Growth of the Adult Goldfish Eye

III. SOURCE OF THE NEW RETINAL CELLS

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ABSTRACT   The manner in which new cells are added to the growing adult goldfish retina was examined using ^{3}H-thymidine radioautography. Cell proliferation leading to the formation of neurons is restricted to the retinal margin at the *ora terminalis*. New retina is added in concentric rings, with slightly more growth dorsonasally. The rate of cell addition is variable, averaging 12,000 cells/day. These new cells account for about 20% of the total increase in retinal area; the remaining 80% is due to hypertrophy, or expansion, of the retina. In contrast to all of the other retinal cells, the rods do not appear to participate in the retinal expansion. This hypothesized immobility of the rods would create a shearing strain between the retinal layers resulting in a shift in their position relative to the other cells. Were they to maintain synaptic contacts with the same horizontal and bipolar cells, the rod axons would have to be elongated peripherally or the post-synaptic cell dendrites displaced centrally. Since neurons with this morphology have not been found in the goldfish retina, these observations suggest that the rods must be changing their synaptic connections as the retina grows.

In the previous paper (Johns and Easter, '77) we presented evidence for cell addition during retinal growth in adult goldfish based on cell counts in animals of different sizes and ages. We also showed that the proportion of rods increased with growth, implying a differential addition of this class of retinal cell. From the normal material we could not determine the source of the new cells, however. To fully understand the growth process it is necessary to determine not only the place of origin of the new cells, but also their final destination and site of differentiation, which may not be the same.

Following an initial early embryonic period during which cells proliferate throughout the developing retina, mitotic activity becomes restricted to a narrow region at the extreme margin of the retina in all vertebrates which have been examined (for example: fish, Müller, '52; chick, Coulombre, '55; mouse, Sidman, '60; amphibian, Straznicky and Gaze, '71). The separation of the retina into a central differentiated region and a marginal proliferative zone was first suggested by histological observations of mitotic figures (Müller, '52; Coulombre, '55; Lyall, '57a; Blaxter and Jones, '67; Mann, '69) and later confirmed

with ^{3}H-thymidine radioautography (Sidman, '60; Denham, '67; Jacobson, '68, '76; Hollyfield, '68, '72; Straznicky and Gaze, '71; Morris et al., '76). The reasons for compartmentation of functional and developmental activities into separate areas of the retina is not known, but is typical of growing tissues (Weiss, '49).

At its simplest, cell proliferation at the retinal margin would result in the appositional addition of concentric rings of new retinal cells. Radioautographic evidence from larval amphibians has confirmed this simple interpretation (Straznicky and Gaze, '71; Gaze and Watson, '68; Jacobson, '76), but this does not preclude the possibility that in some species retinal growth might involve regions other than the periphery. Indeed, during postembryonic growth in certain teleost fish and amphibians the relative proportions of some retinal cells increase in central retinal regions which are devoid of mitotic activity. To explain this, several workers have suggested that one type of retinal cell, either undifferentiated or even differentiated, can be transformed into another (Bernard, 1900; Lyall,

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'57a,b; Blaxter and Jones, '67) or that cells migrate into the central regions from the mitotically active periphery (Glücksman, '40; Hollyfield, '68).

Although some of the cells produced in the marginal germinal zone may migrate into the central retina, clearly not all of them do, since some remain and differentiate in situ. For example, the cones and ganglion cells appear to be added exclusively at the margins in both fish and amphibians (Glücksmann, '40; Lyall, '57a; Jacobson, '68; Hollyfield, '68; Straznicky and Gaze, '71). Retinal growth in teleost fish and larval amphibians may thus involve at least three different mechanisms: (1) appositional addition of concentric annuli of new retina, including all layers and cell types, and differential addition of some cells to central retina via (2) migration of neurons or undifferentiated precursors from the peripheral germinal zone or (3) transformation of one type of differentiated cell into another.

