Proc. R. Soc. Lond. B. **201**, 149–167 (1978)
Printed in Great Britain

The photoreceptors and pigment epithelium of the larval *Xenopus* retina: morphogenesis and outer segment renewal

By Marion S. Kinney† and S. K. Fisher

Department of Biological Sciences, University of California, Santa Barbara, California 93106 *U.S.A.*

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

[Plates 1–5]

Light microscopic autoradiography and electron microscopy were used to examine outer segment renewal and the development of photoreceptors and pigment epithelium in the larval *Xenopus* retina. Following the injection of [^{3}H]-leucine at stages 37/38–40 (when outer segments first develop) or 53–54 (when rod outer segments (r.o.s.) attain adult length), a band of label accumulated at the base of r.o.s. and was displaced sclerally with time, whereas label was diffusely distributed in cone outer segments (c.o.s.). By taking into account the change in shape of r.o.s. from conical to cylindrical around stage 46, and calculating outer segment growth (determined from the rate of band displacement) as *volume* of material added with time, we found a constant rate of membrane addition (1.59 μm/day) from the time of initial outer segment formation. The changes observed in r.o.s. length therefore indicate variations in the rate of disk shedding and phagocytosis, which is minimal before stage 46 and rises to 1.19 μm/day after stages 53–54. Ultrastructural observation showed that although all photoreceptor outer segments form by the repeated evagination of the plasma membrane of the connecting cilium, r.o.s. and c.o.s. are distinguishable by differences in membrane appearance even before they develop divergent membrane topologies. Fibrous granules near the basal body of young receptors may be precursors to the elongating ciliary microtubules. Clusters of cisternae observed near the ciliary base in photoreceptor inner segments may represent a stage in the transport of newly-synthesized opsin to the outer segment base.

Introduction

The formation of photoreceptor outer segments is one of the last major developmental events in the vertebrate retina, followed only by the maturation of synaptic connections (Rodieck 1973). Differentiation begins in photoreceptors in the central part of the retina and proceeds at successively later times in cells located progressively closer to the periphery, new receptors continuing to form around the ciliary margin until the retina attains its adult size (Coulombre 1965). The first stage of outer segment development is marked by the migration of two

† Present address: Department of Physiology, University of California, San Francisco, California 94143 U.S.A.

centrioles to the apical cytoplasm of the receptor inner segment, followed by the elongation of a cilium from one of these centrioles. Outer segment formation occurs by repeated invagination of the plasma membrane of the ciliary stalk, as proposed by Sjöstrand (1959, 1961) and confirmed by Nilsson (1964). In developing *Rana*, the latter found that the outer segments of all photoreceptors form by the same mechanism, although young rod outer segments (r.o.s.) and cone outer segments c.o.s.) have different ultrastructural appearances from the time their membranes begin to invaginate. Shortly thereafter, the outer segments of rods and cones develop the difference in membrane topology characteristic of adult amphibian receptors, i.e., r.o.s. are constructed of a stack of separate enclosed disks, whereas c.o.s. consist of a series of invaginations of the plasma membrane. This difference in outer segment organization is reflected in the different patterns of protein incorporation seen in rod and cone outer segments by autoradiography (Young 1969). The labelling patterns originally reported in adult photoreceptors by Young (1969) have also been found in developing retinae of mouse (LaVail 1973), chick (Godfrey 1974), salamander (Ditto 1975), and cave salamander (Besharse & Hollyfield 1976); i.e., label accumulates into a discrete band in rods but not cones.

In view of the considerable interest in the development of the *Xenopus* visual system (see Gaze 1970; Jacobson 1970; Hollyfield 1971; Straznicky & Gaze 1971; Deuchar 1972; Horder & Spitzer 1973; Deuchar 1975; Jacobson 1976*a*; and reviews by Jacobson 1976*b* and Keating & Kennard 1976), knowledge about the differentiation of its photoreceptors and pigment epithelium (p.e.) is surprisingly limited (Saxén 1954; Lanzavecchia 1960; Dixon & Cronly-Dillon 1972; Grillo & Rosenbluth 1972; Chung, Stirling & Gaze 1975; Witkovsky *et al.* 1976).

In this paper we present our autoradiographic and ultrastructural observations of p.e. and photoreceptor differentiation and outer segment renewal in the retina of *Xenopus* at two periods in larval development: when the outer segments are first forming and shortly thereafter, when rods attain their adult length.

Materials and methods

(*a*) *Rearing of animals*

Xenopus laevis eggs were obtained by inducing breeding of adults by the injection of human chorionic gonadotropin hormone. Developing embryos and larvae were kept at an average temperature of 22 °C under cyclic laboratory lighting conditions, fed a suspension of powdered dried nettle leaves (Indiana Botanic Gardens, Hammond, Ind.), and staged according to Nieuwkoop & Faber (1956). Because animals raised in our laboratory developed at a slower rate than that described by Nieuwkoop & Faber, we report both elapsed time and developmental stage whenever possible.

(*b*) *Autoradiography*

(i) *Labelling at stages* 37/38–40

A number of larvae ranging from stages 37/38 to 40 (3 days old, recently hatched) were each anaesthetized by immersion in sterile Steinberg's solution (Jones & Elsdale 1963) containing 0.2 gm/l MS-222 (tricaine methane sulphonate). They were then injected in the belly with [^{3}H]-leucine at an approximate dosage of 0.2 mCi/g body mass. For injection, a small volume of L-[4,5-^{3}H]-leucine in 2% ethanol (1 mCi/ml, sp act 51 Ci/mmol, 389 Ci/mg) was lyophilized and redissolved in water to a concentration of 6 mCi/ml, and a small amount of neutral red dye was added to the solution to allow it to be observed during injection. Injections were made with a glass micropipette attached by surgical tubing to a micrometer-driven 10 µl syringe, allowing delivery of 0.1 µl volumes to individual larvae. Labelled tadpoles where then maintained for several days in artificial pond water containing 100 units/ml penicillin and 50 µg/ml streptomycin.

Larvae were anaesthetized, staged, and fixed in the light 13 h–14 days after injection by immersion of whole larvae in glutaraldehyde for 2–16 h. In general, subsequent steps of tissue fixation and embedding in araldite were the same as used for autoradiography of adult eyes (see Kinney & Fisher 1978*a*). Half µm sections through the centre of the eyes were collected, processed for light microscopic (l.m.) autoradiography, and observed as previously described (Kinney & Fisher 1978*a*). Measurements of band displacement taken from 20 well aligned rods in the posterior retina of each specimen were used to find the mean for each animal. The average rate of band migration was then determined by the method of least squares. R.o.s. length and width (at the outer segment base) were also measured on 20 rods in the posterior of each retina, and the average rate of r.o.s. elongation was determined by least squares.

(ii) *Labelling at stages* 53–54

Several larvae of stages 53–54 (about 5 weeks old, during incipient metamorphosis) were also anaesthetized and injected in the posterior head region (by a micropipette as before) with [^{3}H]-leucine at an approximate dosage of 10 µCi/g. Individual larvae were anaesthetized and enucleated in the light 1–28 days after injection. The eyes were fixed for 1–4 weeks in 10% formol-saline (10% formaldehyde in 0.12 M sodium chloride made from a stock solution kept at a neutral pH with lithium carbonate), dehydrated, and embedded in paraffin. The tissue was sectioned on an AO rotary microtome. Five µm sections, containing outer segments in the posterior retina oriented parallel to the plane of section were mounted on slides, deparaffinized, and processed for l.m. autoradiography. These autoradiograms were stained with Harris Hematoxylin and counter-stained with Eosin, dehydrated, cleared and mounted by the regressive method (Harris Hematoxylin II; Humason 1972). As before, 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 calculated and the average rates of band migration and r.o.s. elongation were determined by the method of least squares.

(c) *Electron microscopy*

Larvae of stages 32 through 46 and 54 were anaesthetized, and either whole larvae (early stages) or enucleated eyes (stage 54) were fixed and embedded by the same procedure used for autoradiography of adult eyes (Kinney & Fisher 1978*a*). Half μm sections were cut until the central part of the eye was reached, then a pyramid was made. Thin sections were placed on grids, stained with uranyl acetate and lead citrate, and examined by electron microscopy (e.m.).

Results

(a) *Autoradiography*

(i) *Labelling at stages* 37/38–40

At stage 37/38 outer segments are first apparent by l.m. in the posterior *Xenopus* retina. At this stage, as well as stage 40 (about 15 h later), all outer segments are conical, and a clear oil droplet is present in most inner segments.The distribution of radioactive protein in the photoreceptors of *Xenopus* larvae injected with [^{3}H]-leucine at these stages and serially sacrificed is illustrated in figures 1–6, plate 1. Although it is difficult to morphologically distinguish rods from cones by l.m. until stage 44, the receptors could always be separated into two classes on the basis of the labelling pattern of their outer segments. A discrete band of label is present in r.o.s. 13 h after injection (figure 1). At 1 and 4 days after injection (figures 2 and 3) this band of label becomes increasingly displaced from the outer segment base inasmuch as r.o.s. are elongating, but does not get much closer to the tip of the outer segments; r.o.s. are also increasing slightly in width, while the oil droplet disappears from their inner segments. R.o.s. continue to elongate, and at 5, 7 and 14 days after injection (figures 4–6) the band of label is located progressively farther from the base of r.o.s. At these later times, the band is closer to the tip of many r.o.s. and has disappeared from the tip of others. In contrast, c.o.s. retain their typical conical shape and a diffuse labelling pattern, increasing only slightly in length and width; cones also retain their oil droplets. As the r.o.s. grow in length, their tips remain in contact with the apical order of the p.e., while the shorter c.o.s. become increasingly separated from the epithelial border. Although the receptors in the posterior retina develop before those in the periphery, the same labelling pattern and developmental changes were observed in all rods and in all cones, except that rods differentiating from cells in the ciliary margin never have oil droplets.

