower vertebrates is well known; (in fishes: Breucker and Horstmann, 1965; Oksche and Kirschstein, 1967; Rüdeberg, 1968, 1969; Takahashi, 1969; Takahashi and Kasuga, 1971; Murphy, 1971; in amphibians: Eakin *et al.*, 1963; Oksche and Vaupel-von Harnack, 1963, 1965; Kelly and Smith, 1964; Ueck, 1968a; Hendrickson and Kelly, 1971; in reptilians: Collin, 1967; Oksche and Kirschstein, 1968; Petit, 1969). Some of these morphological differences may actually be fixation artifacts, and therefore are not real. In the present study, the transfiguration of the plasma membranes that form the outer segment was avoided by using proper fixation methods. Evidence has been presented to support the hypothesis that the outer segment of the pineal photoreceptor cell shows a degeneration-regeneration cycle as postulated by Holmgren in 1918. It has been demonstrated that the number of the regularly arranged outer segment is smaller in the light- and dark-adapted animals than the control animals. This may indicate that in the outer segment the degenerative process is more dominant than the regenerative process. In addition, large outer segment-like structure has been observed (Figs. 44–46) in the pineal organ of toads which, with their eyes bilaterally removed, were reared under day-night conditions for a month. The inset of Fig. 44 clearly illustrates that lamellated plasma membranes arrange intricately to form a voluminous mass. This suggests that the regenerative process of the outer segment prevails over the degenerative one under that condition, hence resulting in an extraordinarily large outer segment-like structure.

Ueck (1968a) has postulated that degeneration of the outer segments occurs in two ways ultrastructurally: the membrane complexes are discharged from the apex, or lysis begins in the basal portion. It was not determined which degeneration process dominates under the experimental conditions. Kelly and Bunt (1971) have demonstrated autoradiographically that the outer segments of the pineal photoreceptor cell are on a continual renewal process such as Young and his coworkers have postulated in the retinal photoreceptors (Young, 1967; Young and Droz, 1968; Young and Bok, 1969). More precise investigations are necessary to understand thoroughly the relationship between the light (or dark) and the degeneration-regeneration process of the outer segments.

The increase in size and number of the myeloid bodies in the supportive cell of the dark-adapted animals as compared to those of the control and light-adapted animals may give some clue to the function of this organelle in relation to the pineal photoreception.

An increase or decrease in the number and size of the free cells in the pineal lumen and of the myeloid bodies in the supportive cells are dependent on light conditions. This indicates that the anuran pineal organ has a photoreceptive function. However, a secretory function of this organ cannot be excluded. The

large vesicles observed in the pineal lumen of the light-adapted animals strongly suggest apocrine secretion from the supportive cells such as that reported in fish ependymal cells (Marquet *et al.*, 1972). Applications of modern biochemical techniques on the pineal organ has revealed the possible production, storage and/or release of certain chemicals by the pineal organ in anurans (for references, see Wurtman *et al.*, 1968). Using autoradiographical methods, Charlton (1966b) has demonstrated that the pineal organ of *Xenopus laevis* produces melatonin and the photoreceptor cell appears to be the site of its production. On the other hand, Owman *et al.* (1970) demonstrated with the aid of the fluorescent microscope that serotonin, the precursor of melatonin, is present both in the photoreceptor cell and supportive cell in *Rana esculenta* and *R. pipiens*. The presence of well-developed Golgi-complexes in the supportive cell and even in the photoreceptor cell stresses the possibility of the secretory function of these two sites.

Summary

The pineal organ of the African clawed toads, *Xenopus laevis*, kept in different light conditions was investigated by using light and electron microscopes. In the toads kept for a month in continuous darkness the organ contained more abundant free cells in the lumen than the organ in those kept in the usual day-night conditions, LD 12:12 (10,000 and 1,000 lux at the light phase, respectively) or the continuous light (10,000 lux). The increase in number of the free cells was found in animals kept in LD 12:12, with a dim light, 100 lux, at the light phase. Electron microscopically, these free cells were shown to have originated from the pineal parenchymal cells.

The outer segments of the photoreceptor cells were less developed in the light- and dark-adapted animals compared with those in the control animals. The myeloid bodies in the supportive cells increased in size and possibly in number in the dark-adapted animals. In the pineal lumen of the light-adapted animals, large vesicles were present. These vesicles probably originated from the supportive cells and contained numerous presumed glycogen particles.

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Explanation of Plates I-XIII

Abbreviations:

BL,	basal lamina	c,	centriole
Bp,	basal process	g,	Golgi-complex
E,	endothelial cell	lb,	lisosome-like body
F,	free cell	m,	mitochondrion
G,	ganglion cell	mb,	myeloid body
Is,	inner segment	n,	nucleus
NZ,	neuropil zone	nu,	nucleolus
Os,	outer segment	rer,	rough surfaced endoplasmic reticulum
P,	photoreceptor cell		
PL,	pineal lumen	ser,	smooth surfaced endoplasmic
S,	supportive cell		reticulum

All figures except for Figs. 44–46 are of the specimens embedded in Epon 812 and stained with uranyl acetate and/or lead citrate.

Figs. 3 and 4. Fully developed outer segments of photoreceptor cells in control animals. ×16,000.

Fig. 5. Many focus plane electron micrographs of a pineal organ of a control animal. The pineal lumen is crowded with many outer segments, which are consisted of a stack of flattened sacs or of concentrically arranged plasma membranes. ×4,000.

Fig. 6. Membranous remnants (arrows) are observed in the pineal lumen of a dark-adapted animal. ×3,000.

Figs. 7 and 8. Electron micrographs of the connecting piece of photoreceptor cells of the control (Fig. 7) and the dark-adapted animals (Fig. 8). Ciliary fibrils (arrows) are observed running from the centriole through the connecting piece. Note that the membranous remnants in the lumen of the dark-adapted animal occupy a position corresponding to the outer segment of the photoreceptor cell in control animal. Fig. 7, ×16,000; Fig. 8, ×12,800.

Figs. 9 and 10. Outer segments of photoreceptor cells consist of concentrically arranged plasma membranes (Fig. 9) or of thin stack of flattened sacs (Fig. 10) in control animals. ×10,000.

Figs. 11–15. Finger-like cytoplasmic processes of photoreceptor cells in control (Figs. 11, 13 and 14), dark- (Fig. 12) and light- (Fig. 15) adapted animals. The processes apparently come from inner segment (Figs. 11 and 12), closely surrounding outer segment (Figs. 13, 14 and 15). Fig. 11 and 12, ×16,000; Figs. 13, 14 and 15, ×12,500.

Figs. 16 and 17. Inner segment of photoreceptor cell in control (Fig. 16) and light-adapted (Fig. 17) animals. Rough surfaced endoplasmic reticula and Golgi-complexes are well developed in the light-adapted one. ×8,000.

Fig. 18. Nucleated basal part of photoreceptor cell in a light-adapted animal. Microtubules (arrows) are observed running from the inner segment to the basal part through the neck region, where the photoreceptor cell is connected by neighboring supportive cells through desmosomal junctions (arrowheads). ×10,000.

Fig. 19. An electron micrograph of a portion of basal nucleated part of a photoreceptor cell in light-adapted animal, showing huge accumulation of, presumably glycogen particles. ×16,500.

Fig. 20. Distal part of the basal process of a photoreceptor cell in control animal.

Relatively large granules (arrows) are seen among uniformly elaborated minute vesicles. ×20,000.

Fig. 21. Basal process of a photoreceptor cell in a control animal, penetrating the basal lamina. ×10,000.

Figs. 22–27. Myeloid bodies in supportive cell of the control (Figs. 22 and 23), the dark- (Figs. 24, 25 and 26) and the light-adapted (Fig. 27) animals. They are larger in dark-adapted animal than in the light-adapted and control animals. ×16,000.

Fig. 28. Supportive cell of a control animal having well developed Golgi-complexes. ×16,000.

Fig. 29. Myelinated membranous structure in a supportive cell of light-adapted animal. ×12,500.

Fig. 30. Extremely developed lysosome-like bodies in supportive cell of light-adapted animal. ×12,500.

Fig. 31. Electron micrograph of a supportive cell and the outer segment of a photoreceptor cell in light-adapted animal. Note that the supportive cell is closely attached to the photoreceptor cell's outer segment, which is connected to the cell's inner segment by the connecting piece (arrow). ×6,000.

Figs. 32 and 33. Electron micrographs of ganglion cells in the control (Fig. 32) and the light-adapted animals (Fig. 33). Many cored vesicles are seen near the Golgi-complexes in the light-adapted animal. Fig. 32, ×9,300; Fig. 33, ×20,000.