One other source of new retinal cells has been suggested, not in association with development, but with regeneration. Following removal of the neural retina during early embryonic development, the pigmented retinal epithelial cells are capable of regenerating new neural tissue (Coulombre and Coulombre, '65). This capacity is lost early in development in avian and mammalian retinas, and after metamorphosis in anuran amphibians, but is retained in most adult urodeles (Gaze and Watson, '68; Keefe, '73a,b; Levine, '75). Regeneration of the neural retina in teleost fish has not been studied in detail, although it has been noted in the goldfish following retinal lesions (Attardi and Sperry, '63; Schmidt, '76). Although the pigmented retinal epithelium has never been implicated in postembryonic retinal growth, it must be considered as a possible source of cells, particularly in central regions, since it has at least a latent capacity to produce retinal cells.

The following longitudinal growth study employing ^{3}H-thymidine radioautography in adult goldfish was undertaken to determine the source of the new cells, the mechanism of addition of rods to the central retina, and the overall geometry of the retinal growth.

METHODS

Common goldfish (Carassius auratus), 5-7 cm in length tip-to-tip, were purchased from Ozark Fisheries, Stoutland, Missouri, and kept in standard aerated aquaria at room temperature (18-22°C). The tanks were exposed to natural light as well as normal laboratory illumination. The fish were fed with dry commercial fish food (TetraMin) supplemented with live Artemia (brine shrimp) during the first three months and with live Daphnia during the remainder of the experiment.

Experimental groups. The fish were divided into five groups. The first group of 20 fish was injected intraocularly (IO) with ^{3}H-thymidine, specific activity, 50.8 Ci/mmole (New England Nuclear, Boston, Massachusetts). A total of 0.35 μCi in two doses was injected into the right eye of each fish. Three days after the first injection, 6 of the 20 fish were anesthetized by immersion in 0.05% tricaine methanesulfonate and their left eyes (uninjected) removed (group IO-X). The eyes of the remaining 14 fish were left intact (group IO).

The next group of 15 fish (group IP) were injected intraperitoneally with four doses of ^{3}H-thymidine, separated by 12-hour intervals. The total dose was approximately 25 μCi/g.b.w.

Another group of 15 fish (group TdR) was injected intraperitoneally with non-radioactive thymidine according to the same dosage schedule as the IP fish. The total amount of thymidine, labeled or unlabeled, injected into each TdR and IP fish was approximately 12 μg/g.b.w.

The final group of 15 fish (group NC) was injected intraperitoneally with 0.9% NaCl according to the same schedule as the IP and TdR fish. All injections were done between 8-10 A.M. and 8-10 P.M., and all fish were injected during the same 48-hour period.

Histological and radioautographical procedures. Fish were killed at intervals from 1 to 336 days following injection. In all cases, the fish chosen to be killed had body lengths which ranged from the smallest to the largest present in the population at that time. Thus, the selection process did not bias the rate of growth observed. Histological procedures were as described previously (Johns and Easter, '77).