Figure 7 displays the means and ranges of the measurements of band displacement and outer segment length in rods of larvae labelled at stages 37/38–40. This graph demonstrates that both band displacement and r.o.s. length increase with time, the rate of increase in both changing about 3 days after injection (stage 46).

Before this time, the lines drawn between the data points for band location and r.o.s. length are essentially parallel (being best fitted by straight lines of slopes 4.98 and 4.87 μm/day, respectively), indicating that essentially no r.o.s. disk shedding and phagocytosis are occurring. This conclusion was supported by our e.m. observations, according to which phagosomes were first found in the p.e. at 3 days after labelling (stage 46). The continuous line in figure 7 was derived from the method of least squares for band displacement data at 3 or more days after injection; this line has a slope of 1.54 μm/day. The upper dashed line in figure 7 was similarly derived from data on r.o.s. length and has a slope of 1.33 μm/day. Beginning at 3 days after labelling, the rate of band migration is thus slightly greater than that of r.o.s. elongation, indicating that some disk shedding and phagocytosis are occurring during this period. The difference between these rates yields a disk removal rate of about 0.21 μm/day.

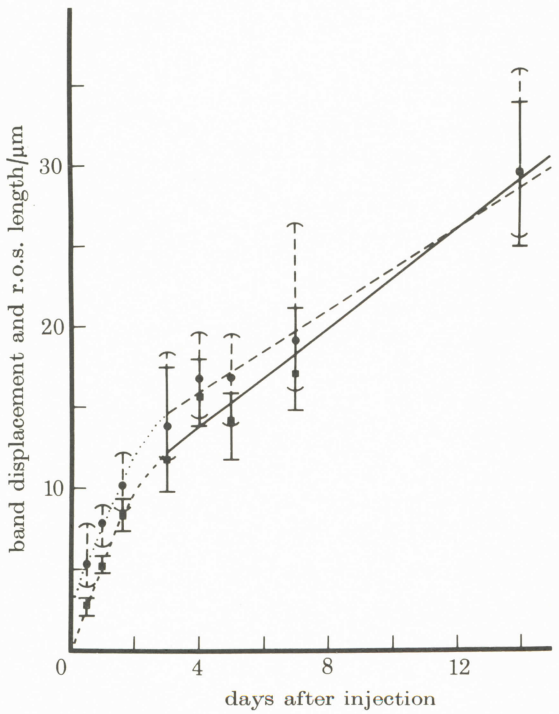


FIGURE 7. R.o.s. renewal in larval *Xenopus* labelled at stages 37/38 to 40. The mean distance of band displacement (■) and r.o.s. length (●) in micrometres are plotted as a function of days after injection. Ranges of these values are indicated by brackets. Separate straight lines were fitted to the data for 0.54–1.67 days and 3 or more days after injection by the method of least squares. At the earliest times, the lines fitted to the data for band displacement and r.o.s. length are essentially parallel, having slopes of 4.98 and 4.87 μm/day (and R^2 values of 97 and 78 %), respectively. The slopes of the lines for 3 or more days after injection indicate that band displacement (continuous line) and r.o.s. length (dashed line) increase at rates of 1.54 and 1.33 μm/day (R^2 = 97 and 87%), respectively.

Since developing r.o.s. undergo a change in shape from conical to cylindrical, it is possible that the *linear* measurement of micrometres of outer segment added per day might not accurately quantify the rate of r.o.s. production. The surface area of disk membrane added per day corresponds more closely to the rate of addition of visual pigment molecules and can be conveniently estimated by calculating r.o.s. volume added per day (Kinney & Fisher 1978*a*). For each specimen, r.o.s. width was measured at the base of the outer segments. From the mean values shown in table 1 it is evident that larval r.o.s. width stopped

TABLE 1. ROD OUTER SEGMENT (R.O.S.) RENEWAL IN LARVAL *XENOPUS* EXPRESSED AS VOLUME

days after injection	diameter† D/μm	height H/μm	length L/μm	volume/μm³ conical‡	cylindrical§	total
0	5.9	2.2	—	14.4	—	14.4
0.54	5.4	5.0	—	38.3	—	38.3
1	5.6	7.4	—	61.2	—	61.2
1.67	6.3	10.4	—	109	—	109
3	7.0	13.9	0	179	0	179
4	7.0	13.9	3.9	179	151	330
5	7.0	13.9	2.6	179	100	279
7	7.0	13.9	5.4	179	208	384
14	7.0	13.9	18.0	179	701	880

† Diameter of cylinder or base of 'cone'.
‡ Conical volume = $\frac{1}{12}\pi D^2 H$.
§ Cylindrical volume = $\frac{1}{4}\pi D^2 L$.

increasing at about 3 days after injection (stage 46). This corresponds to the time when the rate of band migration and increase in r.o.s. length change, i.e., to the deflection of the lines in figure 7. Up to and including 3 days after injection, the volume of outer segment added was calculated by assuming that r.o.s. are conical; after 3 days the r.o.s. were considered to be shaped like a cone on top of a cylinder. We based our calculations on the data for band displacement rather than r.o.s. length, in order to obtain a cumulative measure of total r.o.s. volume added that was independent of disk shedding and phagocytosis. Table 1 presents the mean diameter (D) of the r.o.s. base, the height (H) of conical r.o.s., and the length (L) of the cylindrical portion of r.o.s. after 3 days. At intervals after 3 days, total volume was found by adding the volume of the cylindrical part of the r.o.s. to that of the conical volume present at 3 days.

Figure 8 is a plot of the data on total volume from table 1. The straight line calculated by the least squares method is a good approximation for the data at all times after labelling. Thus, by taking into account the change in r.o.s. shape and determining *volume* rather than *distance* of band displacement, the rate of r.o.s. membrane addition is constant from the time of initial outer segment formation; the apparent change in rate of r.o.s. addition represented by the deflection of the

lines in figure 7 is shown to be misleading. For comparison with r.o.s. synthetic rates during other developmental periods, the rate of membrane addition found by labelling larvae of stages 37/38–40 (61.1 μm^3/day) can be expressed as the equivalent rate of 1.59 μm of outer segment added per day.

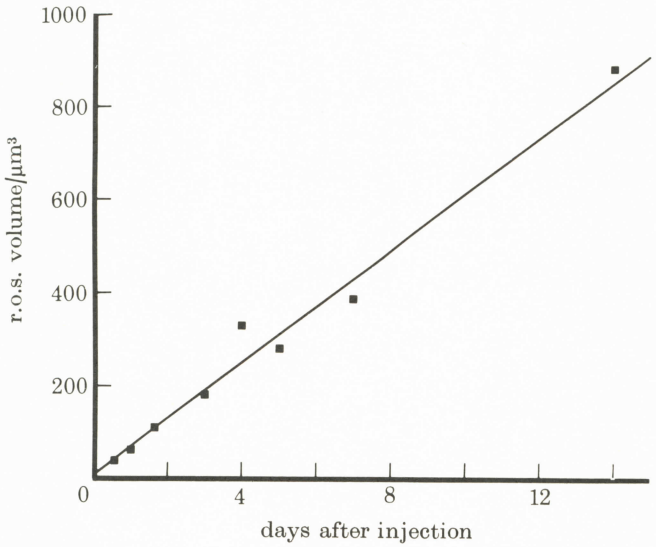


FIGURE 8. Volume (μm^3) of r.o.s. material added as a function of time in larvae injected at stages 37/38 to 40, derived from table 1; see text for details of calculations. A straight (continuous) line fitted to the data by the method of least squares yields a rate of 61.1 μm^3 of outer segment material added per day (R^2 = 98 %), which is equivalent to a band displacement rate of 1.59 μm/day in cylindrical r.o.s. that are 7 μm wide.

(ii) *Labelling at stages* 53–54

The same pattern of labelling was observed in autoradiograms of photoreceptor outer segments during this period as during initial outer segment formation. The mean and range of band displacement measurements and r.o.s. length for each labelling interval are presented in figure 9, from which it is evident that the rate of band displacement is essentially constant and that r.o.s. length is increasing only slightly. As before, straight lines were fitted to the data of band displacement and r.o.s. length (continuous and dashed lines in figure 9, respectively), which yield corresponding rates of 1.58 μm membrane added per day and 0.39 μm increase in r.o.s. length per day. R.o.s. attain their adult length (50 μm) at about stage 54. From the relatively rapid rate of membrane addition and slower rate of increase in r.o.s. length observed during this period, we calculated that the rate of disk shedding and phagocytosis is about 1.19 μm/day.

(*b*) *Electron microscopy*

(i) *Differentiation of photoreceptors and pigment epithelium*

The p.e. and adjacent cells of the neural retina are both morphologically undifferentiated in the tailbud larvae of stage 32 (figure 10, plate 1). The organelles in the p.e. cytoplasm are unlayered and consist of electron dense yolk platelets, lightly staining lipid droplets, numerous mitochondria, free ribosomes, some rough endoplasmic reticulum (r.e.r.), and sparse smooth endoplasmic reticulum (s.e.r.). The cells of the neural retina are separated from the p.e. by the very narrow extracellular space of the optic vesicle lumen, near which adjacent cells of each tissue are joined by zonulae adhaerentes. At this stage, organelles in the cells of the neural retina are randomly distributed and include lipid droplets, yolk platelets, microtubules, numerous ribosomes, a few Golgi bodies, and a small amount of r.e.r.