Fig. 34. Electron micrograph showing the neuropil zone in a control animal. Synaptic bars (arrows) of various size are observed. ×12,000.

Figs. 35 and 36. Free cell in pineal lumen of a dark-adapted (Fig. 35) and a control animals (Fig. 36), showing wide variety of morphology of inclusion. ×6,000.

Fig. 37. "Photoreceptor-like" free cell of control animal, showing nucleus, ellipsoid-like cluster of mitochondria, well developed Golgi-complexes and finger-like cytoplasmic process (arrow). ×6,000.

Figs. 38 and 39. "Photoreceptor-like" free cells of light- (Fig. 38) and dark-adapted animals (Fig. 39). Fig. 38, ×9,000; Fig. 39, ×6,000.

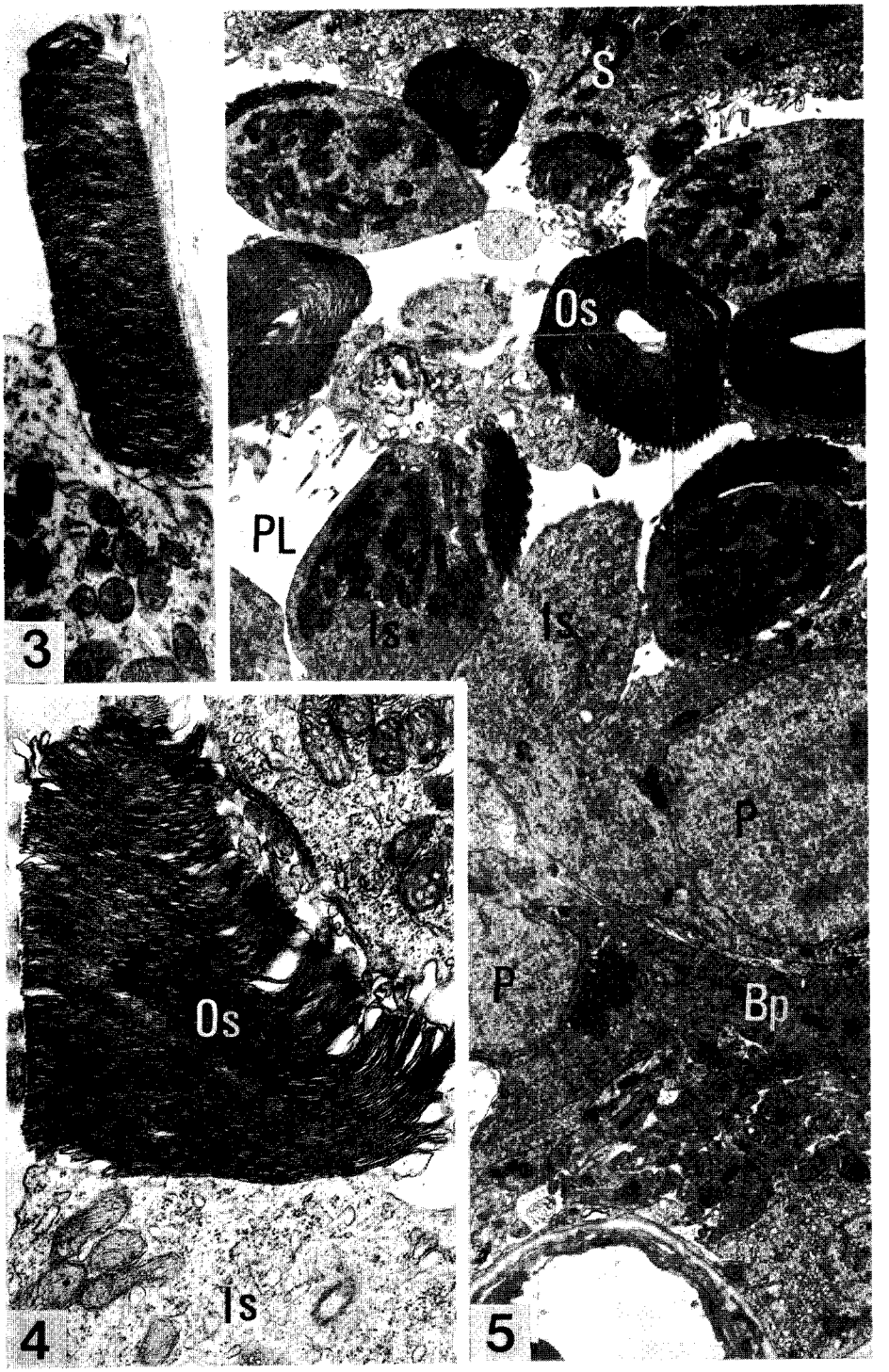
Fig. 40. Dendritic or axonal processes in the pineal lumen of dark-adapted animal. Both mitochondria and microtubules are elongated. ×5,000.

Figs. 41 and 42. Large vesicles in the pineal lumen of light-adapted animal. They contain a large number of particles, presumably of glycogen, with varying electron densities. ×11,500.

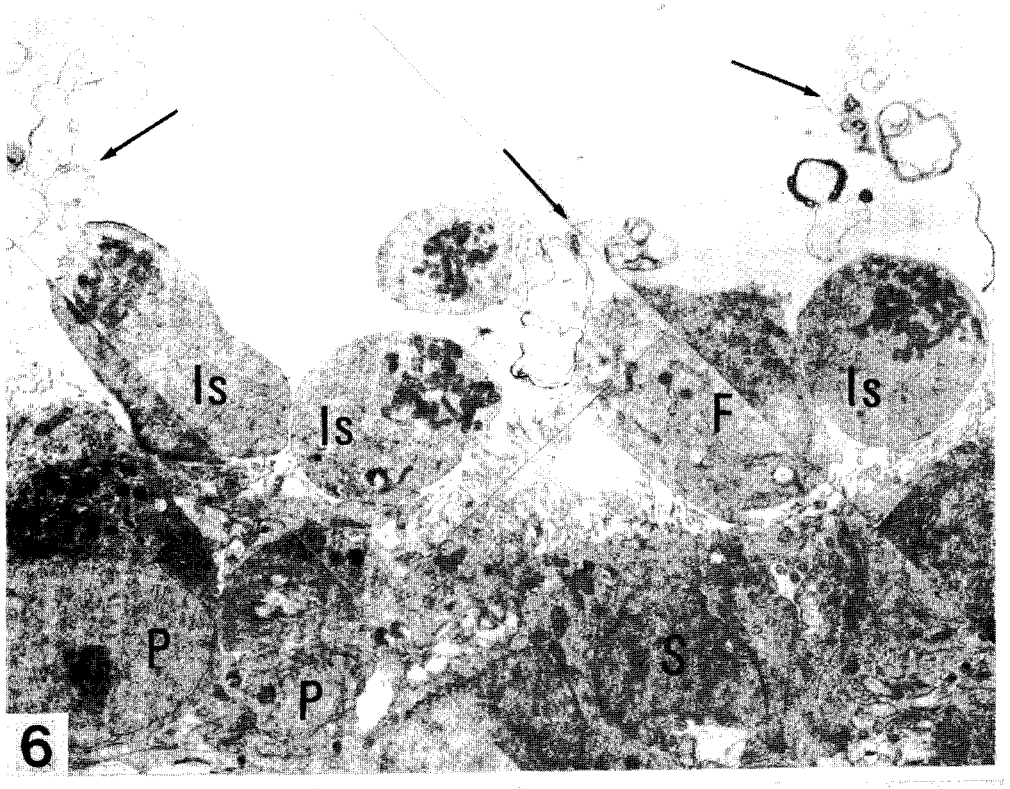
Fig. 43. Vesicles originating from the free surfaced of the supportive cell in light-adapted animal. ×10,000.