The retinas from the IO and IP fish were processed for radioautography according to the technique of Kopriwa and Leblond ('62), using Kodak NTB-2 nuclear emulsion (Eastman Kodak, Rochester, New York) diluted 1:1. The radioautograms were stored at 4°C for five weeks and developed for 3 to 4 minutes in Dektol, diluted 1:1, at 14°C. The sections
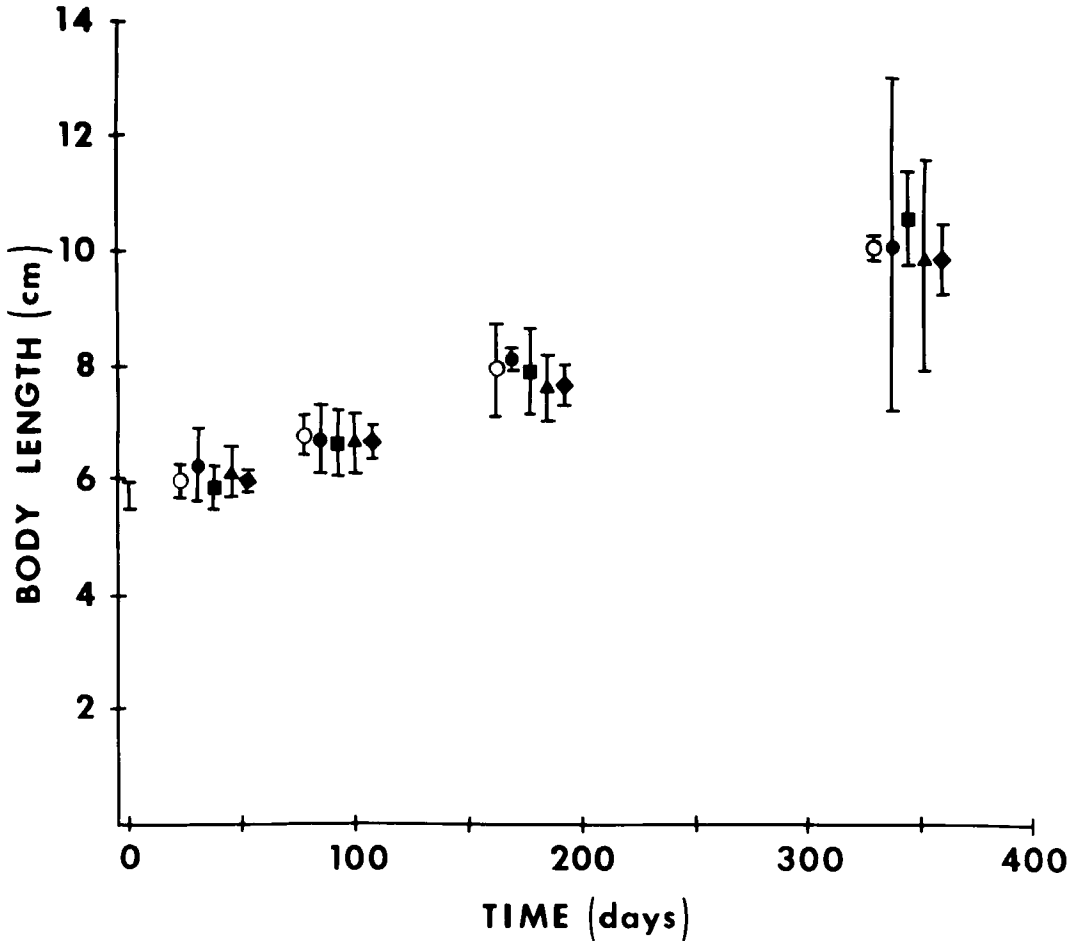
Fig. 1 Growth of adult goldfish. The mean body length ± 2 S.E.M. is plotted as a function of time post-injection. The fish were divided into four groups: IO (open circles), IO-X (filled circles), IP (squares), TdR (triangles), and NC (diamonds). See text for further explanation.

were post-stained through the emulsion with Ehrlich's hematoxylin. The retinas from the TdR and NC fish were handled in the same way, omitting the radioautographic steps.

We will use the term "labeled cells" to mean cells with labeled nuclei, that is, nuclei overlain by silver grains in the developed radioautograms.

Measurements of retinal size and cell number. Retinal cells were counted in radial sections through the center of the eye, the maximum retinal length measured and the retinal area calculated as described previously (Johns and Easter, '77).

The retinal circumference (C, the distance around the *ora terminalis*) and the circumference of other circles defined by the retina and lying in planes parallel to the *ora*, were calculated according to the following formula:

$$C = 2 \pi r \cos (\theta),$$

where θ = the angle between C and the equator of the globe parallel to the pupil, and r = the retinal length.

All retinal dimensions were corrected for an histological shrinkage of 30% (linear) (Johns, '76).