An interesting feature of early retinal development in *Xenopus* is the elimination of the egg pigment granules from the cells of the neural retina. These round, membrane-bounded granules migrate sclerally within the retinal cells, are expelled from the cells into the optic vesicle lumen, and traverse this space until they are

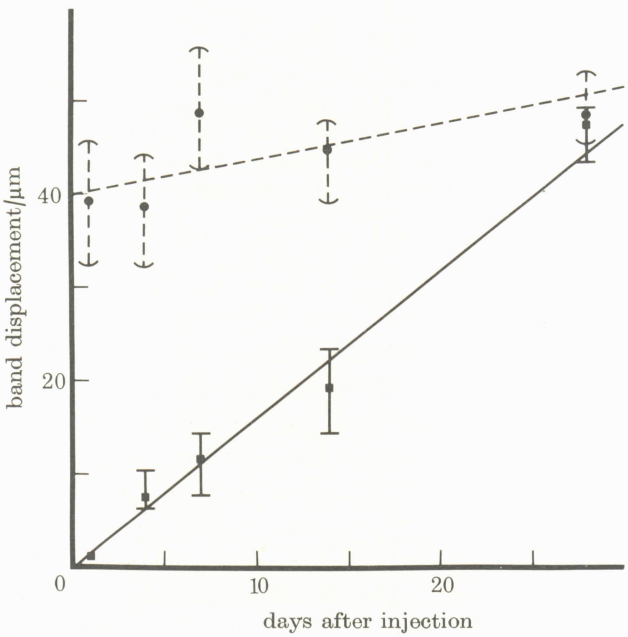


FIGURE 9. R.o.s. renewal data in larvae injected at stages 53 to 54. The mean distance of band displacement (▪) and r.o.s. length (•) plotted as a function of days after injection. Ranges of these values indicated by brackets. By the method of least squares, straight lines were fitted to the data for band displacement (continuous line) and r.o.s. length (dashed line). The slopes of these lines yield a band migration rate of 1·58 μm/day (R^2 = 95%) and increase in r.o.s. length by 0.39 μm/day (R^2 = 32%).

phagocytized by the adjacent retinal p.e. In the periphery of the *Xenopus* retina at stage 35/36 (figure 11, plate 2) such granules (without enclosing membranes) appear within the expanded optic vesicle lumen, at the apical p.e. border, and within the p.e. (where they are again surrounded by a membrane). At this stage of development both p.e. cells and presumptive photoreceptors show morphological evidence of differentiation in that the former no longer contain yolk platelets or lipid droplets, and in the latter the previously numerous and randomly-distributed lipid droplets appear to have aggregated and coalesced into fewer but larger droplets within the apical cytoplasm.

Further receptor development is shown in figure 12, plate 2, where inner segments have formed by the protrusion of cytoplasm beyond the outer limiting membrane. In several of these cells a single large oil droplet, partially surrounded by mitochondria, appears in the inner segment. Although their number and size are decreasing, yolk platelets are still present in the photoreceptors. A few, fine cytoplasmic processes extend from both receptor and p.e. cells into the inter-photoreceptor space. At this time the p.e. cytoplasm contains a few Golgi bodies, abundant mitochondria, ribosomes, r.e.r., and numerous pigment granules.

Figure 13, plate 3, illustrates the appearance of the p.e. at a stage still later in development (stage 46), following the differentiation of photoreceptor outer segments. The presence of several phagosomes in the p.e. indicates that disk shedding and phagocytosis of outer segment membranes have begun. This section is from a larva labelled for autoradiography at stage 37/38–40 and sacrificed 3 days later; this was the earliest retina in which phagosomes were found. A basement membrane is present on the basal p.e. border, and this epithelium displays numerous fine apical cell processes and extensive cytoplasmic membranes including s.e.r., Golgi bodies, and membrane arrays which may be early myeloid bodies. The p.e. cytoplasm also contains many pigment granules of different morphologies; the majority of them appear elongated, while some are spherical and a few appear to be aggregates of several granules.

(ii) *Differentiation of photoreceptor outer segments*

The first morphological signs of outer segment differentiation are evident in the posterior *Xenopus* retina at stage 35/36. In some photoreceptors at this time, one of the centrioles in the apical inner segment cytoplasm gives rise to a modified cilium enclosed within a budlike extension of the plasma membrane (figure 14, plate 3). Cytoplasmic protrusions then form near the base of the cilium, extending out from it at right angles (figure 15, plate 3). A striking feature of the inner segment cytoplasm in many young receptors is an accumulation, near the ciliary base, of small membrane bounded cisternae which always appear to be empty and sometimes are observed closely apposed to the plasma membrane near the base of the cilium (figures 14–16, plate 3). Similar cisternae are observed near the Golgi apparatus in the myoid region of inner segments and are occasionally found in the cytoplasm of the ellipsoid, between the Golgi body and the base of the developing outer segment.

Outer segments increase in size by incorporating both additional cytoplasm and membrane in a very organized pattern, that is by the formation of layer after layer of membrane foldings or evaginations on one side of the cilium. Figures 17–20, plate 4 show progressive stages of this process in developing cones. In each of these cells, the membrane is continuous around the bends at the outer segment perimeter and a separate plasma membrane encloses the outer segment only in the area of the ciliary stalk. The membranes of c.o.s. are slighly wavy but in general very parallel to each other, and the extracellular distance between adjacent membranes is slightly less than the cytoplasmic distance between neighbouring membranes. The most basal membrane folds extend a shorter distance from the connecting cilium than those above them. In these young cones (as well as the receptors in figures 14–16), the ciliary matrix contains amorphous granular cytoplasm and axonemal microtubules, but no additional organelles. Cytoplasmic protrusions of the inner segments which project toward the p.e. near the edge of these young outer segments probably represent early stages of calycal process formation. The cytoplasm between the base of the cilium and the oil droplet in these cells commonly contains cisternae as well as mitochondria and numerous ribosomes. Various numbers of electron-dense, fibrous granules (70–90 Å† in diameter) frequently occur near the

---

Description of plate 1

Figures 1–6. Light microscopic autoradiographs from posterior retinae of larvae injected with [^{3}H]-leucine at stages 37/38 to 40 and serially sacrificed. Note band of label in r.o.s. (arrows). Rod (R); Cone (C); Pigment epithelium (p.e.). Toluidine blue. (Magn. × 2000.)

Figure 1. Stage 41. Thirteen hours after injection, a band of label is present near base of the outer segment in rods but not cones; a large, round oil droplet is apparent in the inner segment of both kinds of receptors, and both r.o.s. and c.o.s. are conical, although the latter are smaller.

Figure 2. Stage 42. After 1 day, the bands of label are located farther from the base of the r.o.s. but the same distance from their tips. R.o.s. have wider bases but are still conical. Note that the tips of all outer segments contact the p.e.

Figure 3. Stage 46–47. After 4 days, r.o.s. have lengthened and are less tapered, and their bands of label are displaced sclerally. The oil droplet has disappeared from rod inner segments.

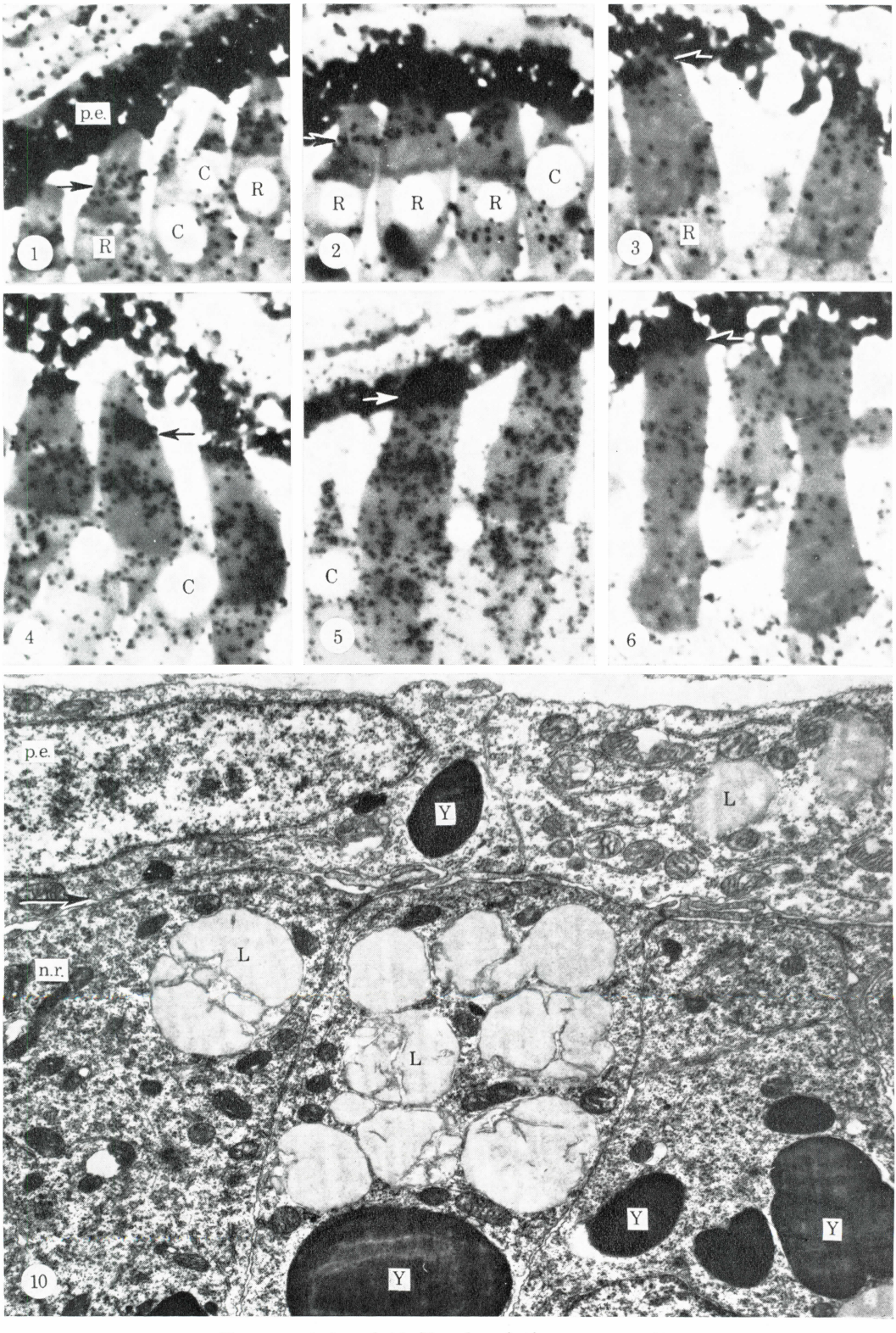
Figure 4. Stage 47. After 5 days, the band of label in some r.o.s. is still the same distance from the tip as in figure 1, but considerably farther from the base.

