

Changes in length and disk shedding rate of *Xenopus* rod outer segments associated with metamorphosis

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(Communicated by B. B. Boycott, F.R.S. - Received 27 July 1977)

[Plates 1-3]

Histological examination of the retinae of *Xenopus* tadpoles undergoing the extensive transformations of metamorphic climax revealed a progressive and dramatic decrease in the length of rod outer segments (r.o.s.) (by 1.22 $\mu\text{m}/\text{day}$), which was reversed after the completion of metamorphosis, when r.o.s. grew longer (by 1.11 $\mu\text{m}/\text{day}$). The rate of r.o.s. disk addition during these two periods was determined by examining the incorporation of [^3H]-leucine by light microscopic autoradiography. The band of labelled protein in r.o.s. was displaced sclerally at a rate of 1.70 $\mu\text{m}/\text{day}$ during the first half of metamorphic climax, and of 1.56 $\mu\text{m}/\text{day}$ in young juveniles during the second month after metamorphosis. The similarity of the rate of band displacement at these times indicates that the changes in r.o.s. length associated with metamorphosis result from major changes in the rate of disk shedding and/or phagocytosis, which was about 2.92 $\mu\text{m}/\text{day}$ pre-metamorphically and 0.45 $\mu\text{m}/\text{day}$ post-metamorphically. E.m. observation at these stages and during the final stages of metamorphic climax revealed no significant alterations in the cellular organization or ultrastructure of rods or pigment epithelium, even though some r.o.s. were only 3 μm long. This large change in r.o.s. length undoubtedly influences the animal's scotopic sensitivity and the relative mesopic activity of its rods and cones, and may have important effects on the animal's visual physiology.

INTRODUCTION

During metamorphosis, amphibians undergo extensive behavioural, physiological, and morphological changes whose cellular and molecular basis remains incompletely understood. In most respects, visual development during this period represents a continuation of earlier developmental phenomena (Saxén 1954), however several modifications of the amphibian visual system have been reported to occur in association with metamorphosis (Kollros 1958; Wilson, 1971; Straznický & Gaze 1971; Bridges 1972; Beazeley, Keating & Gaze 1972; L. G. Fisher 1972, 1976; Pomeranz 1972; Chung, Stirling & Gaze 1975; Keating & Kennard 1976).

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Although the African clawed frog *Xenopus* undergoes many transformations during metamorphosis, including major changes in morphology, locomotion, and feeding, it exhibits fewer changes than most other anurans, (Deuchar 1972, 1975). Since this frog remains aquatic as an adult, its vision does not require modifications for terrestrial life (e.g., a change in visual pigment) as does that of many anurans (Deuchar 1972). It was originally reported that larval *Xenopus* photoreceptors contained both rhodopsin and porphyropsin (Crescitelli 1973), but more recent evidence shows that porphyropsin is the sole visual pigment present throughout this animal's life cycle (Bridges, Hollyfield, Witkovsky & Gallin 1977). In *Xenopus*, metamorphosis has been associated with several functional and morphological changes in the visual system. These changes include a gradual elongation of the rod outer segments (r.o.s.) (Saxén 1954; Witkovsky *et al.* 1976), a gradual change in synaptic organization and width of the inner plexiform layer (Hollyfield 1971; Fisher 1976; Tucker & Hollyfield 1977), the appearance of a new ganglion cell type (Chung *et al.* 1975), a continuation of the developmental shift in the retinotectal projection (Gaze, Keating & Chung 1974), and a change in orientation of the eyes accompanied by appearance of a binocular visual field (Beazeley *et al.* 1972).

In this paper we describe the morphology of *Xenopus* photoreceptors and pigment epithelium (p.e.), as well as the process of outer segment renewal, from the beginning of metamorphic climax to shortly after the completion of metamorphosis.

MATERIALS AND METHODS

(a) Rearing of animals

As described previously (Kinney & Fisher 1978b), *Xenopus laevis* larvae obtained by induced breeding of adults were fed a suspension of powered dried nettle leaves, kept under cyclic lighting conditions at about 22 °C, and staged according to Nieuwkoop & Faber (1956). Beginning at the final stages of metamorphic climax the animals were fed minced beef liver.

(b) Autoradiography

(i) Labelling at stage 57

Several larvae at stage 57 (last stage before the beginning of metamorphic climax; Altman & Dittmer 1972) were anaesthetized and injected in the posterior head region with [³H]-leucine at an approximate dosage of 0.3 mCi/g (see Kinney & Fisher 1978b for details). Larvae were again anaesthetized, staged, and enucleated in the light at $\frac{1}{2}$, $5\frac{1}{2}$, 7 and 9 days after injection. The eyes were fixed, embedded in araldite, and processed for light microscopic (l.m.) autoradiography, as described previously (Kinney & Fisher 1978a). Measurements of band displacement taken from 20 well aligned rods in the posterior retina of each specimen were used to determine the mean for each specimen, and the method of least squares was used to calculate the average rate of band migration during this period. From

similar measurements of r.o.s. length, the mean r.o.s. length for each retina was determined and used to calculate the average rate of r.o.s. shortening.