RESULTS

Intergroup comparisons of growth. No differences were found among the average growth rates for the five groups of fish. This is illustrated in figure 1, in which the mean body lengths ± 2 S.E.M. for each group of fish at each time point are given. All confidence
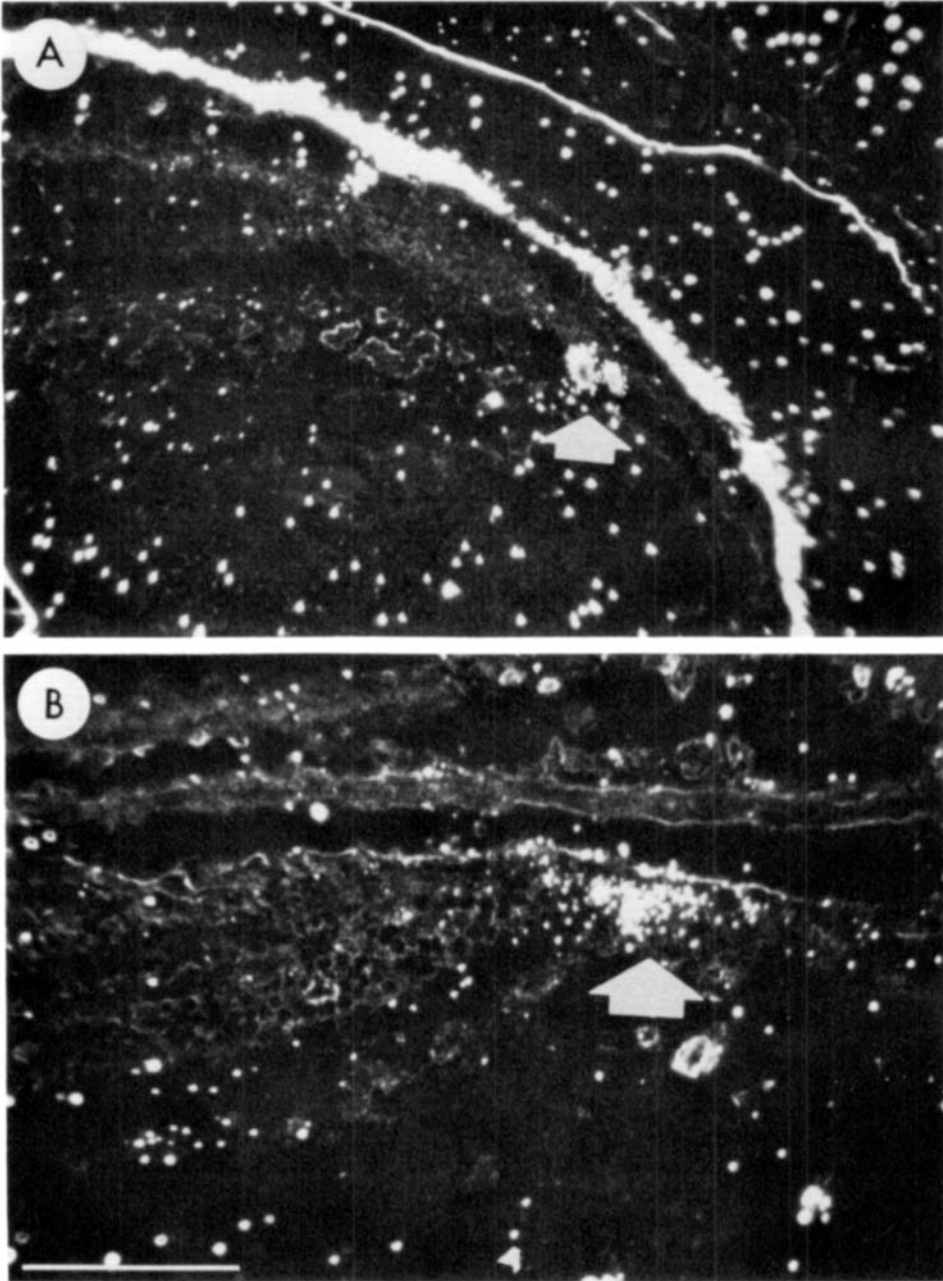
Fig. 2 Radioautograms at short survival times.

A   Dark field photomicrograph showing two heavily labeled cells (arrow) at the *ora terminalis* of the retina from a fish killed 12 hours following a single intraocular injection of ³H-thymidine. The retina extends leftward from the arrow; its laminar structure is seen better in B. The bright curved line is not label, but the reflection from a pigmented layer behind the retina.

B   This fish was killed four days following a series of intraperitoneal injections of ³H-thymidine. The labeled cells (arrow) are still confined to the *ora*, and have increased in number. Calibration bar: 50 μm.

intervals overlapped extensively leading to the conclusion that growth was not affected by the injections, or by the unilateral enucleations in the IO-X fish. The increased variability in the body lengths at 336 days resulted from variability in individual growth rates. The average length at the start of the experiment was 5.6 cm, and at the conclusion (336 days later) it was 10.1 cm. The average eye diameter increased from 3.9 to 5.6 mm.