Figure 5. Stage 47–48. After 7 days, r.o.s. are longer and their bands of label are displaced farther sclerally and located closer to their tips. Cones do not contact the apical p.e. border; their outer segments still display diffuse labelling and an oil droplet persists in their inner segments.

Figure 6. About stage 49. After 14 days, the r.o.s. have assumed a cylindrical shape and are considerably elongated; the band of label is very close to their tips.

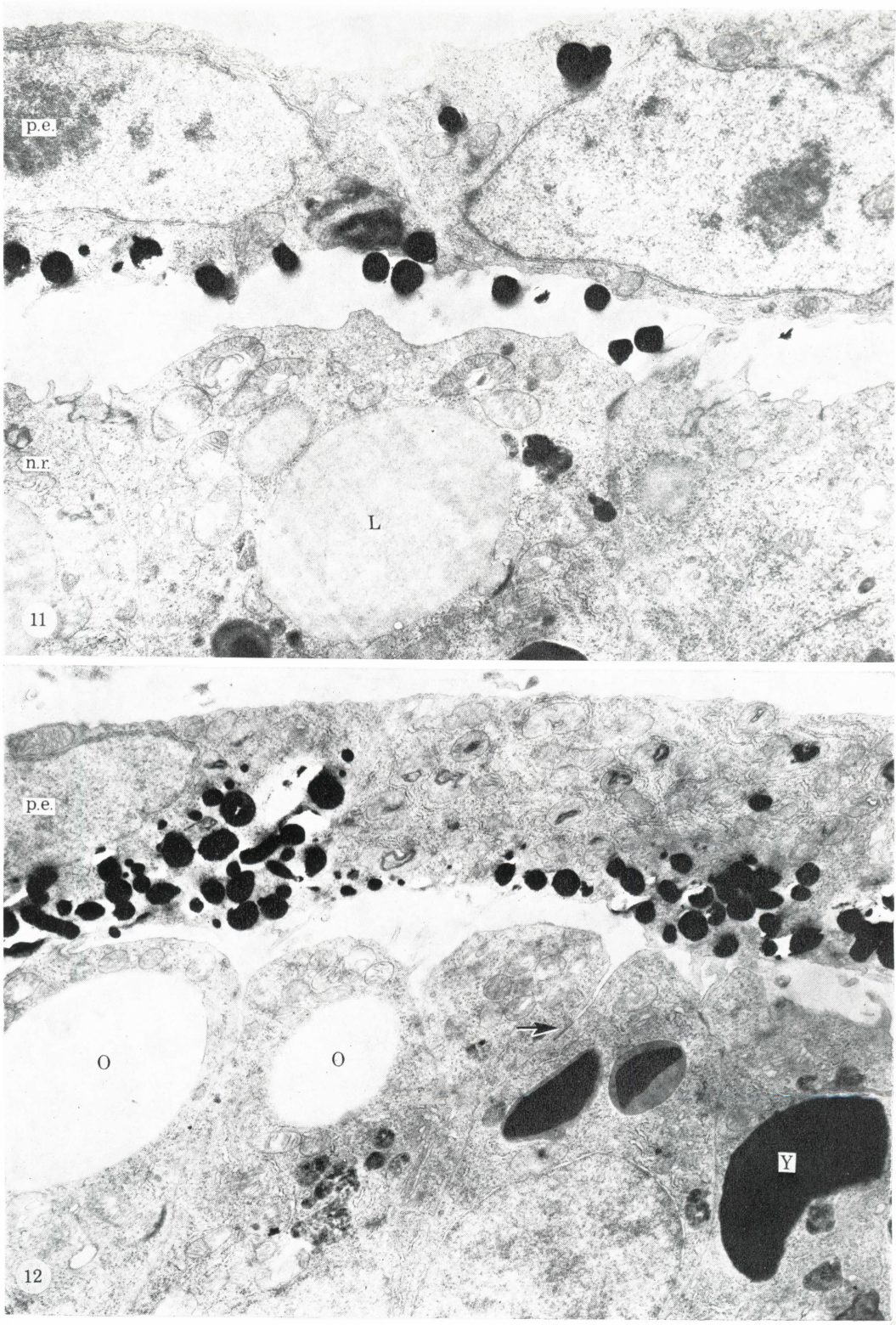
Figure 10. Electron micrograph of developing *Xenopus* retina at stage 32. Cells of the p.e. and neural retina (n.r.) are morphologically undifferentiated and contain yolk platelets (Y) and lipid droplets (L); they are separated by a narrow optic vesicle lumen (arrow). (Magn. × 9000.)

† 1 Å = 0.1 nm = 10^{-10} m.



FIGURES 1–6 and 10. For description see opposite.

(*Facing p.* 158)



FIGURES 11–12. For description see opposite.

Description of plate 2

Figures 11 and 12. Electron micrographs of developing *Xenopus* p.e. and photoreceptors. (Magn. × 9000.)

Figure 11. In the peripheral retina at stage 35/36, egg pigment granules expelled from the neural retina (n.r.) are traversing the expanded optic vesicle lumen and being phagocytized by the p.e. Lipid droplets (L) appear to be aggregating and coalescing into larger droplets in the apical cytoplasm of presumptive photoreceptor cells.

Figure 12. At stage 37/38 photoreceptor inner segments protrude beyond junctions (arrow) between adjacent cells, and often contain a single large oil droplet (O). Yolk platelets (Y) are still present in the receptor cytoplasm and a few fine processes extend from the apical cytoplasm. The p.e. contains numerous pigment granules and also sends a few processes into the optic vesicle lumen.

Description of plate 3

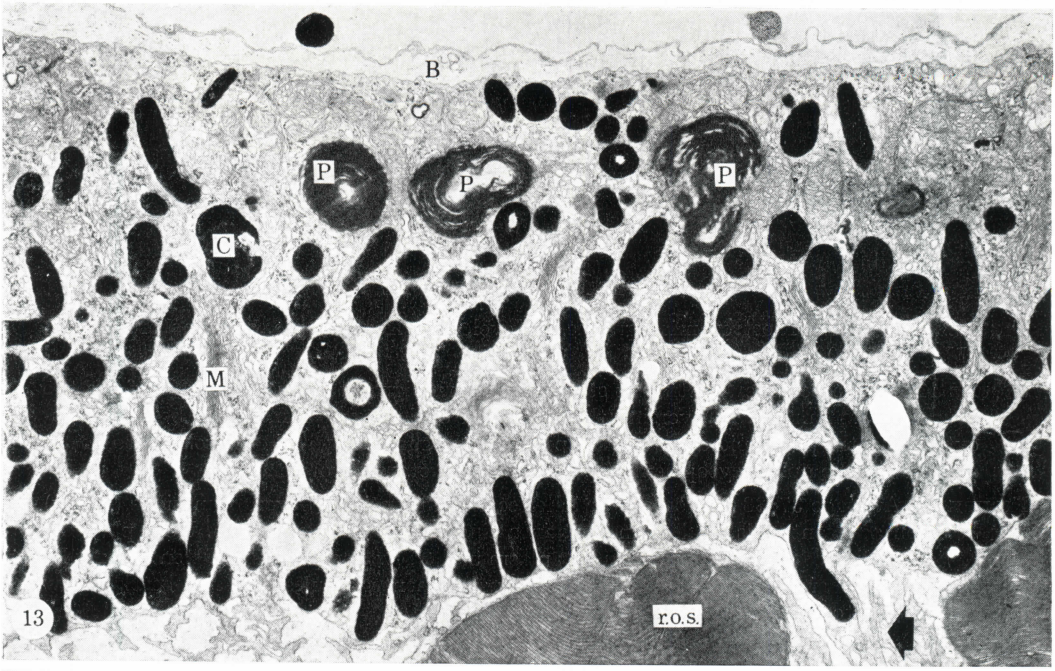
Figure 13. By stage 46, photoreceptor outer segments have developed and groups of disks shed from r.o.s. appear as phagosomes (P) within the p.e. The epithelial cytoplasm also contains developing myeloid bodies (M) and round, elongated, and composite (C) pigment granules. Numerous processes extend from the apical p.e. cytoplasm (arrow) to surround the r.o.s., and a basement membrane (B) is present at its basal border. (Magn. × 9000.)

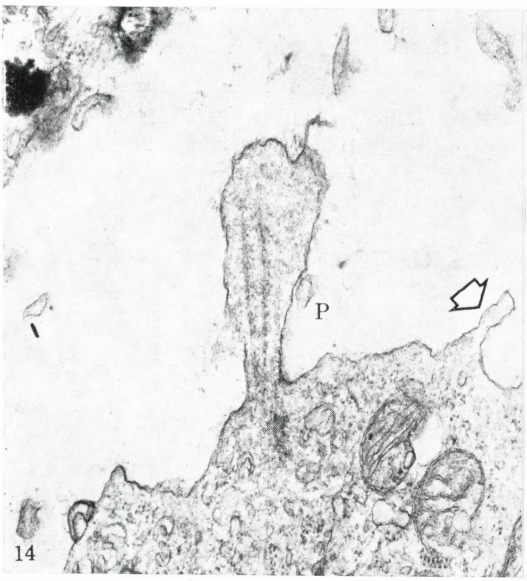
Figures 14–16. Early stages in the development of photoreceptor outer segments. Note extensions of receptor cytoplasm near the base of the cilium (clear arrows) and fine processes (P) around the outer segments, many of which were traced from the p.e. (Magn. × 30 000.)