(ii) *Labelling of post-metamorphic juveniles*

Several juveniles (average mass 0.36 g) which had completed metamorphic climax approximately one month earlier were anaesthetized and injected intraperitoneally with [^3H]-leucine at an approximate dosage of 0.06 mCi/g. The animals were later anaesthetized and enucleated in the light at 6, 13, 20, and 27 days after injection. The eyes were fixed, embedded, and processed for l.m. autoradiography as previously described (Kinney & Fisher 1978*a*). Measurements were taken of band displacement and r.o.s. length on 20 rods in the central part of each retina. The means of these values were determined, and the average rates of band migration and r.o.s. elongation were calculated by the method of least squares.

(b) *Morphology*

The eyes of stages 63 through 66 larvae (final stages of metamorphic climax) and juveniles (up to 2 weeks post-metamorphic) were fixed and embedded by the same procedure used for autoradiography of adult eyes (Kinney & Fisher 1978*a*). Half μm sections containing well aligned r.o.s. in the posterior retina were collected, stained with 1% toluidine blue in 1% sodium borate, and observed by l.m.

For electron microscopy (e.m.), thin sections of stage 64 and 66 retinae were placed on grids, stained with uranyl acetate and lead citrate. The fine structure of the retinae prepared for l.m. autoradiography was also examined.

RESULTS

(a) *Autoradiography*

(i) *Labelling at stage 57*

Autoradiographs from larvae injected with [^3H]-leucine at stage 57 are illustrated in figures 1-3, plate 1. One-half day after injection (figure 1) a distinct band of radioactive material appears at the base of the r.o.s., after which it is displaced sclerally (figure 2, 5½ days) until, at 9 days (figure 3) it disappears from the outer segment and appears in phagosomes in the p.e. (figure 3, inset). The most dramatic observation during this period (the first half of metamorphic climax) is, however, progressive shortening of r.o.s. (figures 1-3). With this shortening, the apical border of the p.e. approaches the rod inner and cone outer segments (c.o.s., which display only diffuse labelling).

The mean and range of radioactive band displacement and r.o.s. length data are displayed graphically in figure 4, demonstrating that although the band of label migrates sclerally as at other stages (Kinney & Fisher 1978*a, b*), the r.o.s. are becoming shorter. Straight lines, generated by the method of least squares for the data of band displacement (continuous line) and r.o.s. length (dashed line), have slopes representing rate of 1.70 $\mu\text{m}/\text{day}$ for band displacement and 1.22 $\mu\text{m}/\text{day}$

for rod shortening. Combining these rates, one obtains a rate of disk shedding and phagocytosis of about $2.92 \mu\text{m}/\text{day}$ during this period.

(ii) *Labelling of post-metamorphic juveniles*

Figure 5-7, plate 1, are autoradiographs from animals injected with [^3H]-leucine about one month after metamorphosis. During this experiment, r.o.s. elongated and the apical border of the p.e. separated from the rod inner segments

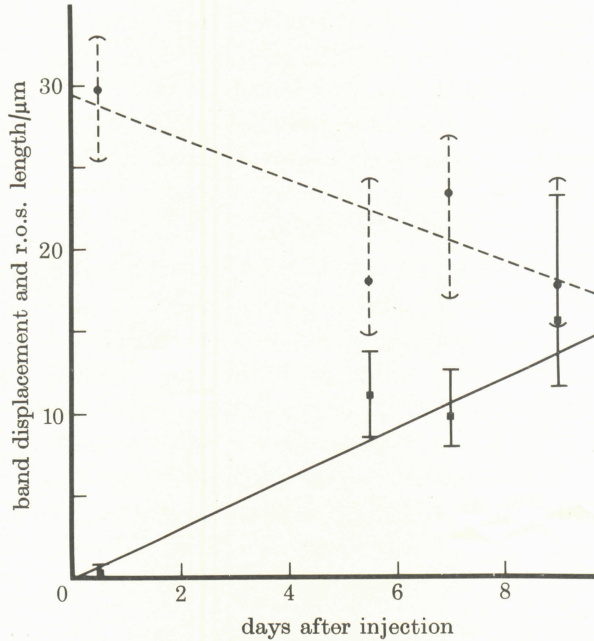


FIGURE 4. R.o.s. renewal in larval *Xenopus* injected at stage 57. The mean distance of band displacement (■) and r.o.s. length (●) are plotted as a function of days after injection. Ranges of these values are indicated by brackets. By the method of least squares, straight lines were fitted to the data for band displacement (continuous line) and r.o.s. length (dashed line). The slopes of these lines yield a band migration rate of $1.70 \mu\text{m}/\text{day}$ ($R^2 = 86\%$) and decrease in r.o.s. length by about $1.22 \mu\text{m}/\text{day}$ ($R^2 = 55\%$).