Radioautograms at short survival times (12 hours-4 days). Following intraocular or intraperitoneal injections of ^{3}H-thymidine most labeled retinal cells were found at the *ora terminalis,* as shown in the photomicrographs in figures 2A and B. Twelve hours following a single injection, usually only one or two cells were labeled at each retinal edge in radial sections, as in figure 2A. The number of labeled cells per section in general increased both with time and continuing injections, as a comparison of figures 2A and B illustrates.

Occasional heavily labeled cells were also found scattered throughout the retina, particularly in the IO fish. They were most prevalent in the optic fiber layer, especially near the optic disk, but were also found in other retinal layers. The morphology of their nuclei could not be distinguished due to the high density of overlying silver grains. These cells did not make any permanent contribution to the retinal cell population, since they could not be found at the later time intervals (see below). We therefore believe them to be nonneuronal elements; those in the optic fiber layer were undoubtedly macroglia or vascular endothelial cells, and the others may have been microglia. Labeled cells were also seen scattered throughout the pigmented retinal epithelium (PRE) in the radioautograms from IP fish. They were not seen in the IO radioautograms, suggesting that the retina acts as a barrier to diffusion of the thymidine.

Radioautograms at longer survival times (28-336 days). Labeled cells were not found anywhere in the central retina or in the PRE after 28 days. The only cells still labeled were in the peripheral retina; these were displaced progressively from the *ora* with increasing survival times. Figures 3 and 4 indicate that the labeled cells in the ganglion cell and inner nuclear layers (INL) and the labeled cone nuclei were confined to a circumscribed zone or band oriented perpendicularly to the layers of the retina. The cytological characteristics of these nuclei were those of retinal neurons,

leading to the conclusion that the cell proliferation at the margin was in fact neurogenesis. The most heavily labeled cells were at the edge of the band farthest from the *ora,* and formed a sharp boundary between labeled and unlabeled retina. Less heavily labeled cells extended for a short distance toward the periphery, rendering the edge of the band on this side indistinct. Cells both peripheral and central to this region were unlabeled.

In contrast to the other cells, the labeled nuclei in the outer nuclear layer (ONL) proximal to the ELM (believed to be the rod nuclei) were not confined to this narrow zone. Labeled rods spread inward toward the center for a variable distance beyond the most central, heavily labeled cells in the INL (figs. 3B, 4). At no time, however, were labeled rod nuclei found more than about 1 mm from the zone of labeled cells in the other layers, leaving a central region about 5 mm in diameter free from labeled cells of any kind.

Cytological characteristics of the germinal zone. The radioautograms indicated that cell proliferation leading to the formation of long-lived retinal cells occurred only at the extreme periphery of the goldfish retina. In normal histological preparations this germinal zone showed several unique morphological characteristics, the most striking of which were the changes in cone morphology. The cones at the peripheral edge of the retina were small, and at the *ora* their distal processes were completely lacking (figs. 5A,B). Toward the central retina, these processes appeared and increased in size until some 100-200 μm inward they were indistinguishable from those in the central retina (fig. 5C).

In the most peripheral 100 to 200-μm segment of retina, the INL was thicker, and the density of its cells was increased by 25-30%. The cone nuclei were also more dense here than elsewhere, although they were still in a layer only one nucleus thick. In contrast, the layer of rod nuclei became thinner at the margin and the cell density decreased by 20-30%. Cells in the ganglion cell layer at the extreme margin were sometimes more dense, on the average by about 15%, but this varied from section to section.