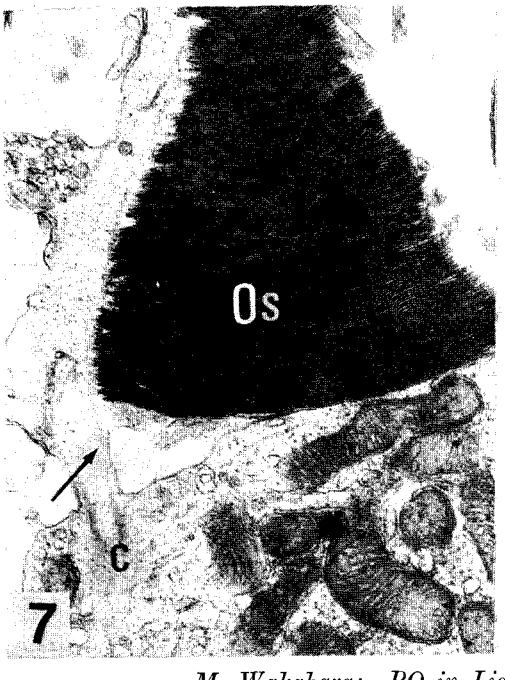
Fig. 44. A large outer segment-like structure in pineal lumen of an animal whose eyes had been removed bilaterally and kept in normal day-night conditions for a month. ×5,000. Inset: higher magnification (×24,000) of a section through the larger outer segment-like structure. Osmium tetroxide-fixed, styrene methacrylate-embedded, uranyl acetate-stained.

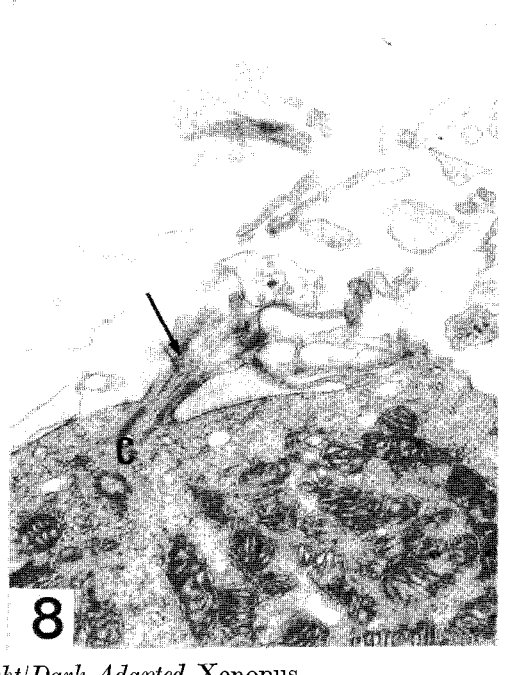
Figs. 45 and 46. Microphotographs of larger outer segment-like structures in blinded animals. Arrowheads, outer segments of photoreceptor cells; arrows, large outer segment-like structures. Bouin-fixed, paraffin-embedded. Fig. 45, AF-stained, ×900; Fig. 46, Heidenhain's iron hematoxylin-stained. ×900.



M. Wakahara: PO in Light/Dark-Adapted Xenopus







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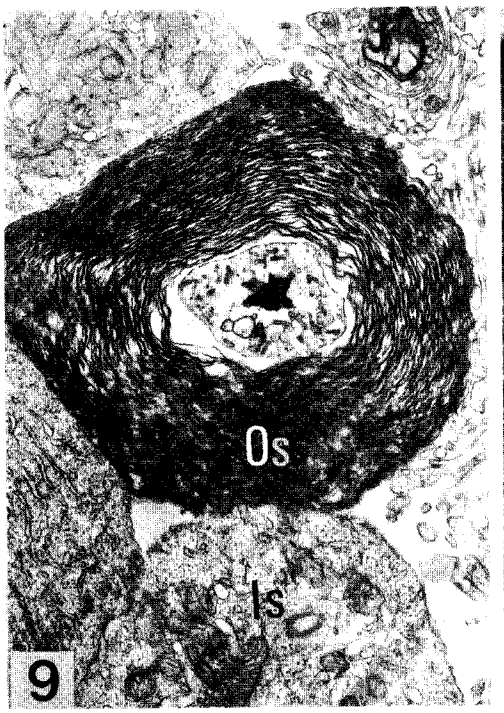
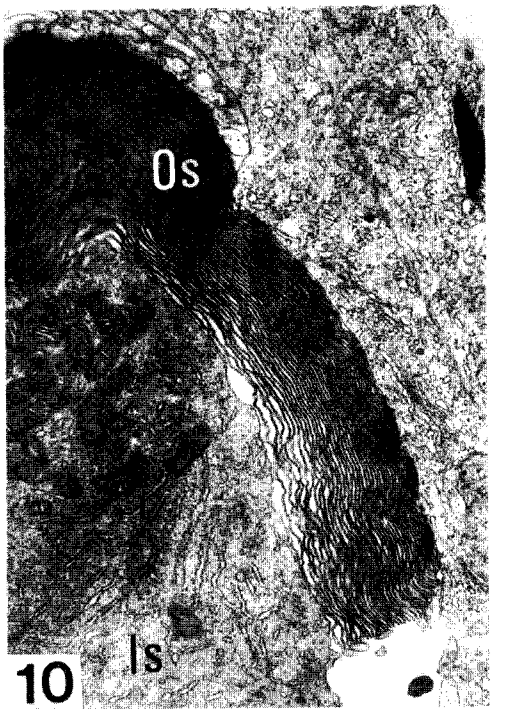
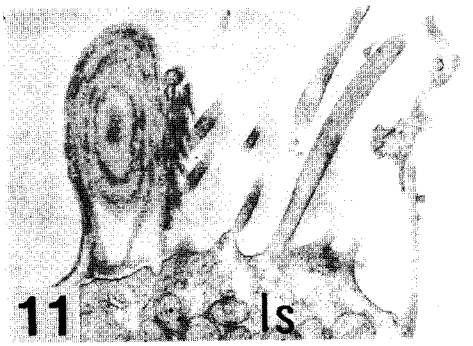
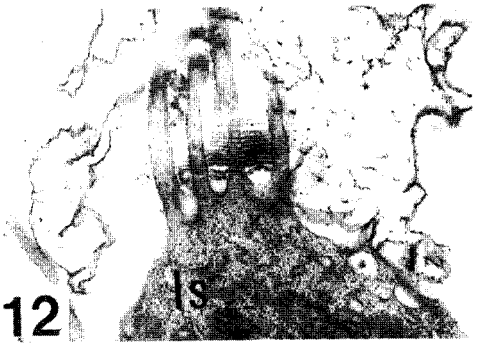
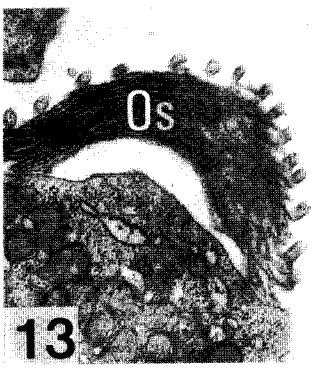
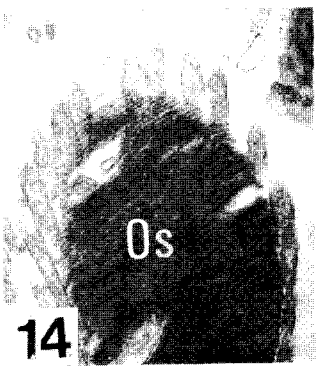