and c.o.s. The data from this experiment are displayed in figure 8, which shows the rate of band displacement ($1.56 \mu\text{m}/\text{day}$) to be slightly greater than the rate of r.o.s. elongation ($1.11 \mu\text{m}/\text{day}$), their difference yielding a rate of disk shedding and phagocytosis of $0.45 \mu\text{m}/\text{day}$.

(b) *Morphology*

R.o.s. length varied across the retina, with the most peripheral outer segments being generally longer than those located more centrally. Since the shortest central r.o.s. were found by l.m. to occur during the final metamorphic stages (64-66), emphasis was placed on studying retinæ from this period by e.m., after the

pre- and post-metamorphic retinæ used for autoradiography were found to have normal fine structure. The outer segments of a cone and rod at stage 64 are illustrated in figure 9, plate 2. The ultrastructure and organization of these outer segments is normal, except that the r.o.s. is only about 5 μm long. General photo-receptor morphology at stage 64 is shown in figure 10, plate 3; these inner segments, nuclei, and synaptic terminals appear normal (compare to fig. 8, plate 2, in Kinney & Fisher 1978a), with the possible exception that higher numbers of autophagic vacuoles occur in the receptor inner segments during metamorphosis. Figure 11,

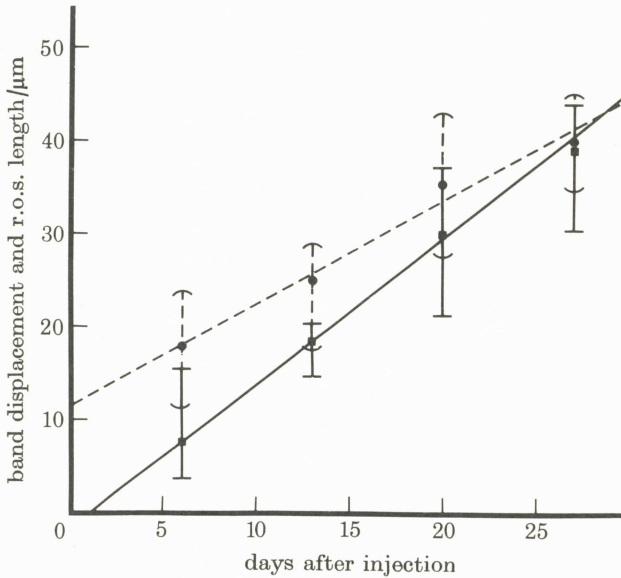


FIGURE 8. R.o.s. renewal in juvenile *Xenopus* injected about one month after metamorphosis. The mean distance of band displacement (■) and r.o.s. length (●) plotted as a function of days after injection. Ranges of these values indicated by brackets. By the method of least squares, straight lines were fitted to the data for band displacement (continuous line) and r.o.s. length (dashed line). The slopes of these lines yield a band migration rate of 1.56 $\mu\text{m}/\text{day}$ ($R^2 = 94\%$) and increase in r.o.s. length by 1.11 $\mu\text{m}/\text{day}$ ($R^2 = 86\%$).

plate 3, demonstrates the expanded width of the p.e. at this stage relative to non-metamorphic stages. The p.e. displays the usual complement of organelles as well as numerous large phagosomes and residual bodies. Long cell processes extend towards the outer segment tips from the apical p.e. border which is now more closely apposed to the receptor inner segments than at preceding stages. At stage 66 (figures 12 and 13, plate 4) r.o.s. likewise have normal fine structure and are considerably shortened (the r.o.s. in figure 12 is only 3 μm long).