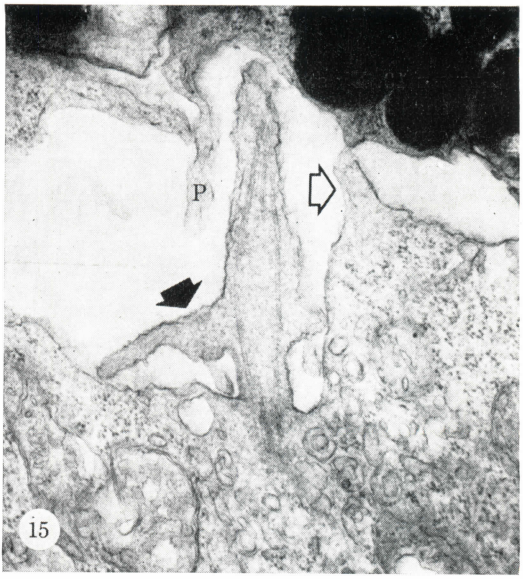
Figure 14. A very early photoreceptor outer segment, in which a short cilium forms a bud-like extension of the apical plasma membrane of the inner segment.

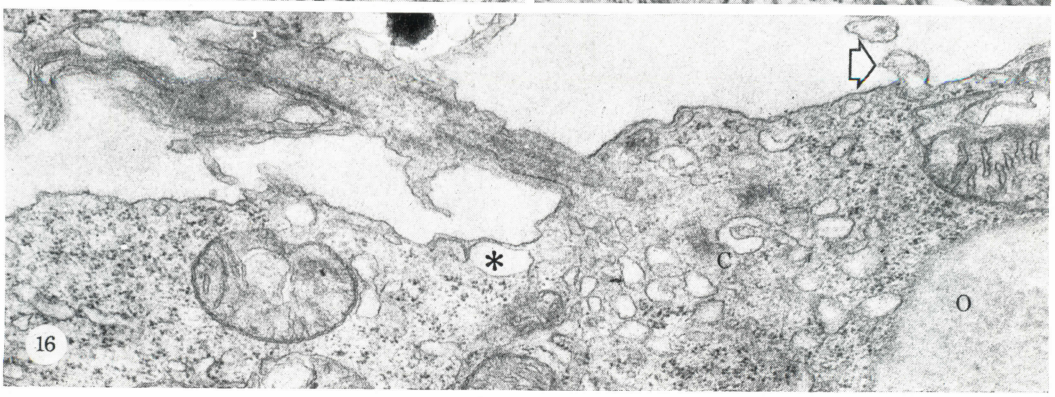
Figure 15. An early outer segment in which a single cytoplasmic evagination has formed near the base of the cilium (dark arrow).

Figure 16. A developing receptor outer segment containing a conspicuous accumulation of cisternae (C) in the cytoplasm immediately surrounding the base of the early outer segment. One such cistern (asterisk) is closely apposed to the plasma membrane. Oil droplet (O).

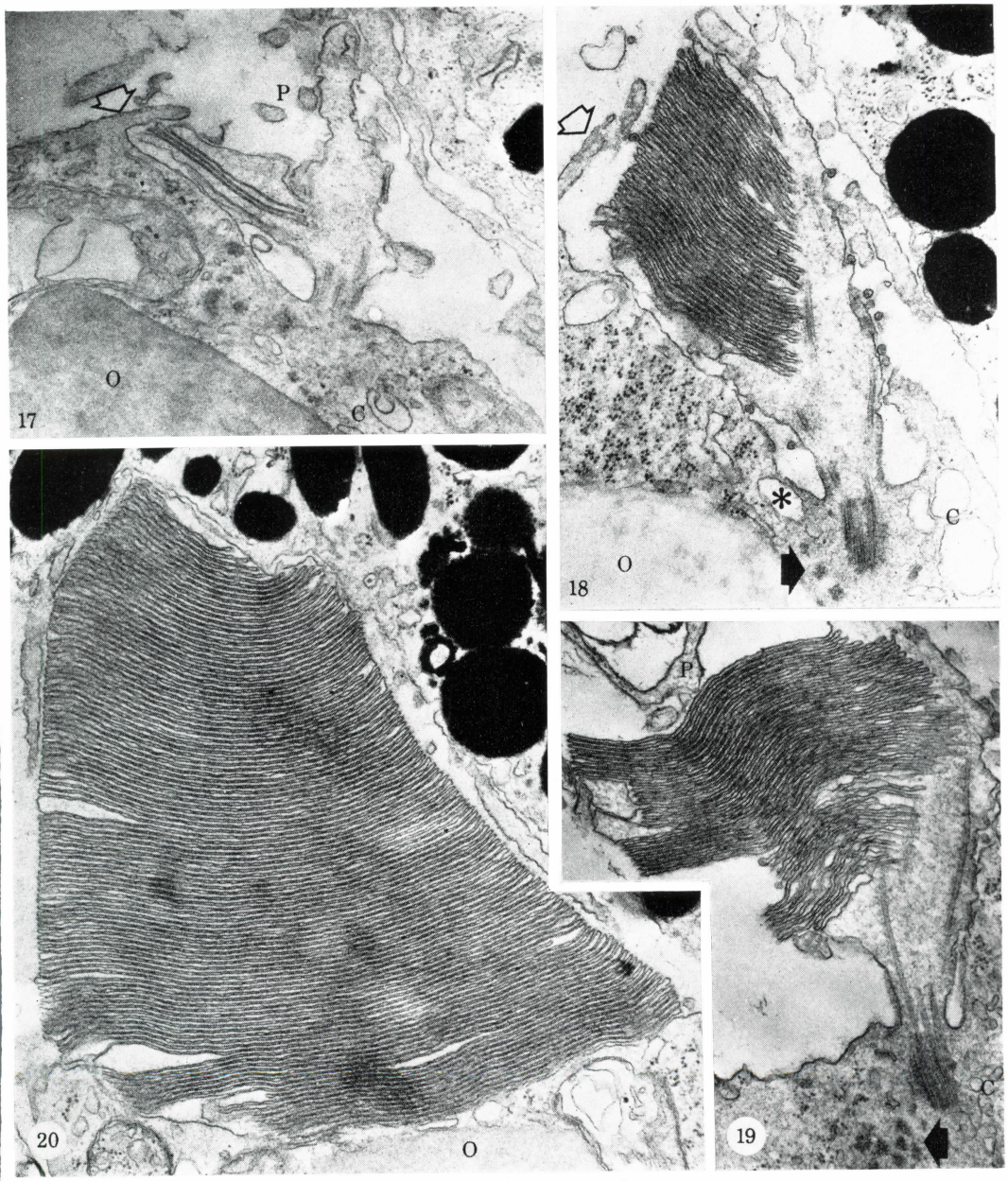






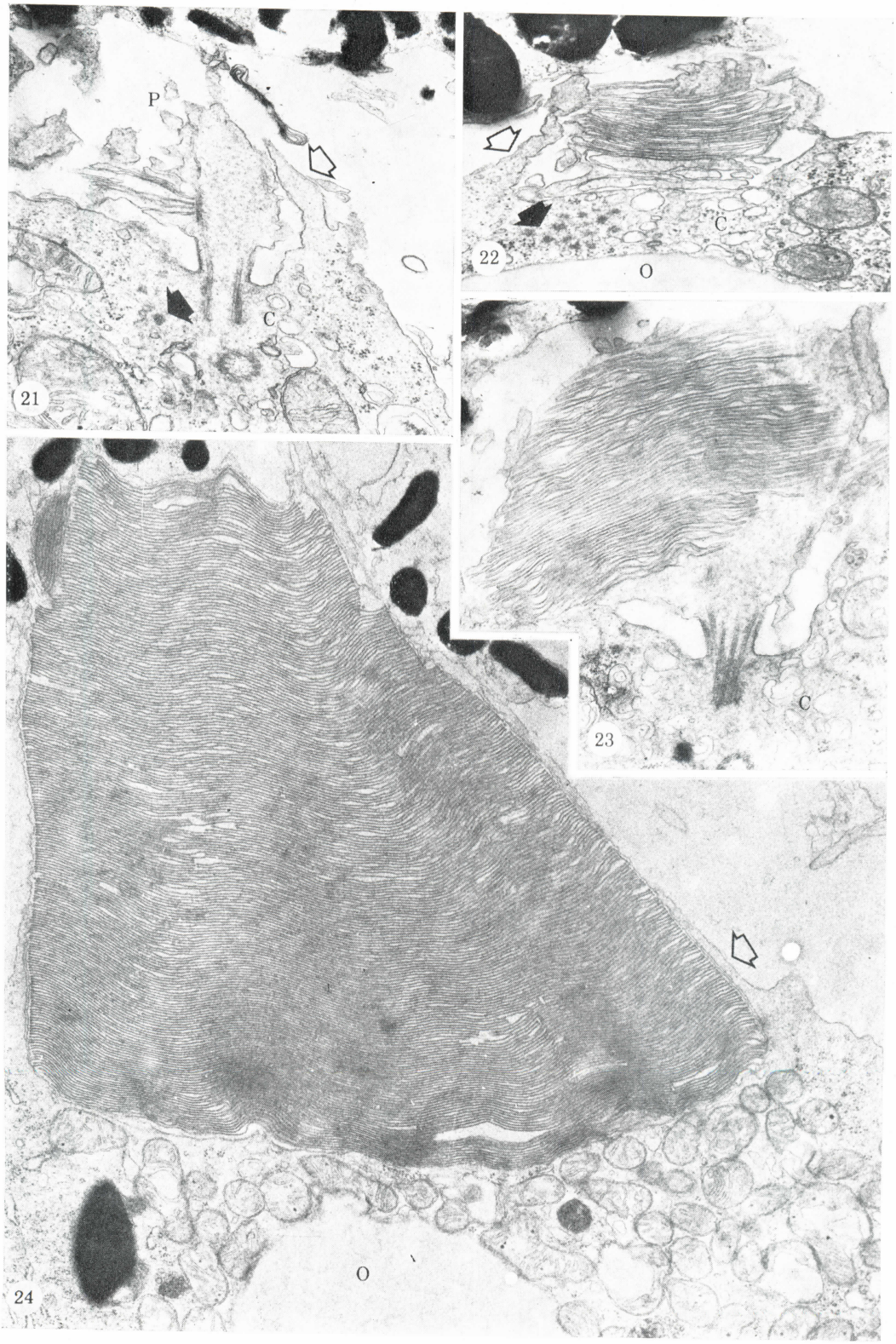


FIGURES 13–16. For description see previous page.