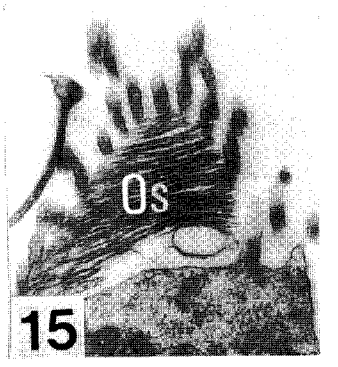




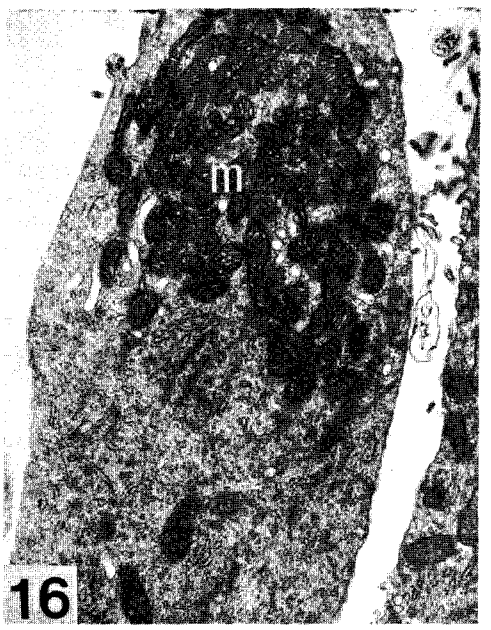


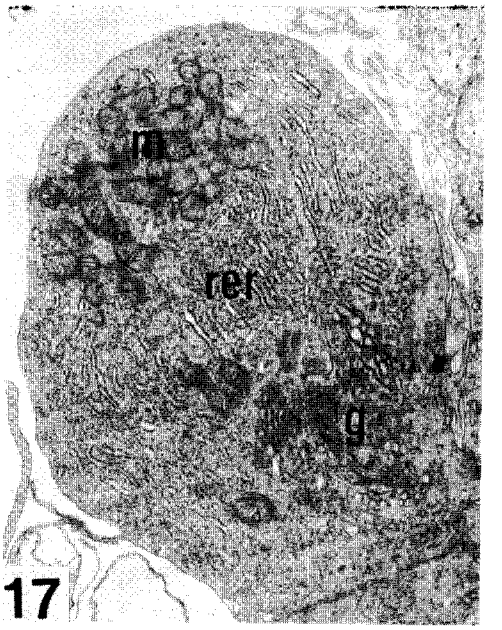


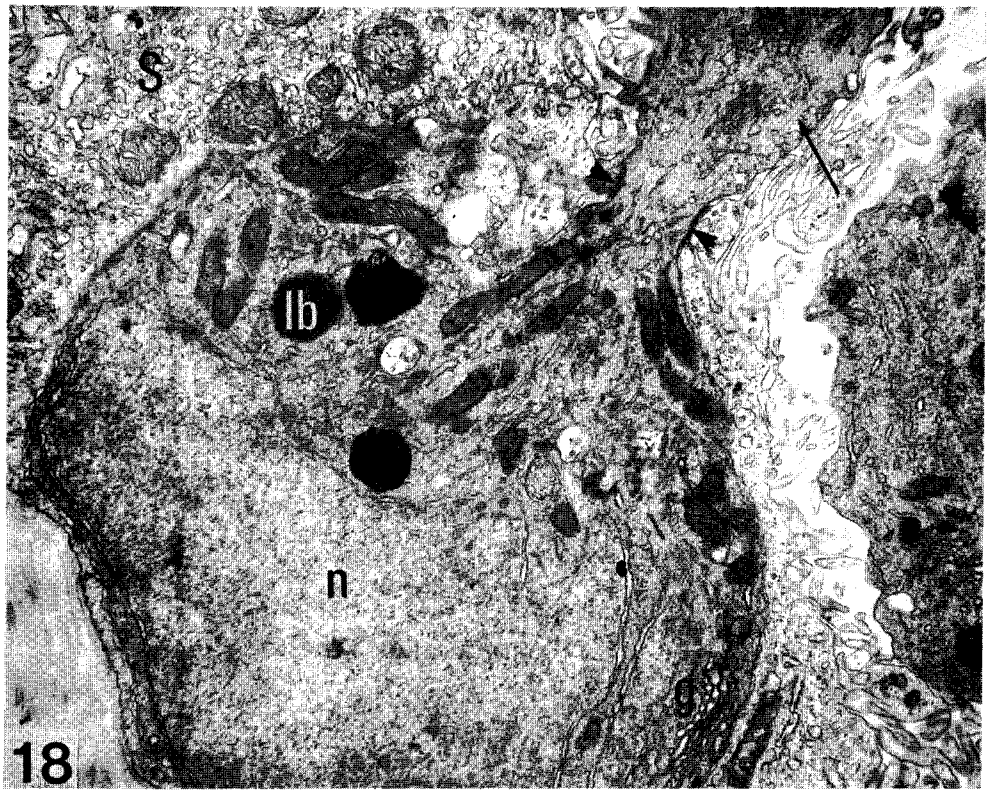




M. Wakahara: PO in Light/Dark-Adapted Xenopus







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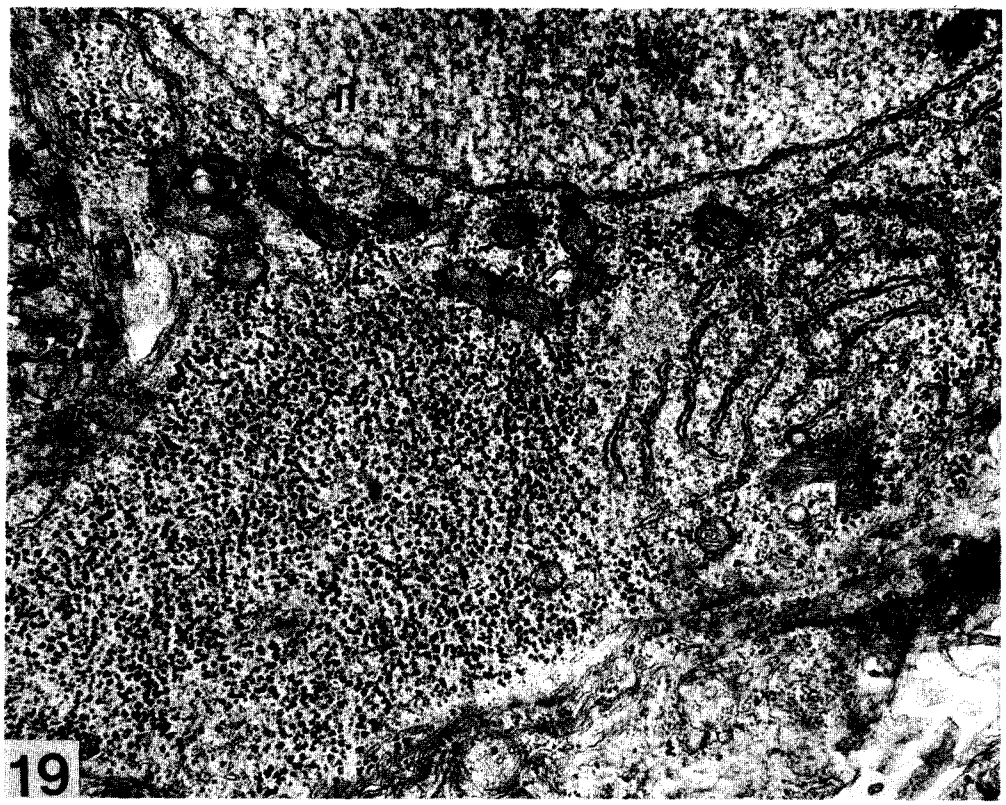