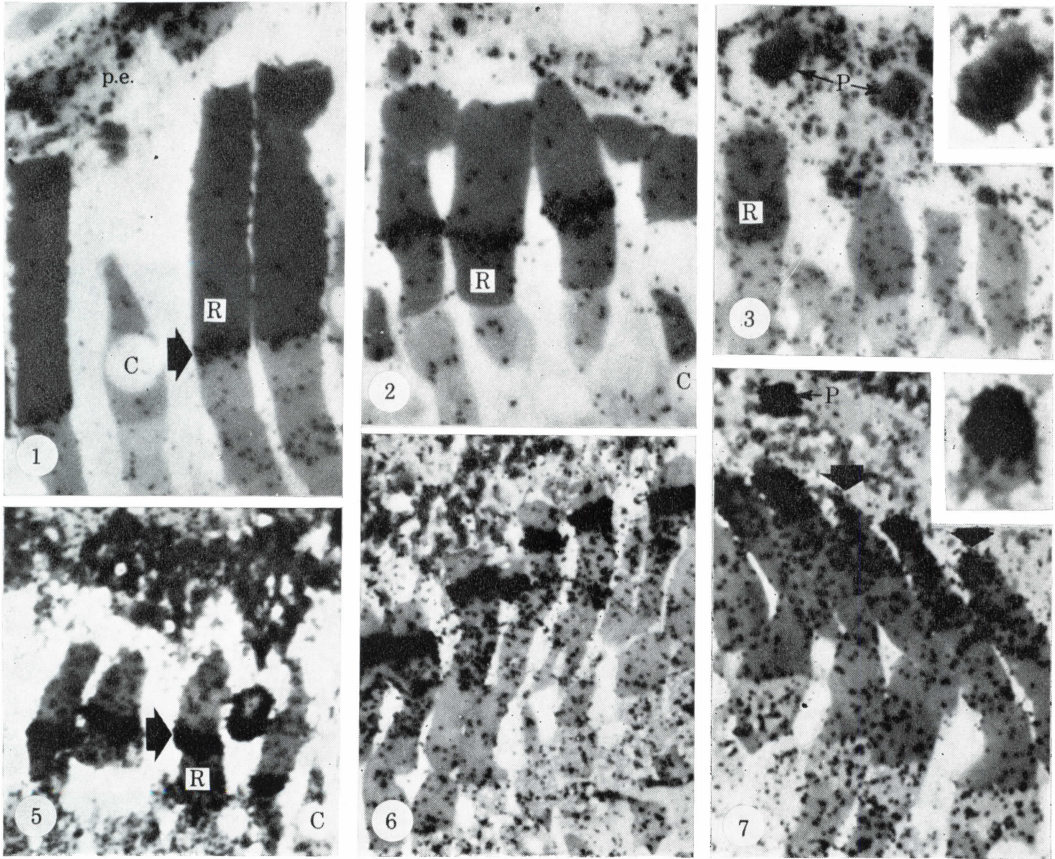
DISCUSSION

The dramatic, pre-metamorphic shortening and subsequent, post-metamorphic elongation of r.o.s. reported here has not been described previously. Other reports of *Xenopus* r.o.s. development (Saxén 1954; Chung *et al.* 1975; Fisher 1976; Witkovsky *et al.* 1976) do not actually present data on r.o.s. length during successive stages of metamorphosis to substantiate their assertion that r.o.s. continue to grow in length through this period. It is clear that the shortening phenomenon could easily be missed if the appropriate stages are not examined or that it may be overlooked because apparent r.o.s. length varies considerably with the plane of section and because the phenomenon is less pronounced in peripheral r.o.s. A similar change in *Xenopus* r.o.s. during metamorphosis has now been observed in another laboratory (Dr Gail S. Tucker, personal communication).

The rates of radioactive band displacement in *Xenopus* r.o.s. during both the first half of metamorphic climax (1.70 $\mu\text{m}/\text{day}$) and one month after its completion (1.56 $\mu\text{m}/\text{day}$) are similar to those during larval development (1.59 $\mu\text{m}/\text{day}$; Kinney & Fisher 1978*b*) and in the adult (1.86 $\mu\text{m}/\text{day}$; Kinney & Fisher 1978*a*). Thus it appears that the rate of disk addition to the base of *Xenopus* r.o.s. does not vary greatly during development. It follows that the changes observed in r.o.s. length during metamorphosis must reflect changes in the rate of disk shedding and/or phagocytosis. Indeed, the rate of disk removal following labelling at stage 57 (2.92 $\mu\text{m}/\text{day}$) is considerably higher than that observed at any other time of development, whereas about one month after metamorphosis it is much lower (0.45 $\mu\text{m}/\text{day}$) than that observed at any time other than during initial outer segment elongation. There is little reason to believe that metamorphic r.o.s. shortening represents a metabolic deficiency (specifically starvation or a vitamin A deficiency) in as much as the animals in this study showed no morphological or behavioural signs of such deficiencies (Witkovsky *et al.* 1976), and their diet was the commonly-recommended one for larval *Xenopus* (Gasche 1944; Nieuwkoop & Faber 1956; Gurdon 1967; Witkovsky *et al.* 1976).