FIGURES 17–19. Successive stages in the development of c.o.s. See text for description. Cisternae (C); Oil droplets (O); p.e. cell processes (P); Developing calycal processes (clear arrows); Fibrous granules (dark arrows); Cistern near plasma membrane (asterisk). (Magn. × 30 000.)

FIGURE 20. A more developed c.o.s. from a stage 41 larva. (Magn. × 27 500.)



FIGURES 21–24. For description see opposite.

basal body (figures 16–19, plate 4). A few fine p.e. cell processes are often found in association with early outer segments but do not appear to be closely involved in their formation.

The outer segments of rods likewise form by the repeated evagination of the plasma membrane of the ciliary stalk, but their ultrastructural appearance differs from c.o.s. from a very early stage of development. As shown in figures 21–24, plate 5, by our fixation the membranes of developing r.o.s. are considerably more irregular and less parallel to one another than those of c.o.s. Although the number of membranes per unit distance is similar in all developing outer segments (42 'disks'/μm), the rod discs are considerably flattened and are thereby separated by a relatively larger cytoplasmic distance. These features serve to identify young r.o.s., whose topology is initially the same as that of c.o.s. (e.g., compare figure 17 and figure 21). Soon thereafter the topology of the r.o.s. changes, as all but the few most basal membranes lose continuity with each other at the edge opposite the connecting cilium to form isolated discs enclosed in the outer plasma membrane (figures 23 and 24). Developing r.o.s., like c.o.s., have inner segment oil droplets, calycal processes, cisternae, fibrous granules, and associated p.e. cell processes. The larger r.o.s. in figure 24 has the button-like edges characteristic of adult rods but lacks scallops, as suggested by the absence of aligned membrane discontinuities in this section (Kinney & Fisher 1978*a*). At subsequent stages of development, the outer segments of rods assume a cylindrical form and their inner segment oil droplets disappear.

Discussion

(*a*) *Autoradiography*

Though at stage 37/38 all developing *Xenopus* photoreceptors have oil droplets and conical outer segments, rods and cones can be readily distinguished by observing successive stages of their morphological development. Cones retain their oil droplets, and their outer segments increase slightly in length while maintaining a conical shape. In contrast, rods exhibit more dramatic changes during development: their outer segments increase considerably in both length and width, then cease to increase in diameter while continuing to elongate, assuming a cylindrical form but retaining a conical tip. At stage 46 phagosomes are first observed in the p.e., many r.o.s. tips are no longer conical, and the oil droplets (which had

Description of plate 5

Figures 21–23. Successive stages in the development of r.o.s. See text for description. Cisternae (C); Oil droplets (O); Developing calycal processes (clear arrows): Fibrous granules (dark arrows). (Magn. × 24000.)

Figure 24. A rod from a stage 41 larva displaying many ultrastructural features of adult r.o.s., which nevertheless resembles a cone (compare to figure 20) in that its inner segment has an oil droplet (O) and its outer segment is conical. Calycal process (clear arrow). (Magn. × 16000.)

previously been decreasing in size) have disappeared from rod inner segments. Similar aspects of receptor development in *Xenopus* have been described previously (Saxén 1954; Witkovsky *et al.* 1976).

The contrast in morphological development between rods and cones was accentuated when we examined the pattern of incorporation of radioactive protein into their outer segments with autoradiography. In larvae labelled at stages 37/38 to 40, a distinct band of label accumulated near the base of r.o.s. and was displaced sclerally with time, whereas a diffuse distribution of label was found in all c.o.s. Although both rod and cone outer segments were elongating at the beginning of this period, e.m. revealed that their outer segments already had divergent membrane topologies (the receptors in figure 1, plate 1, the cone in figure 20, plate 4, and the rod in figure 24, plate 5 are all from stage 41 larvae). These same distributions of label in *Xenopus* r.o.s. and c.o.s. are also found by labelling at stages 53–54, as well as shortly before and after metamorphic climax (Kinney & Fisher 1978*b*), and in the adult (Kinney & Fisher 1978*a*). Moreover, the same labelling patterns have been reported in the developing and adult receptors of all vertebrates examined (see Introduction and Kinney & Fisher 1978*a*). Because in all of these cases receptor outer segments likewise exhibit divergent membrane topologies, it is likely that the difference between rods and cones in autoradiographic labelling pattern reflects the difference in their structural organization.

The rate of r.o.s. disk addition has generally been expressed as rate of band displacement in disks/day or μm/day (Young & Droz 1968; Young 1971; LaVail 1973; Besharse & Hollyfield 1976). When the rate of membrane addition in young larval *Xenopus* rods was analysed by plotting band displacement/μm as a function of time after labelling, a deflection in the line at 3 days after injection (stage 46) appeared to indicate a change in this rate. Because r.o.s. stopped increasing in width and began to assume a cylindrical shape at stage 46, it was hypothesized that this apparent variation in rate of disk addition might result from the change in r.o.s. shape. To test this hypothesis, we calculated the volume of outer segment material present as a function of time, neglecting phagocytosis and assuming that r.o.s. were initially conical and subsequently shaped like a cone on top of a cylinder. These calculations fall along a straight line (see figure 7), demonstrating a constant rate of increase in r.o.s. volume for two weeks following the appearance of outer segments, which most likely reflects a constant rate of addition of membrane and visual pigment molecules.

During early development r.o.s. were elongating at the same rate at which new membrane was added to their bases. R.o.s. disk shedding and phagocytosis were not detected until about stage 46, some 3 days after initial outer segment formation. Beginning at this time (and for the next several days), disk removal occurred at the low rate of 0.21 μm/day.

In *Xenopus* larvae labelled later in development (at stages 53–54, when the r.o.s. were cylindrical and were attaining adult length) a constant rate of r.o.s. membrane addition was found; this rate (1.58 μm/day) was the same as that during

early larval development (1.59 μm/day), and quite similar to that measured in the adult (1.86 μm/day; Kinney & Fisher 1978*a*). The rate of disk shedding and phacotytosis (about 1.19 μm/day) at this time (following stages 53–54) was somewhat lower than the adult rate (1.86 μm/day; Kinney & Fisher 1978*a*).

LaVail (1973) measured the kinetics of r.o.s. renewal during rod elongation in the mouse retina. Because of the narrow diameter of mouse r.o.s., he did not take measurements of band location and r.o.s. length from individual r.o.s., but from lines drawn across the retina approximating these parameters for a large number of cells. LaVail reported changes in the rates of both disk addition and removal during early development, whose net difference accounted for observed variations in the rate of rod elongation. Although r.o.s. diameter and volume presumably increase in mice during early development (as reported here in *Xenopus* and in rats by Dowling & Sidman 1962), LaVail expressed the rate of disk addition as μm/day. Additionally, he noted that the membranes of young mouse r.o.s. were often aligned parallel, rather than perpendicular, to the long axis of the cell. Some of the variation in the rate of disk synthesis which he observed could reflect changes in the diameter or alignment of developing r.o.s. It is thus possible that from the beginning of r.o.s. genesis the actual rate of addition of membrane and visual pigment molecules in the mouse proceeds at a constant rate as described here for *Xenopus*.

(*b*) *Electron microscopy*

(i) *Differentiation of pigment epithelium*

Morphological differentiation of the retinal p.e. begins in the posterior pole of the *Xenopus* retina at about stage 33/34. At this stage, yolk platelets and lipid droplets disappear from the p.e. and its phagocytic capability is demonstrated by its engulfment of egg pigment granules which had been extruded into the optic vesicle lumen by neural retina cells. Analogous observations were noted in *Xenopus* (Grillo & Rosenbluth 1972) and were more fully described in *Rana* by Hollyfield (1973). Subsequent indicators of p.e. differentiation in *Xenopus* (at stage 37/38) include the appearance of increased numbers of mitochondria, more abundant r.e.r. and s.e.r., elongated pigment granules, as well as a few apical cell processes extending around the developing photoreceptor outer segments. Still later, at stage 46, the p.e. exhibits extensive apical cell processes, and its cytoplasm contains Golgi bodies, extensive s.e.r., r.o.s. phagosomes, and what may be early myeloid bodies. The presence of numerous elongated and composite pigment granules at this stage presumably demonstrates that the *Xenopus* p.e. has begun synthesizing melanin (Eppig 1970; Hollyfield 1973). These observations of p.e. development in *Xenopus* are similar to descriptions of this process in *Rana* (Eppig 1970; Hollyfield 1973; Hollyfield & Ward 1974).

(ii) *Differentiation of photoreceptors*

Photoreceptor development in *Xenopus* occurs in parallel with the differentiation of the adjacent p.e., and, as in other vertebrate retinae (Nilsson 1964; Coulombre 1965; Rodieck 1973), is marked by the gradual acquisition of specialized morphological features. These include the appearance of an apical oil droplet, the protrusion of the inner segment beyond the outer limiting membrane, the elongation of an apical cilium, and the final generation of the outer segment by evagination and folding of the ciliary membrane. No membrane vesiculation was found within early outer segments, supporting the contention (Nilsson 1964; Rodieck 1973) that vesicles seen in some developing photoreceptors represent artifacts of fixation rather than a stage in outer segment membrane formation.