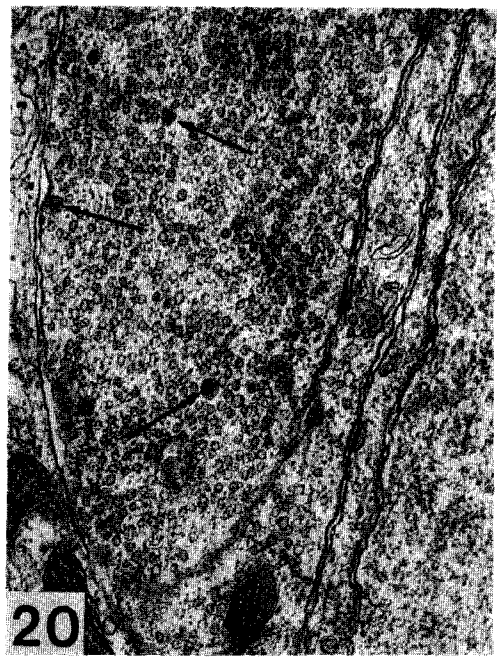

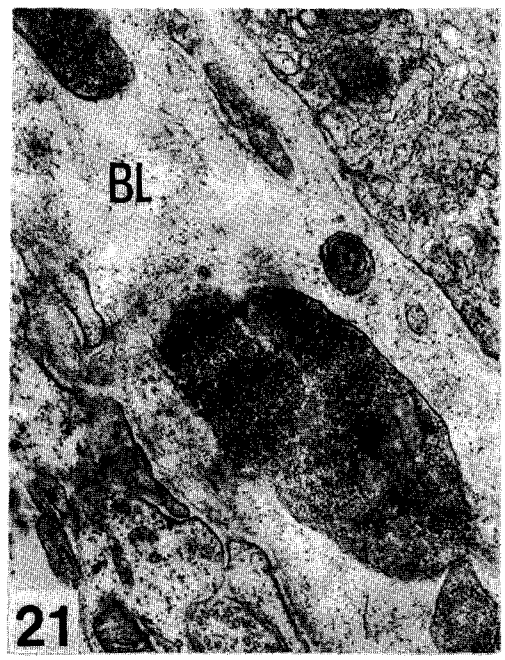

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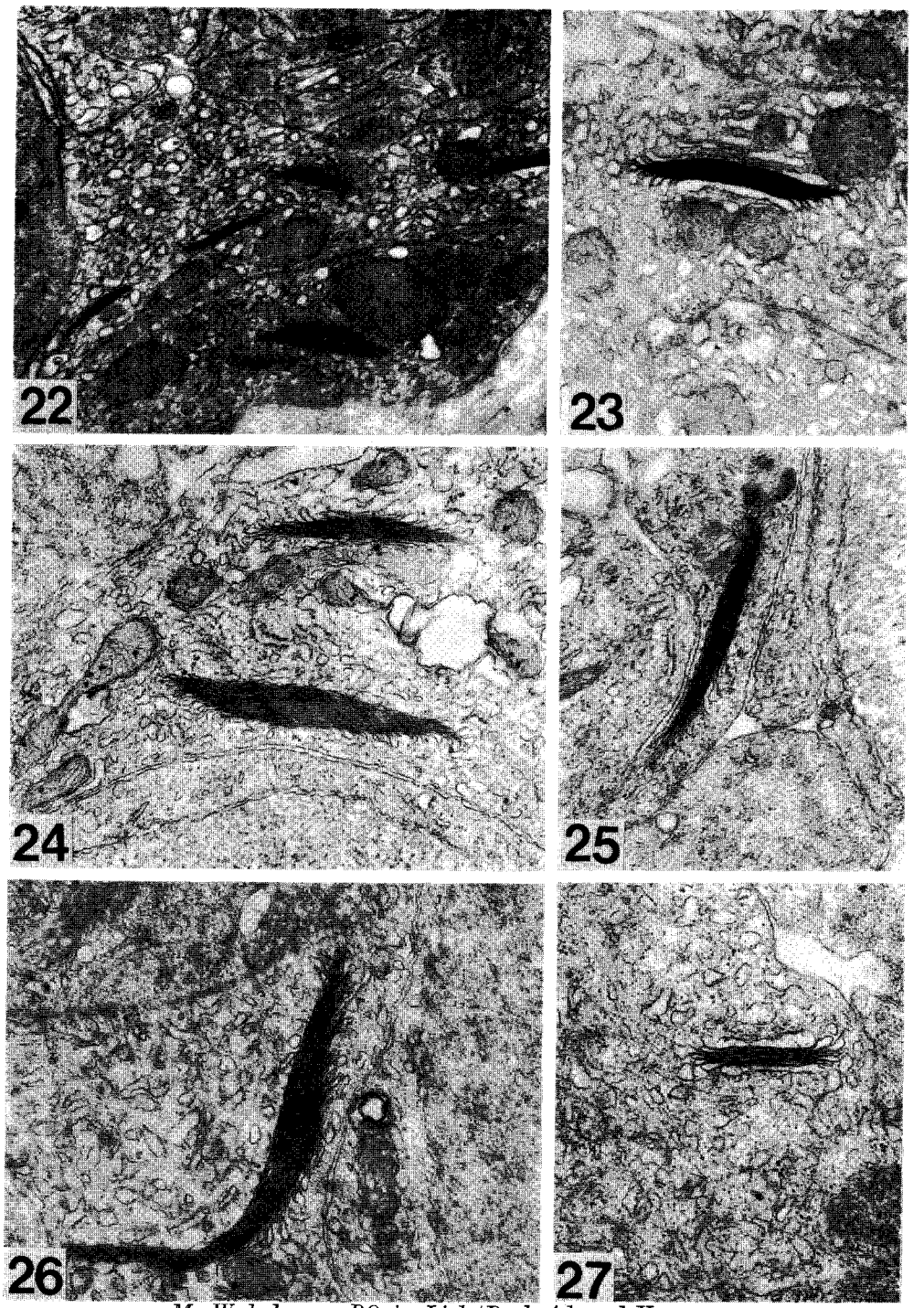

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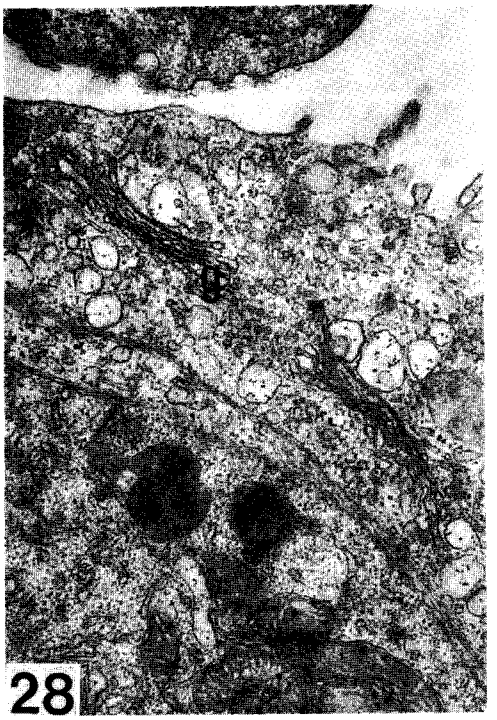