At the *ora* the three nuclear layers merged, the plexiform layers were obliterated, and the nuclear morphology of the cells became nonspecific (fig. 5A). These undifferentiated nuclei were the ones which were labeled in the radioautograms at zero to four days. Farther
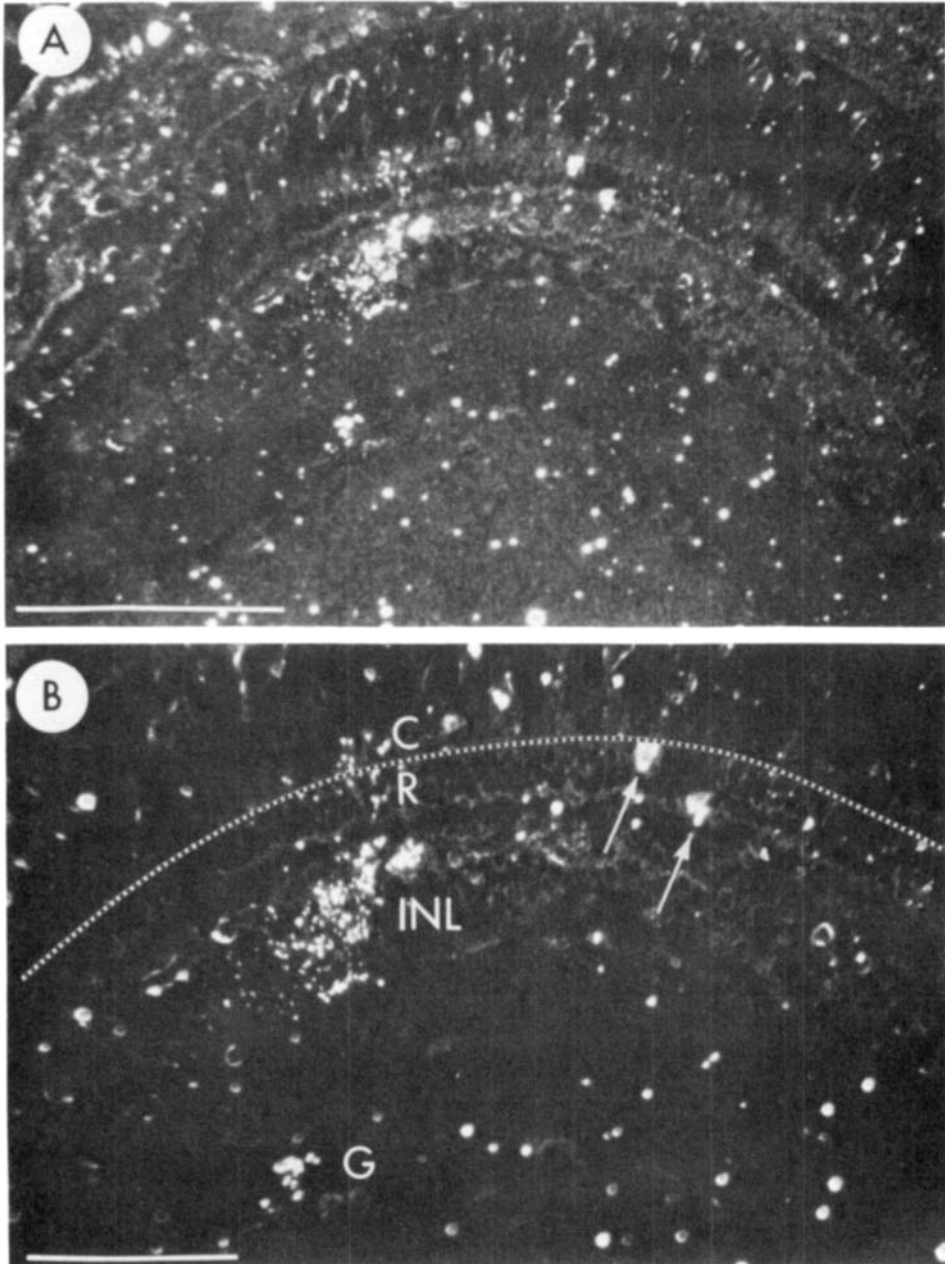
Fig. 3   Radioautograms at long survival times.
    A   This is a low power, darkfield view of the retina from a fish killed 168 days after injection of ^{3}H-thy-
midine. The *ora terminalis* is at the left margin of the photomicrograph. Calibration bar: 100 μm.
    B   A higher power view of the same section. The ganglion cell (G) and inner nuclear (INL) layers and