Ultrastructural examination of these retinæ revealed that the drastic changes in r.o.s. length were not accompanied by significant alterations in cellular organization or ultrastructure of rods. Although the p.e. cells had increased slightly in width when r.o.s. were shortest, there was no significant alteration in their fine structure.

R.o.s. shortening during metamorphosis in *Xenopus* resembles the post-embryonic degeneration reported in cave salamander photoreceptors (Besharse & Brandon 1974) in as much as both phenomena begin after embryonic r.o.s. elongation and entail a decrease in r.o.s. length despite continued disk addition; they differ, however, in that the latter is only loosely associated with metamorphosis, involves disruption of r.o.s. structure, and is followed by extensive, irreversible retinal degeneration (Besharse & Brandon 1974; Besharse & Hollyfield 1976).



FIGURES 1-3. Light microscopic autoradiographs from posterior retinæ of larvae injected with [^3H]-leucine at stage 57. Toluidine blue. Rod (R), cone (C). (Magn. $\times 1500$.)

FIGURE 1. Stage 57; $\frac{1}{2}$ day after injection, a distinct band of label (arrow) is present near the base of the outer segment in rods but not cones. The r.o.s. tips extend to the apical border of the pigment epithelium (p.e.).

FIGURE 2. Stage 58; $5\frac{1}{2}$ days after injection, the band of label is displaced sclerally within the r.o.s., which are considerably shorter.

FIGURE 3. Stage 61; 9 days after injection, a band of label is no longer present in most r.o.s., but appears in many phagosomes (P) within the p.e. (phagosome at left is enlarged in the inset). The r.o.s. are further shortened and the apical p.e. border is close to the receptor inner segments. (Inset magn. $\times 2500$.)

FIGURES 5-7. Light microscopic autoradiographs from posterior retinæ of juveniles injected with [^3H]-leucine about one month after metamorphosis. Toluidine blue. Rod (R), cone (C). (Magn. $\times 1500$.)

FIGURE 5. Six days after injection, a distinct band of label appears part way up the outer segment of rods (arrow) but is not present in cones. R.o.s. are about the same length as in figure 3, and the apical p.e. border is near the receptor inner segments.

FIGURE 6. After 20 days, the bands of label have been displaced sclerally in the r.o.s. which have also elongated.

FIGURE 7. After 27 days, the band of label has migrated to the tips of many r.o.s. and has disappeared from others (arrow). Many labelled phagosomes (P and inset) appear in the p.e. (Inset magn. $\times 2500$.)