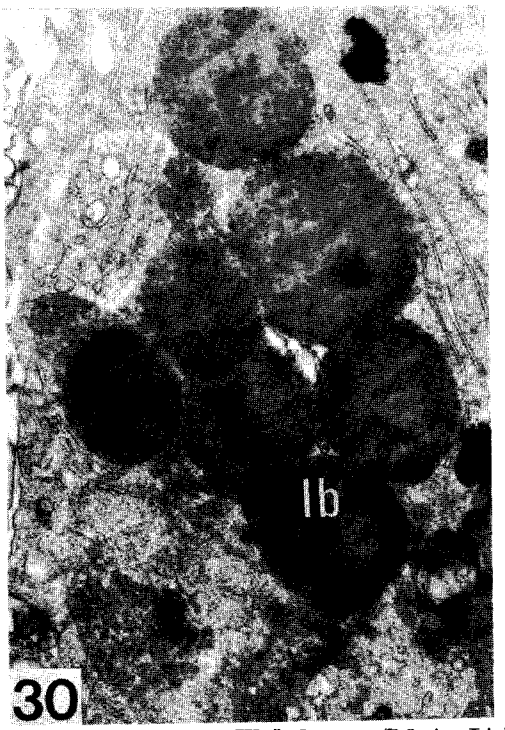

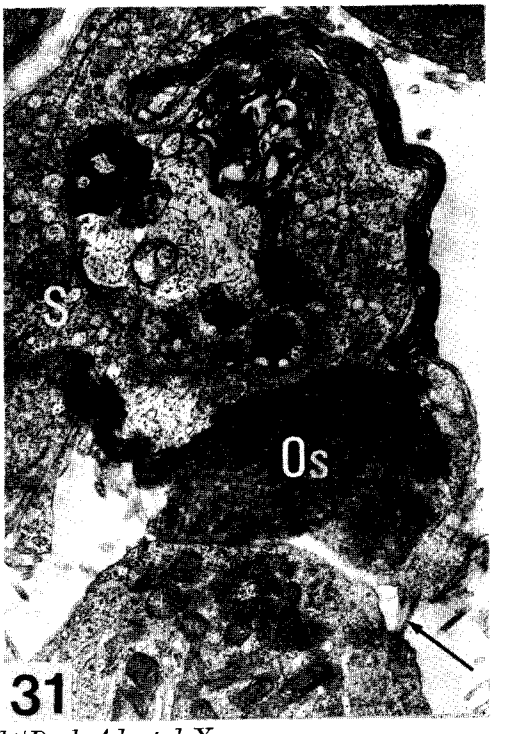

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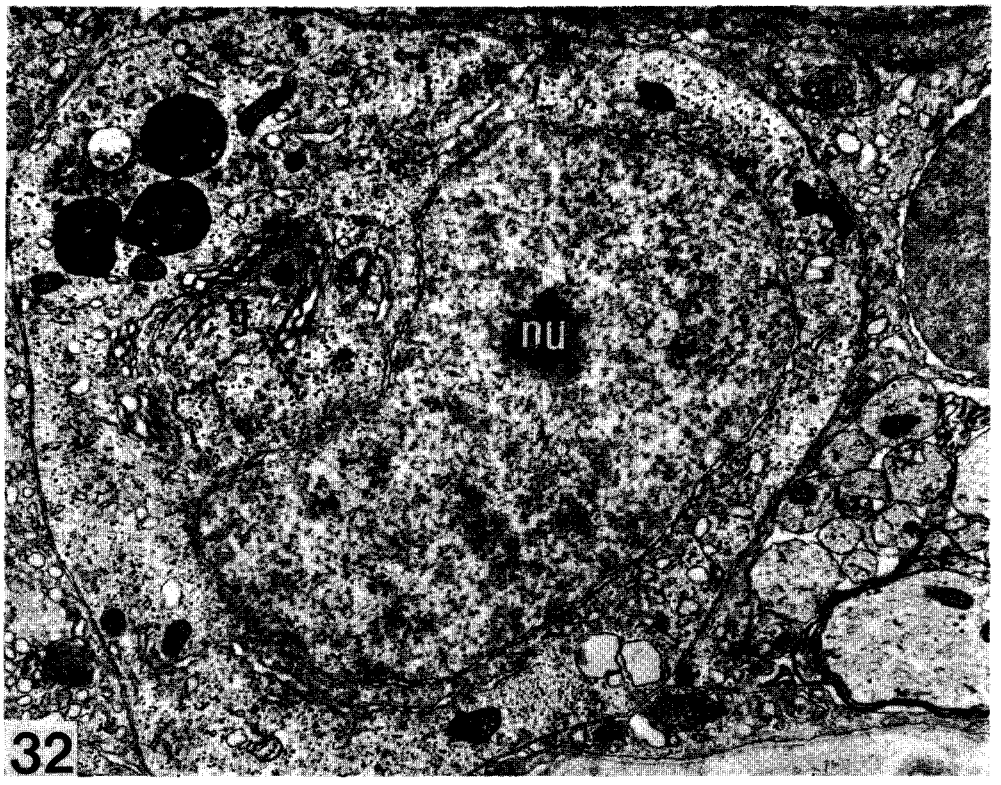

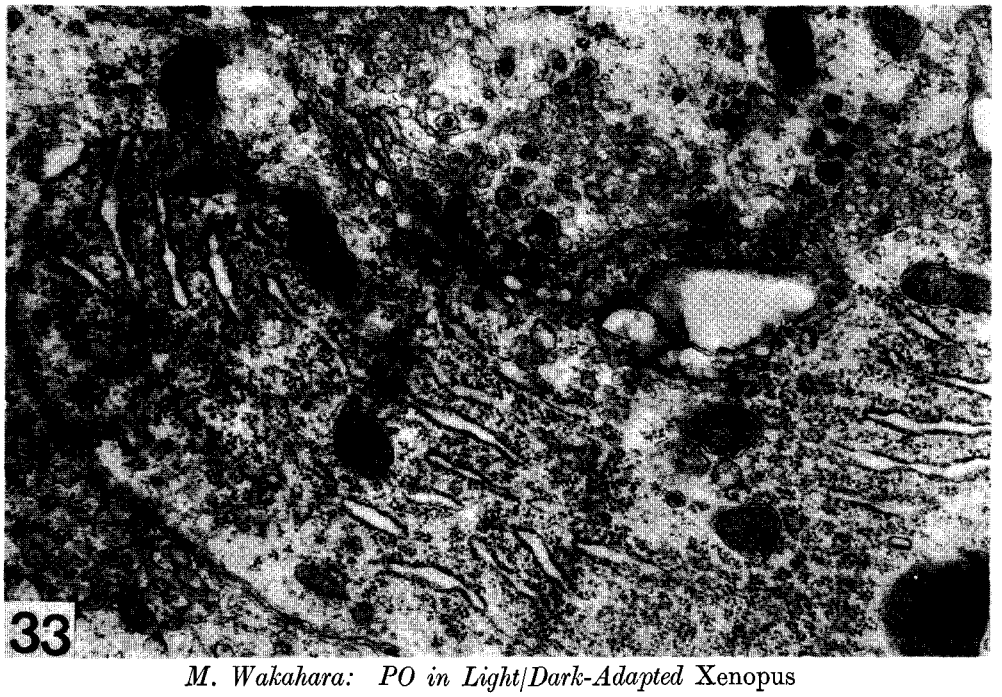

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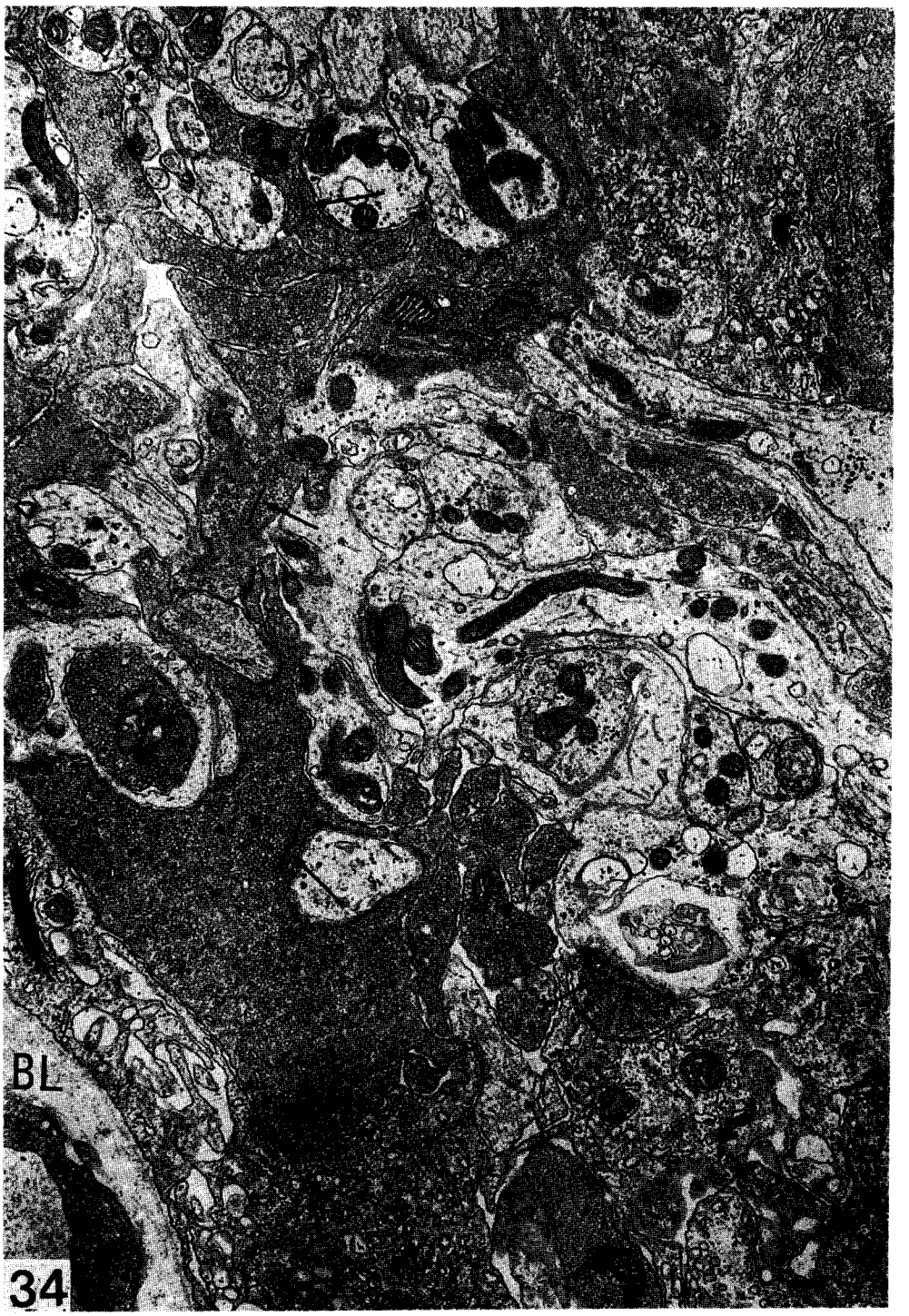

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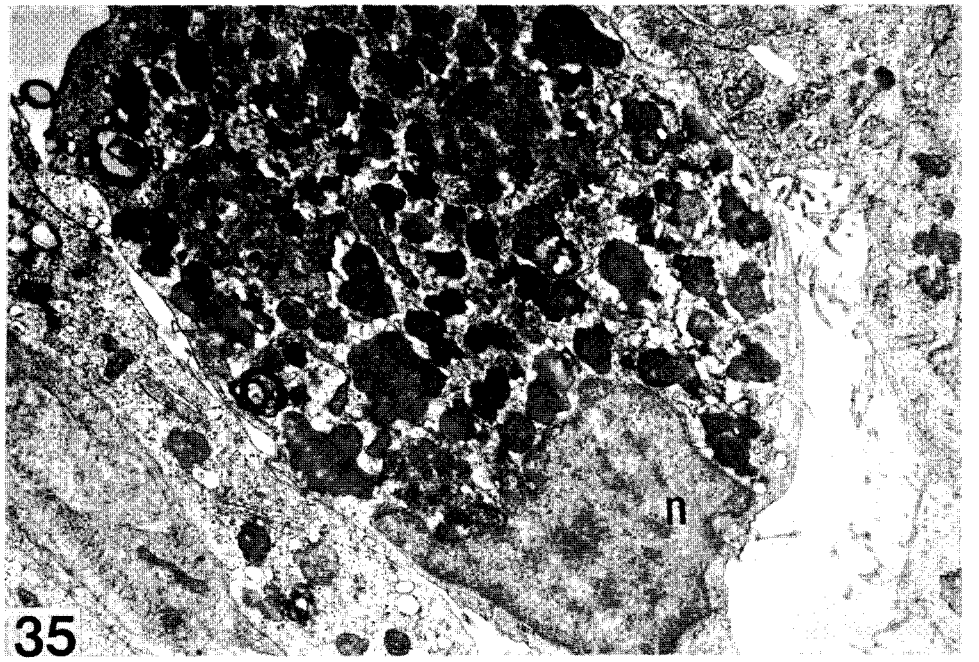