Although rod and cone outer segments have the same membrane topology during the first few membrane folds, the two cell types are readily distinguishable (even at this early stage) by the ultrastructural appearance of their outer segment membranes. Nilsson (1964) made analogous observations in developing *Rana* photoreceptors, but the fine structural appearance of the cell membranes in his study were somehwat different from ours. In *Xenopus* (as in *Rana*; Nilsson 1964), developing r.o.s. soon acquire the topology characteristic of adult rods, as all but the most basal membranes pinch off to form disks separated from the surrounding plasma membrane. In contrast, the membranes of developing c.o.s. retain continuity with one another. These topological distinctions persist during subsequent larval development, as r.o.s. and c.o.s. attain their mature dimensions.

An interesting feature of developing *Xenopus* photoreceptors was the occurrence of electron dense fibrous granules in the inner segment cytoplasm near the basal body. Similar granules occur near basal bodies in photoreceptors of young rats (Weidman & Kuwabara 1968, 1969) and mice (Feeney 1973*a*) and in a number of other tissues in association with the formation of centrioles and/or cilia (Steinman 1968; Dirksen 1971; Anderson & Brenner 1971; Sandoz & Boisvieux-Ulrich 1976). Several lines of experimental evidence (Steinman 1970; Staprans & Dirksen 1974; Dirksen & Staprans 1975) support the assumption that these granules represent protein precursors (probably tubulin) to the microtubules of centrioles and cilia. Because the formation of photoreceptor outer segments does not appear to involve centriole morphogenesis but does require the genesis and elongation of the connecting cilium, it seems likely that the fibrous granules we have observed are axonemal precursors which become assembled into the ciliary microtubules. This conclusion is consistent with our failure to find such granules at the base of cilia in more developed photoreceptors, in which the cilium is no longer elongating.

Another intriguing observation in the receptors of larval *Xenopus* is the presence of cisternae in the apical cytoplasm of the inner segment, which are typically clustered near the base of the connecting cilium and occasionally found closely apposed to the neighbouring plasma membrane. Similar structures present in

adult and developing photoreceptors of various species (DeRobertis 1956, 1960; Nilsson 1964; Young 1968, 1969; Richardson 1969; Godfrey 1974), including humans (Hogan, Alvarado & Weddell 1971) have (when mentioned) generally been thought to be endoplasmic reticulum, whereas such cisternae observed in other cells in which cilia (Steinman 1968) and flagella (Bouck 1971) were elongating have been suggested to contribute to the development of the ciliary and flagellar membranes.

Though much is known about the process by which r.o.s. renew their membrane and constituent visual pigment molecules, the intracellular route by which newly-synthesized opsin is transported from the Golgi apparatus, through the connecting cilium, and into new membrane assembled at the outer segment base is incompletely understood (Hall, Bok & Bacharach 1969; Young 1974,1976; Papermaster, Converse & Siu 1975; Papermaster, Converse & Zorn 1976). Recent biochemical research has shown that newly-synthesized opsin is transported in rod inner segments as a water-insoluble, membrane-bound molecule (Papermaster *et al.* 1975) and that rhodopsin is present in the plasma membrane of r.o.s., the connecting cilium, and probably part of the inner segment (Dewey, Davis, Blaise & Barr 1969; Jan & Revel 1974; Basinger, Bok & Hall 1976). The limits of resolution of e.m. autoradiography preclude the conclusive demonstration of whether labelled opsin passes through the membrane or cytoplasm of the connecting cilium (Young 1968). The former route is more likely because freeze-fracture studies suggest that visual pigment molecules are present in the ciliary membrane (Matsusaka 1974; Jan & Revel 1974; Rohlich 1975) and despite considerable ultrastructural research on photoreceptors, there are no reported observations of the movement of membranous structures through the cytoplasm of the ciliary matrix.

Our observation of cisternae near the ciliary base in receptor inner segments suggests a possible route for the migration of newly-synthesized, opsin-containing membrane between its site of synthesis in the myoid and the base of outer segments. These cisternae may be composed of membranes pinched off from the Golgi body which travel through the perimitochondrial cytoplasm, and are inserted into the cell membrane by fusing with the inner segment plasma membrane in the area immediately surrounding the base of the connecting cilium. This new membrane would then migrate sclerally, flow around the ciliary microtubules, and become part of the outer segment by the folding of its basal membrane. This mechanism is consistent with present knowledge of protein synthesis (Palade 1975; Rothman & Lenard 1977), Golgi function (Dauwalder, Whaley & Kephart 1972), membrane biogenesis (Hirano *et al.* 1972; Trump 1975) and asymmetry (Rothman & Lenard 1972), and exocytosis (Allison & Davies 1974) in other cell types, and is similar to a proposed route for addition of flagellar membrane in an alga (Bouck 1971). The proposed mechanism is entirely compatible with evidence regarding photoreceptor outer segment membrane synthesis (reviewed by Young 1976); these cisternae do, in fact, conform to the description of transport vesicles for visual pigment ('schleppersomes') which Papermaster *et al.* (1976) formulated on the basis of biochemical data.

Cisternae were found at the base of the cilium in both classes of developing receptors, as expected because the outer segments of both rods and cones elongate during early development. Although we first noticed them in very young photoreceptors, we have also observed them in both rods and cones at all stages examined, including the adult. Such observations, coupled with recent evidence that mature cones may continue to renew their membranes (see Kinney & Fisher 1978*a*), support our expectation that membrane may be added near the ciliary base of all vertebrate photoreceptors in a qualitatively similar manner. By investigating developing visual cells in *Xenopus*, we were fortunately examining cells where such cisternae are particularly conspicuous, since the rate of r.o.s. membrane renewal is particularly high in this species (see Kinney & Fisher 1978*a*) and the small size of developing outer segments maximizes the probability of observing the connecting cilium and the area of inner segment cytoplasm surrounding the ciliary base.

Another function for these cisternae seems possible in light of the secretory role of the Golgi apparatus in many cells (Dauwalder *et al.* 1972) and the fact that photoreceptor outer segments are embedded in a mucopolysaccharide matrix which may be produced by the photoreceptors (Hall & Heller 1969; Rohlich 1970; Feeney 1973*a*, *b*). There is evidence that components of this matrix are synthesized in the photoreceptor myoid, transported through the Golgi complex, and extruded to the interphotoreceptor space; this matrix appears during ontogeny at the time of outer segment development and seems to undergo renewal at a rate similar to that of r.o.s. (Ocumpaugh & Young 1966; Hall & Heller 1969; Feeney 1973*a*, *b*). These cisternae may, in fact, be transporting the matrix components for exocytosis near the inner segment–outer segment junction. Evidence for this second function remains circumstantial since the techniques of fixation and staining used in this study do not demonstrate material within these cisternae or in the interphotoreceptor space.

The authors wish to thank Mr R. Gill for his technical assistance, Dr E. L. Triplett for his advice on raising *Xenopus* larvae, Dr J. F. Case, Dr G. H. Jacobs, and Dr R. H. Steinberg for their critical comments on the manuscrupt. 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 Caifornia by M. S. K. as partial fulfilment of the requirements for the Ph.D. degree.

References

Allison, A. C. & Davies, P. 1974 Mechanisms of endocytosis and exocytosis. *Symp. Soc. exp. Biol.* **28**, 419–446.

Anderson, R. G. W. & Brenner, R. M. 1971 The formation of basal bodies (centrioles) in the rhesus monkey oviduct. *J. Cell Biol.* **50**, 10–34.

Basinger, S., Bok, D. & Hall, M. 1976 Rhodopsin in the rod outer segment plasma membrane. *J. Cell Biol.* **69**, 29–42.

Besharse, J. C. & Hollyfield, J. G. 1976 Renewal of normal and degenerating photoreceptor outer segments in the ozark cave salamander. *J. exp. Zool.* **198**, 287–302.

Bouck, G. B. 1971 The structure, origin, isolation, and composition of the tubular mastigonemes of the *Ochromonas* flagellum. *J. Cell. Biol.* **50**, 362–384.

Chung, S. H., Stirling, R. V. & Gaze, R. M. 1975 The structural and functional development of the retina in larval *Xenopus*. *J. Embryol. exp. Morph.* **33**, 910–940.

Coulombre, A. J. 1965 The eye. In *Organogenesis* (ed. R. L. De Haan & H. Ursprung), pp. 219–251. New York: Holt, Rinehart & Winston.

Dauwalder, M., Whaley, W. G. & Kephart, J. E. 1972 Functional aspects of the golgi apparatus. *Sub-Cell. Biochem.* **1**, 225–275.

DeRobertis, E. 1956 Electron microscope observations of the retinal rods. *J. biophysic. biochem. Cytol.* **2**, 319–337.

DeRobertis, E. 1960 Some observations on the ultrastructure and morphogenesis of photoreceptors. *J. gen. Physiol.* **43**, 1–13.

Deuchar, E. M. 1972 *Xenopus laevis* and developmental biology. *Biol. Rev.* **47**, 37–112.

Deuchar, E. M. 1975 *Xenopus: the south African clawed frog.* London: John Wiley & Sons.

Dewey, M. M., Davis, P. K., Blaisie, J. K. & Barr, L. 1969 Localization of rhodopsin antibody in the retina of the frog. *J. molec. Biol.* **39**, 395–405.

Dirksen, E. R. 1971 Centriole morphogenesis in developing ciliated epithelium of the mouse oviduct. *J. Cell Biol.* **51**, 286–302.

Dirksen, E. R. & Straprans, I. 1975 Tubulin synthesis during ciliogenesis in the mouse oviduct. *Devl Biol.* **46**, 1–13.