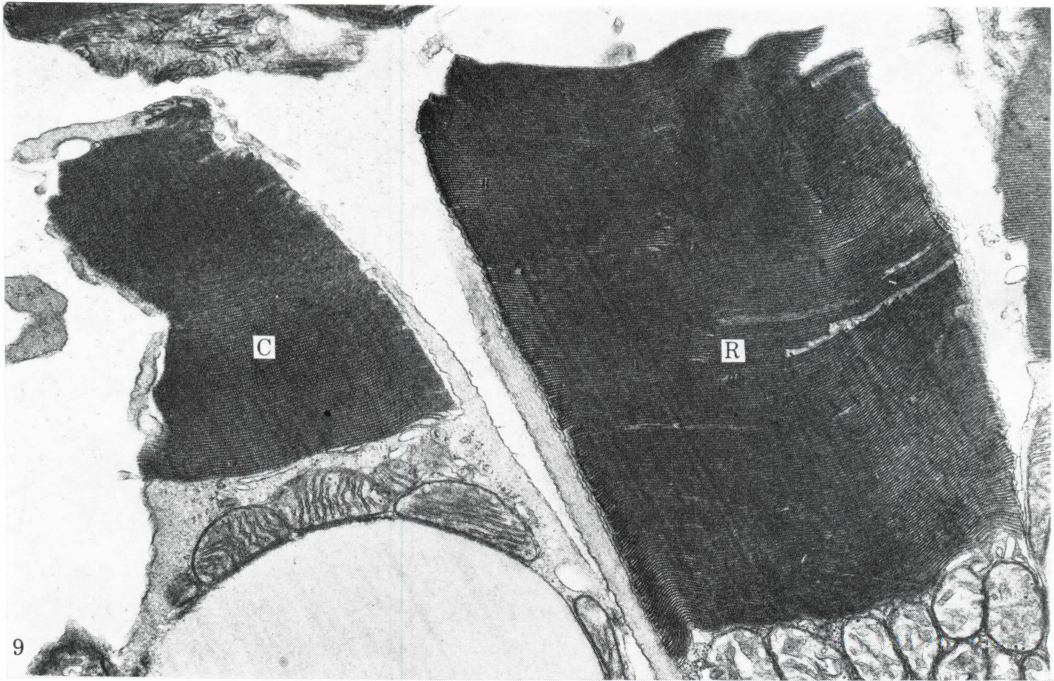
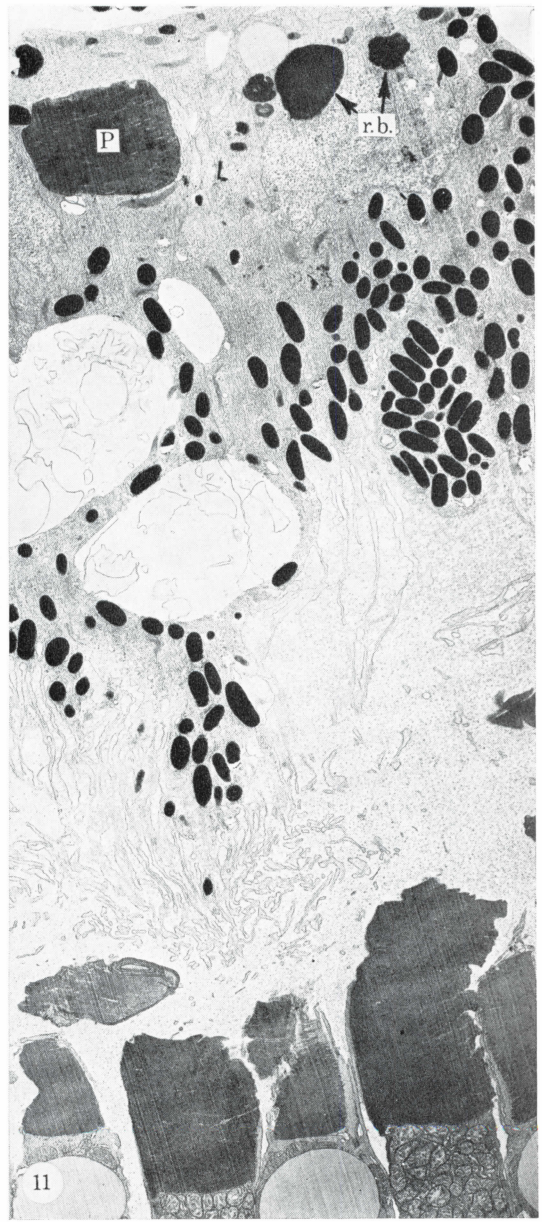
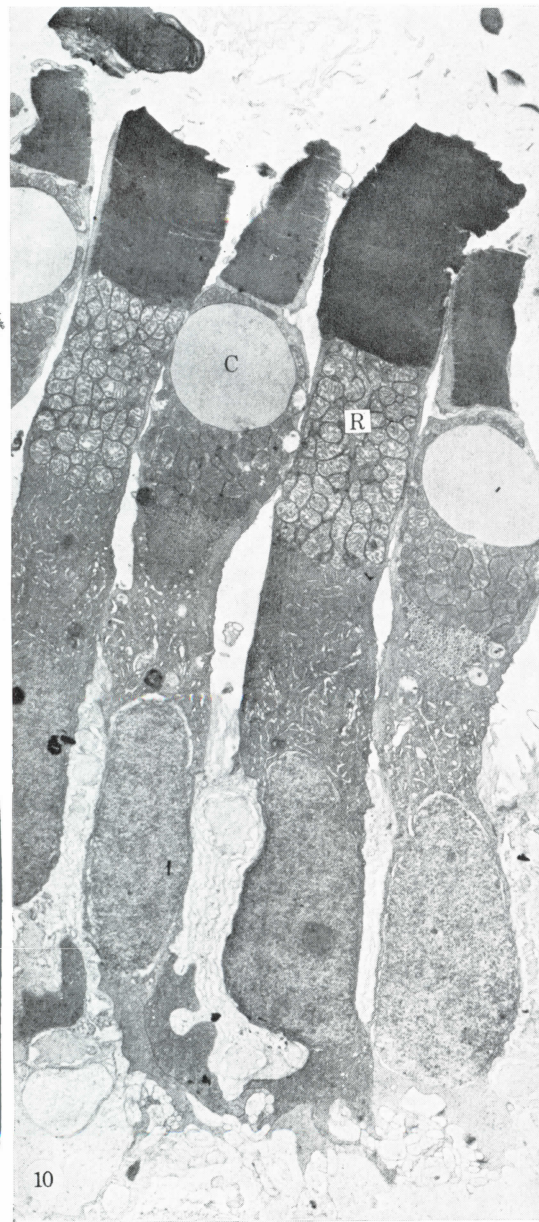