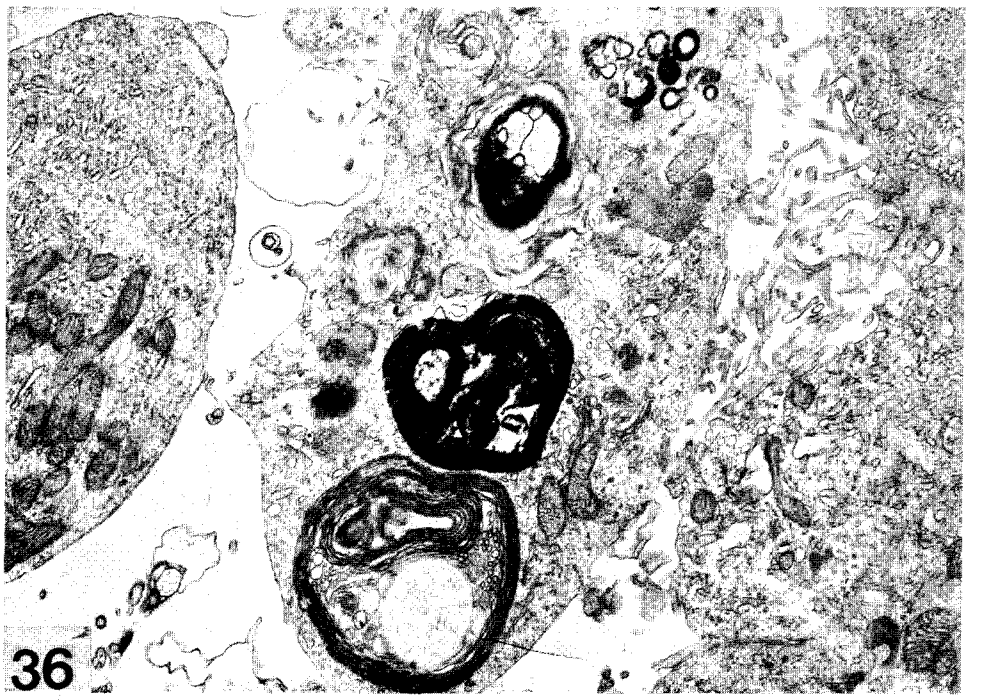

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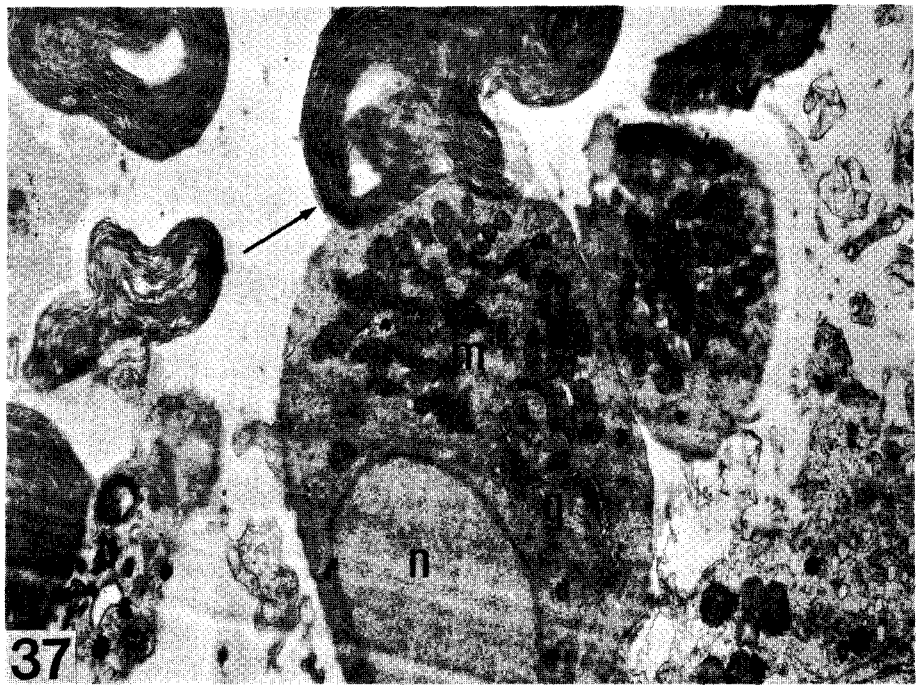