Ditto, M. 1975 A difference between developing rods and cones in the formation of outer segment membranes. *Vision Res.* **15**, 535–536.

Dixon, J. S. & Cronly-Dillon, J. R. 1972 The fine structure of the developing retina in *Xenopus laevis*. *J. Embryol. exp. Morph.* **28**, 659–666.

Dowling, J. E. & Sidman, R. L. 1962 Inherited retinal dystrophy in the rat. *J. Cell Biol.* **14**, 73–109.

Eppig, J. J. 1970 Melanogenesis in amphibians. *Z. Zellforsch.* **103**, 238–246.

Feeney, L. 1973*a* The interphotoreceptor space. I. Postnatal ontogeny in mice and rats. *Devl. Biol.* **32**, 101–114.

Feeney, L. 1973*b* The interphotoreceptor space. II. Histochemistry of the matrix. *Devl Biol.* **32**, 115–128.

Gaze, R. M. 1970 *The formation of nerve connections.* London: Academic Press.

Godfrey, A. J. 1974 The photoreceptors of the chick. Ph.D. dissertation, University of California at Los Angeles.

Grillo, M. A. & Rosenbluth, J. 1972 Ultrastructure of developing *Xenopus* retina before and after ganglion cell specification. *J. comp. Neurol.* **145**, 131–140.

Hall, M. O., Bok, D. & Bacharach, A. D. E. 1969 Biosynthesis and assembly of the rod outer segment membrane system. *J. molec. Biol.* **45**, 397–406.

Hall, M. O. & Heller, J. 1969 Mucopolysaccharides of the retina. In *The retina* (ed. B. R. Straatsma, M. O. Hall, R. A. Allen, & F. Crescitelli), pp. 211–224. Berkeley: University of California Press.

Hirano, H., Parkhouse, B., Nicolson, G. L., Lennox, E. S. & Singer, S. J. 1972 Distribution of saccharide residues on membrane fragments from a myeloma-cell homogenate: its implications for membrane biogenesis. *Proc. natn. Acad. Sci. U.S.A.* **63**, 233–238.

Hogan, M. J., Alvarado, J. A. & Weddel, J. E. 1971 *The histology of the human eye.* Philadelphia: W. B. Saunders Co.

Hollyfield, J. G. 1971 Differential growth of the neural retina in *Xenopus laevis* larvae. *Devl Biol.* **24**, 264–286.

Hollyfield, J. G. 1973 Elimination of egg pigment from developing ocular tissues in the frog *Rana pipiens*. *Devl Biol.* **30**, 115–128.

Hollyfield, J. G. & Ward, A. 1974 Phagocytic activity in the retinal pigment epithelium of the frog *Rana pipiens*. *J. ultrastruct. Res.* **46**, 327–338.

Horder, T. J. & Spitzer, J. L. 1973 Absence of cell mobility across the retina in *Xenopus laevis* embryos. *J. Physiol., Lond.* **233**, 33–34P.

Humason, G. L. 1972 *Animal tissue techniques*. San Francisco: W. H. Freeman & Co.

Jacobson, M. 1970 *Developmental neurobiology*. New York: Holt, Rinehart & Winston.

Jacobson, M. 1976*a* Histogenesis of retina in the clawed frog with implications for the pattern of development of retinotectal connections. *Brain Res.* **103**, 541–545.

Jacobson, M. 1976*b* Neuronal recognition in the retinotectal system. In *Neuronal recognition* (ed. S. H. Barondes), pp. 2–23. New York: Plenum Press.

Jan, L. Y. & Revel, J. 1974 Ultrastructural localization of rhodopsin in the vertebrate retina. *J. Cell Biol.* **62**, 257–273.

Jones, K. W. & Elsdale, T. R. 1963 The culture of small aggregates of amphibian embryonic cells *in vitro*. *J. Embryol. exp. Morph.* **11**, 135–154.

Keating, M. J. & Kennard, C. 1976 The amphibian visual system as a model for developmental neurobiology. In *The amphibian visual system* (ed. K. V. Fite), pp 267–315. New York: Academic Press.

Kinney, M. S. & Fisher, S. K. 1978*a* The photoreceptors and pigment epithelium of the adult *Xenopus* retina: morphology and outer segment renewal. *Proc. R. Soc. Lond.* B **201**, 131–147.

Kinney, M. S. & Fisher, S. K. 1978*b* Changes in length and disk shedding rate of *Xenopus* rod outer segments associated with metamorphosis. *Proc. R. Soc. Lond.* B **201**, 169–177.

Lanzavecchia, G. 1960 Ultrastuturra dei coni e dei bastoncelli nella retina di *Xenopus laevis*. *Archo. ital. Anat. Embriol.* **65**, 417–435.

LaVail, M. M. 1973 Kinetics of rod outer segment renewal in the developing mouse retina. *J. Cell Biol.* **58**, 650–661.

Matsusaka, T. 1974 Membrane particles of the connecting cilium. *J. ultrastruct. Res.* **48**, 305–312.

Nieuwkoop, F. W. & Faber, J. 1956 *Normal table of Xenopus laevis Daudin*. Amsterdam: North Holland Publishing Co.

Nilsson, S. E. G. 1964 Receptor cell outer segment development and ultrastructure of the disc membranes in the retina of the tadpole (*Rana pipiens*). *J. ultrastruct. Res.* **11**, 581–620.

Ocumpaugh, D. E. & Young, R. W. 1966 Distribution and synthesis of sulfated mucopolysaccharides in the retina of the rat. *Invest. Ophthal.* **5**, 196–203.

Palade, F. 1975 Intracellular aspects of the process of protein synthesis. *Science, N.Y.* **189**, 347–358.

Papermaster, D. S., Converse, C. S. & Siu, J. 1975 Membrane biosynthesis in the frog retina: opsin transport in the photoreceptor cell. *Biochemistry, N.Y.* **14**, 1343–1352.

Papermaster, D. S., Converse, C. A. & Zorn, M. 1976 Biosynthetic and immunochemical characterization of a large protein in frog and cattle rod outer segment membranes. *Expl. Eye Res.* **23**, 105–115.

Richardson, T. M. 1969 Cytoplasmic and ciliary connections between the inner and outer segments of mammalian visual receptors. *Vision Res.* **9**, 727–731.

Rodieck, R. W. 1973 *The vertebrate retina*. San Francisco: W. H. Freeman & Co.

Rohlich, P. 1970 The interphotoreceptor matrix: electron microscopic and histochemical observations on the vertebrate retina. *Expl Eye Res.* **10**, 80–96.

Rohlich, P. 1975 The sensory cilium of retinal rods is analogous to the transitional zone of motile cilia. *Cell Tissue Res.* **161**, 421–430.

Rothman, J. E. & Lenard, J. 1977 Membrane assymmetry. *Science, N.Y.* **195**, 743–753.

Sandoz. D. & Boisvieux-Ulrich, E. 1976 Ciliogenèse dans les cellules à mucus de l'oviducte de caille. *J. Cell Biol.* **71**, 449–459.

Saxén, L. 1954 The development of the visual cells. *Suomal Tiedeakat. Toim.* (*Sarja IV. Biologica*) **23**, 1–107.

Sjöstrand, F. 1959 Fine structure of cytoplasm: the organization of membranous layers. In *Biophysical science – a study program* (ed. J. L. Oncley), pp. 301–318. New York: John Wiley & Sons.

Sjöstrand, F. S. 1961 Electron microscopy of the retina. In *The structure of the eye* (ed. G. K. Smelser), pp. 1–28. New York: Academic Press.

Staprans, I. & Dirksen, E. R. 1974 Microtubule protein during ciliogenesis in the mouse oviduct. *J. Cell Biol.* **62**, 164–174.

Steinman, R. M. 1968 An electron microscopic study of ciliogenesis in developing epidermis and trachea in the embryo of *Xenopus laevis*. *Am. J. Anat.* **122**, 19–56.

Steinman, R. M. 1970 Inhibitory effects of colchicine on ciliogenesis in ectoderm of *Xenopus laevis*. *J. ultrastruct. Res.* **30**, 423–440.

Straznicky, K. & Gaze, R. M. 1971 The growth of the retina in *Xenopus laevis*: an autoradiographic study. *J. Embryol. exp. Morph.* **26**, 67–79.

Trump, B. F. 1975 The network of intracellular membranes. In *Cell membranes* (ed. G. Weissman & R. Claiborne), pp. 123–133. New York: Hospital Practice Publishing Co.

Weidman, T. A. & Kuwabara, T. 1968 Postnatal development of the rat retina. *Archs Ophthal., N.Y.* **79**, 470–484.

Weidman, T. A. & Kuwabara, T. 1969 Development of the rat retina. *Invest. Ophthal.* **8**, 60–69.

Witkovsky, P., Gallin, E., Hollyfield, J. G., Ripps, H. & Bridges, C. D. B. 1976 Photoreceptor thresholds and visual pigment levels in normal and vitamin A-deprived *Xenopus* tadpoles. *J. Neurophysiol.* **39**, 1272–1287.

Young, R. W. 1968 Passage of newly formed protein through the connecting cilium of retinal rods in the frog. *J. ultrastruct. Res.* **23**, 462–473.

Young, R. W. 1969 A difference between rods and cones in the renewal of outer segment protein. *Invest. Ophthal.* **8**, 222–231.

Young, R. W. 1971 The renewal of rod and cone outer segments in the rhesus monkey. *J. Cell Biol.* **49**, 303–318.

Young, R. W. 1974 Biogenesis and renewal of visual cell outer segment membranes. *Expl. Eye Res.* **18**, 215–223.

Young, R. W. 1976 Visual cells and the concept of renewal. *Invest. Ophthal.* **15**, 700–725.

Young, R. W. & Droz, B. 1968 The renewal of protein in retinal rods and cones. *J. Cell Biol.* **39**, 169–184.