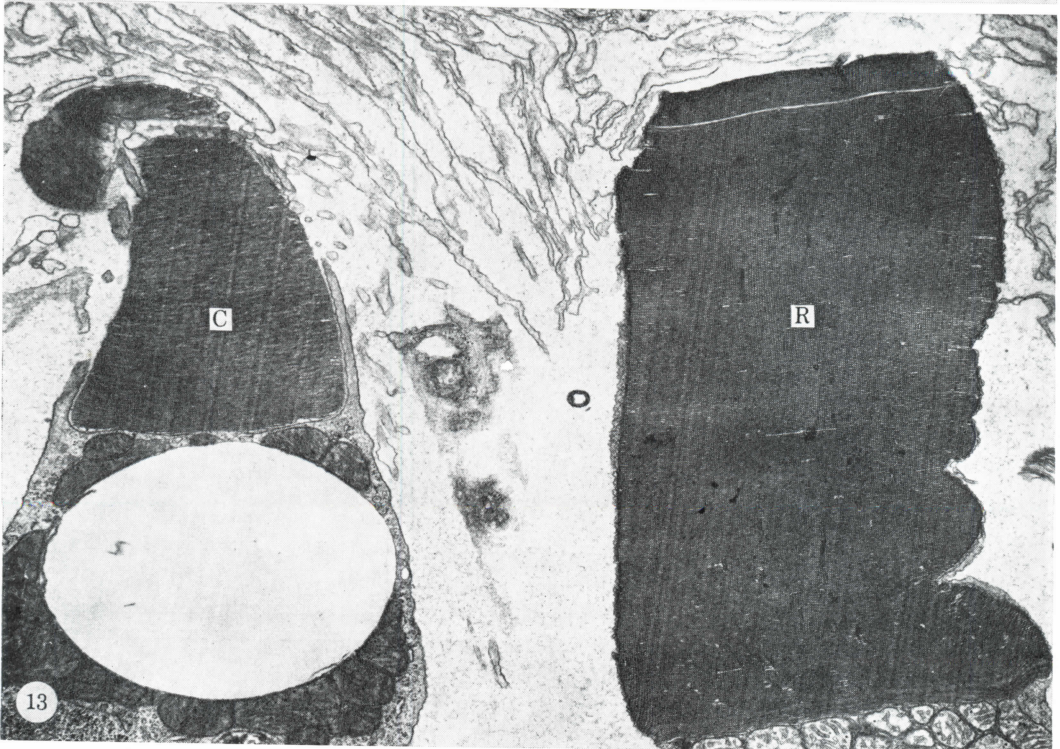
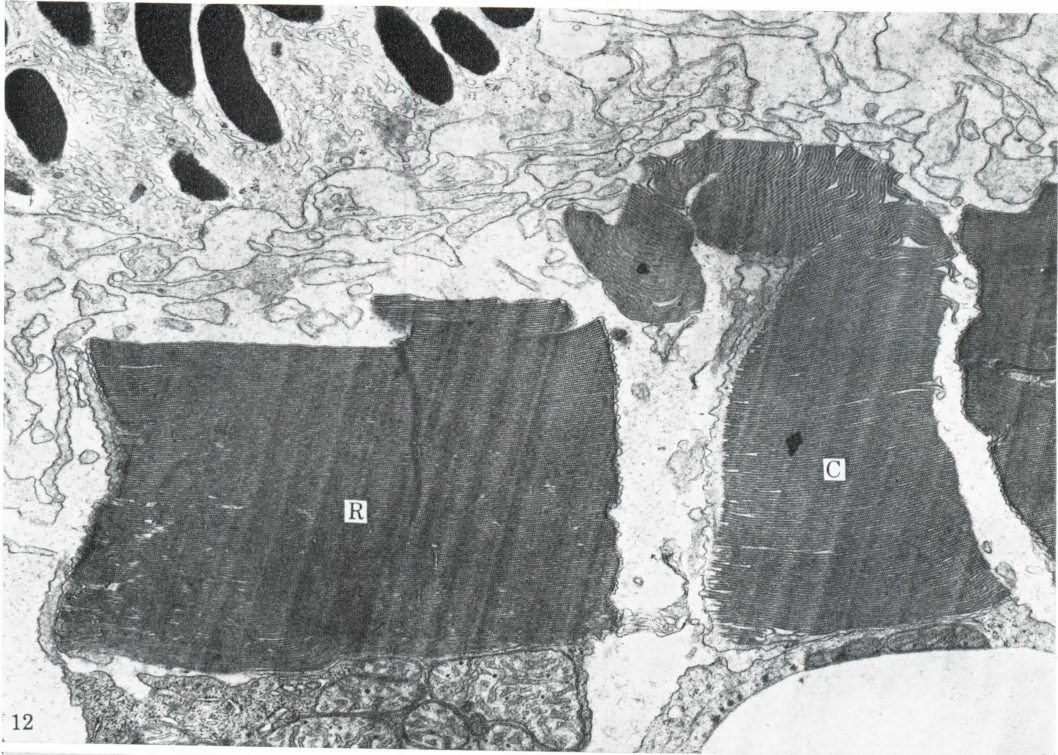
FIGURE 9. Electron micrograph of photoreceptors in *Xenopus* at stage 64 (during metamorphic climax). A rod and cone outer segment displaying normal fine structural features; this r.o.s. is about 5 μm long. Rod (R), cone (C). (Magn. $\times 15000$.)