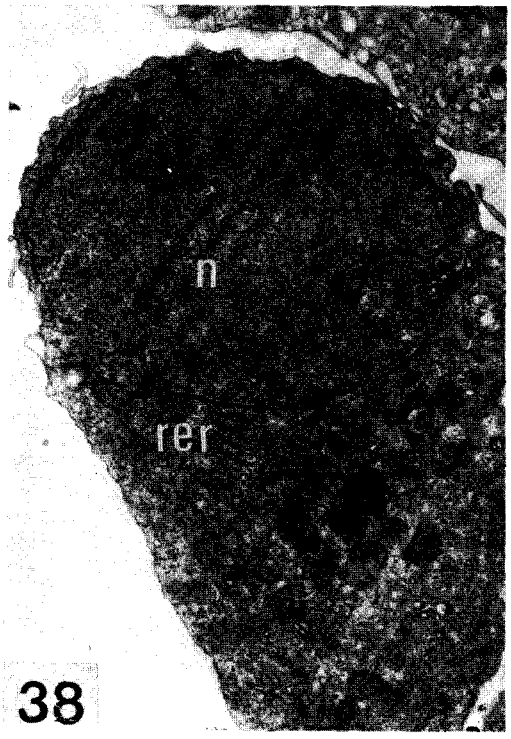

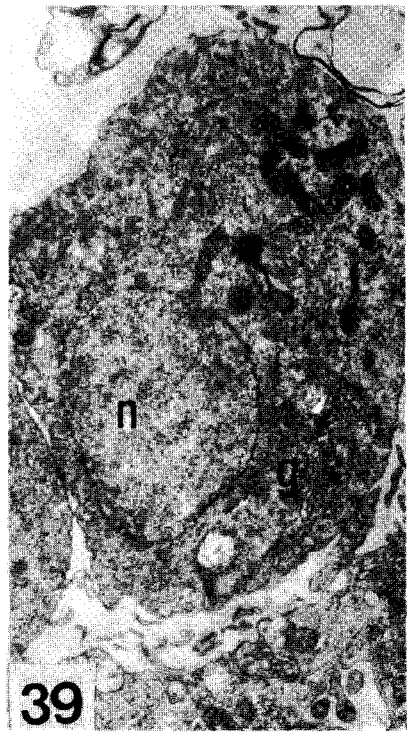

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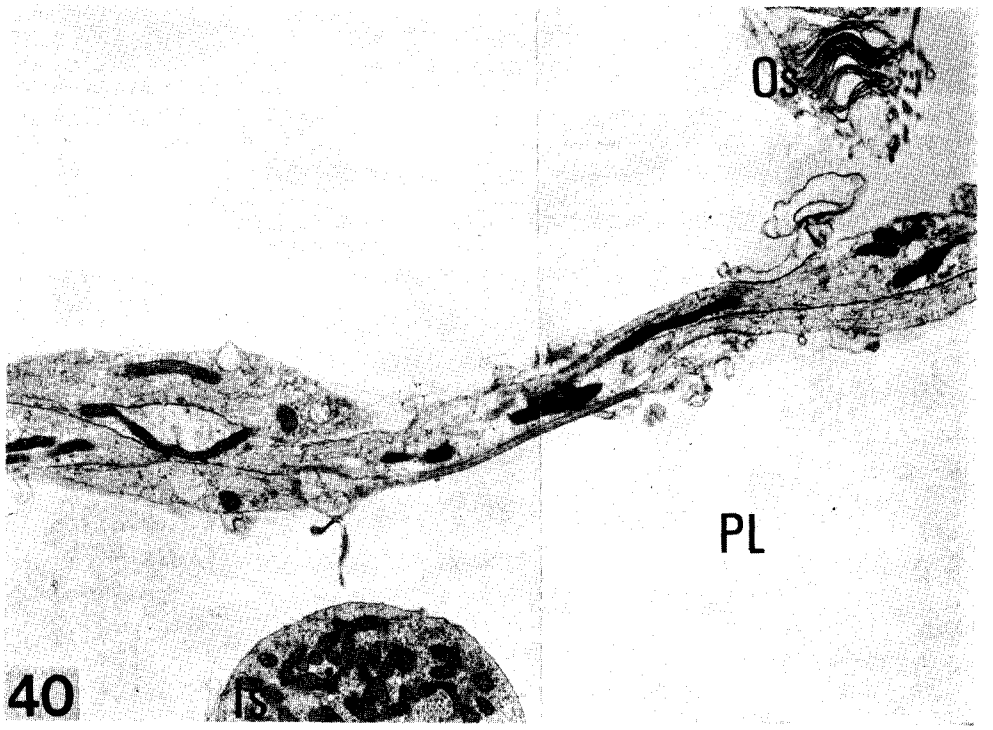
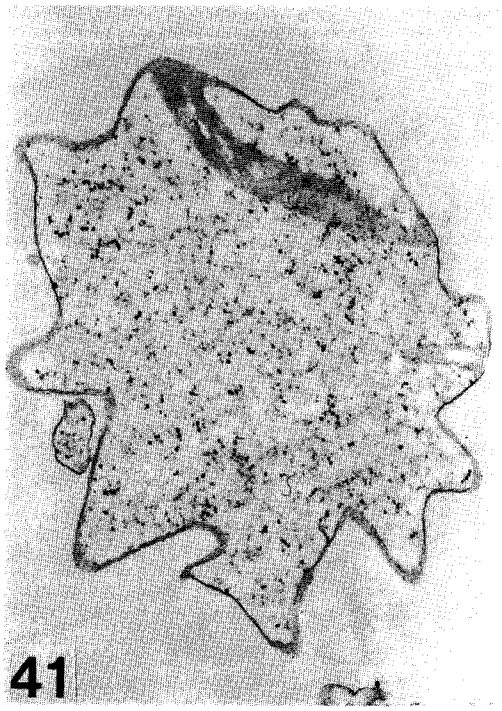
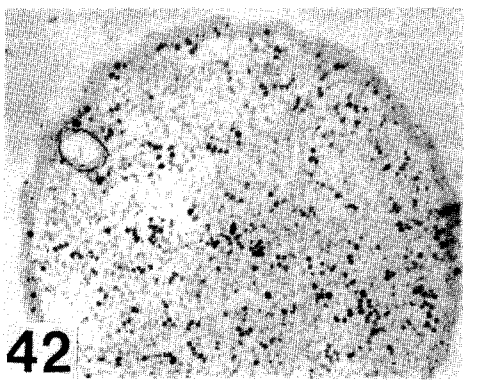
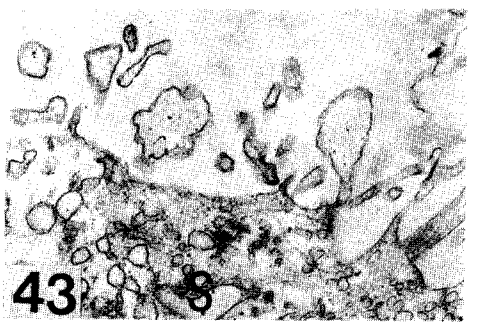
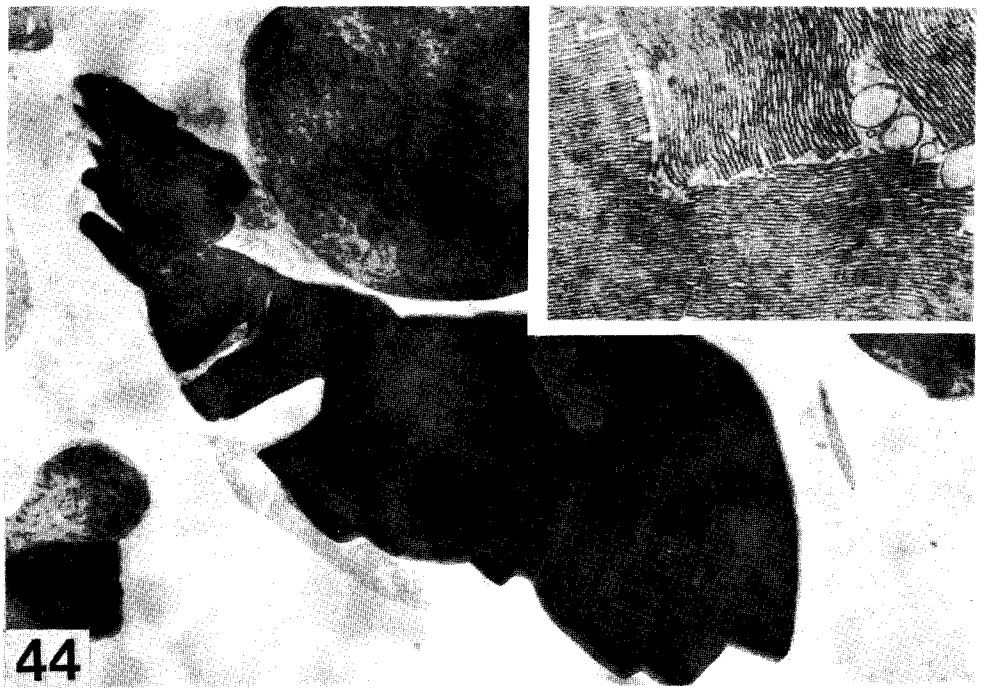


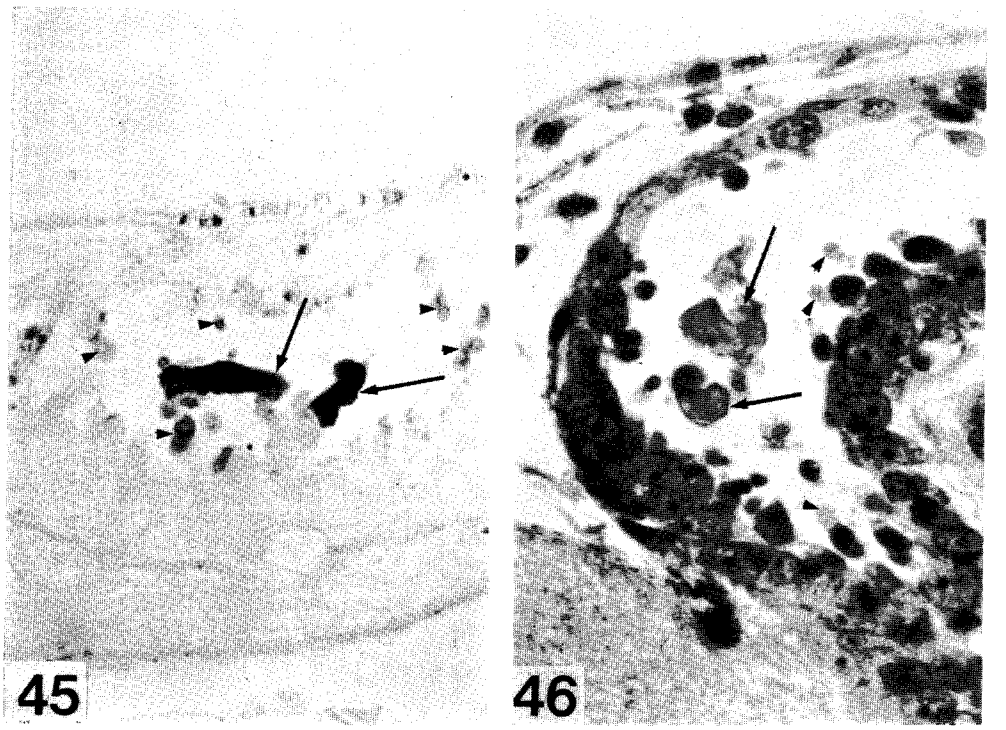






M. Wakahara: PO in Light/Dark-Adapted Xenopus





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