FIGURES 10 AND 11. Electron micrographs of photoreceptors and p.e. at stage 64. (Magn. $\times 3900$.)

FIGURE 10. Photoreceptors at low magnification illustrating that their general morphologies are normal although many autophagic vacuoles are present in their inner segments. Rod (R), cone (C).

FIGURE 11. Receptor outer segments and p.e. from a section adjacent to that in figure 10. Phagosome (P), residual bodies (r.b.)



FIGURES 12 AND 13. For description see opposite.

It seems likely that the extensive changes in r.o.s. length observed in metamorphic *Xenopus* would influence scotopic visual threshold, as well as the relative activity of rods and cones under mesopic conditions. A direct relationship has been demonstrated between scotopic sensitivity and the fraction of light absorbed during rod elongation in larval *Xenopus* (Witkovsky *et al.* 1976). On the basis of the results of that study, one can calculate that rod threshold would be raised by about 0.75 log unit between stages 54 and 64, assuming that the number of rods in the retina remains constant and that all r.o.s. shorten from 45 to 5 μm (Kinney 1977). The metamorphic changes in r.o.s. length may have an important effect on subsequent levels of the *Xenopus* visual system. Considering the paucity of information available on visual functions in this animal, it is premature to speculate on how this might occur.

It is difficult to imagine an adaptive value for metamorphic r.o.s. shortening, since it is expected to lead to decreased visual sensitivity. In their natural habitat, however, *Xenopus* tadpoles in the late stages of metamorphosis usually hide in the mud (Nieuwkoop & Faber 1956); in the laboratory they stop feeding (Deuchar 1972), become sluggish, and keep to the bottom of the tank (New 1966). During this period of limited activity and environmental interaction the animals may be less dependent on sensory information (e.g., the *Xenopus* lateral line system undergoes extensive reorganization during metamorphosis; Shelton 1970), and a decrease in visual sensitivity may not represent a significant adaptive disadvantage.

Some hormonal mechanism for the observed changes in the rate of r.o.s. disk shedding and phagocytosis would seem likely considering its temporal association with metamorphosis. Many of the transformations of amphibian metamorphosis are due to changes in the levels of thyroid hormones and/or pituitary growth hormones (prolactin and somatotropin) (Etkin 1968; Frieden 1968; Kaltenbach 1968; Frieden & Just 1970; Tata 1970, 1971; Frye, Brown & Snyder 1972; Turakulov *et al.* 1975). Since thyroid hormones affect many metamorphic tissues (such as the tail of *Xenopus*; Robinson 1970, 1972) by stimulating phagocytic activity, the metamorphic changes in r.o.s. length may reflect a similar hormonal stimulation of phagocytosis in the p.e. Because work by La Vail (1976) indicates that pineal hormones may play a rôle in r.o.s. disk shedding, it is interesting that in *Xenopus* the frontal organ (which is part of the pineal complex) attains its

DESCRIPTION OF PLATE 4

FIGURES 12 and 13. Electron micrographs of photoreceptor outer segments at stage 66 (the final stage of metamorphic climax). Rod (R), cone (C).

FIGURE 12. The ultrastructure of this c.o.s. and r.o.s. is normal, although the latter is only 3 μm long; note proximity of apical p.e. border to receptor inner segments. (Magn. \times 13 000.)

FIGURE 13. Another illustration of the normal fine structure of a shortened r.o.s. (9 μm in length). (Magn. \times 9500.)

maximum size during metamorphosis after which the organ and its photoreceptors degenerate (von Haffner 1951). The metamorphic changes in rate of r.o.s. disk shedding and phagocytosis discussed above may be a result of changes in the secretion of thyroid, pituitary, and/or pineal hormones.

The authors wish to thank Mr R. Gill for his technical assistance, Dr J. F. Case, Dr G. H. Jacobs, and Dr R. H. Steinberg for their critical reading of the manuscript. The work was supported by a U.S. Public Health Service N.I.H. grant no. EY00888 to S.K.F. Work contained in this paper was submitted to the University of California by M.S.K. as partial fulfilment of the requirements for the Ph.D. degree.

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