the layers of rod (R) and cone (C) nuclei are indicated. The external limiting membrane is shown by the dashed
line. Note that all of the labeled cells are confined to a restricted band except the labeled rods (arrows) which
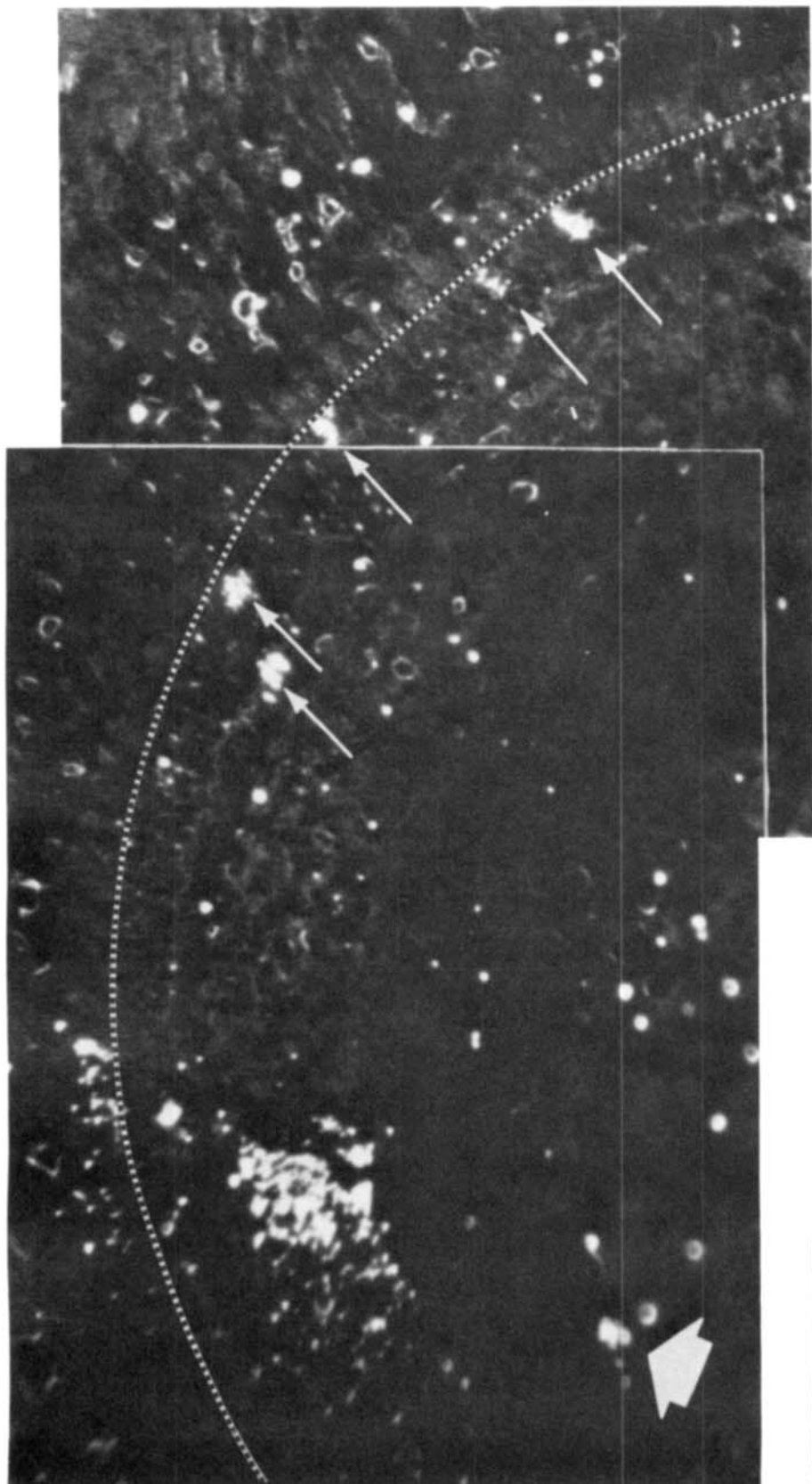are displaced toward the central retina. Calibration bar: 50 μm.

Fig. 4 Displacement of labeled rods. This is the same retina as in figure 3, but a different section. The band of labeled retinal cells is indicated by the large arrow; the ora is out of view toward the left. Again, the external limiting membrane is shown by the dashed line. Five labeled rod nuclei, displaced centrally from the other labeled cells, are indicated by the small arrows. Calibration bar: 50 μm.
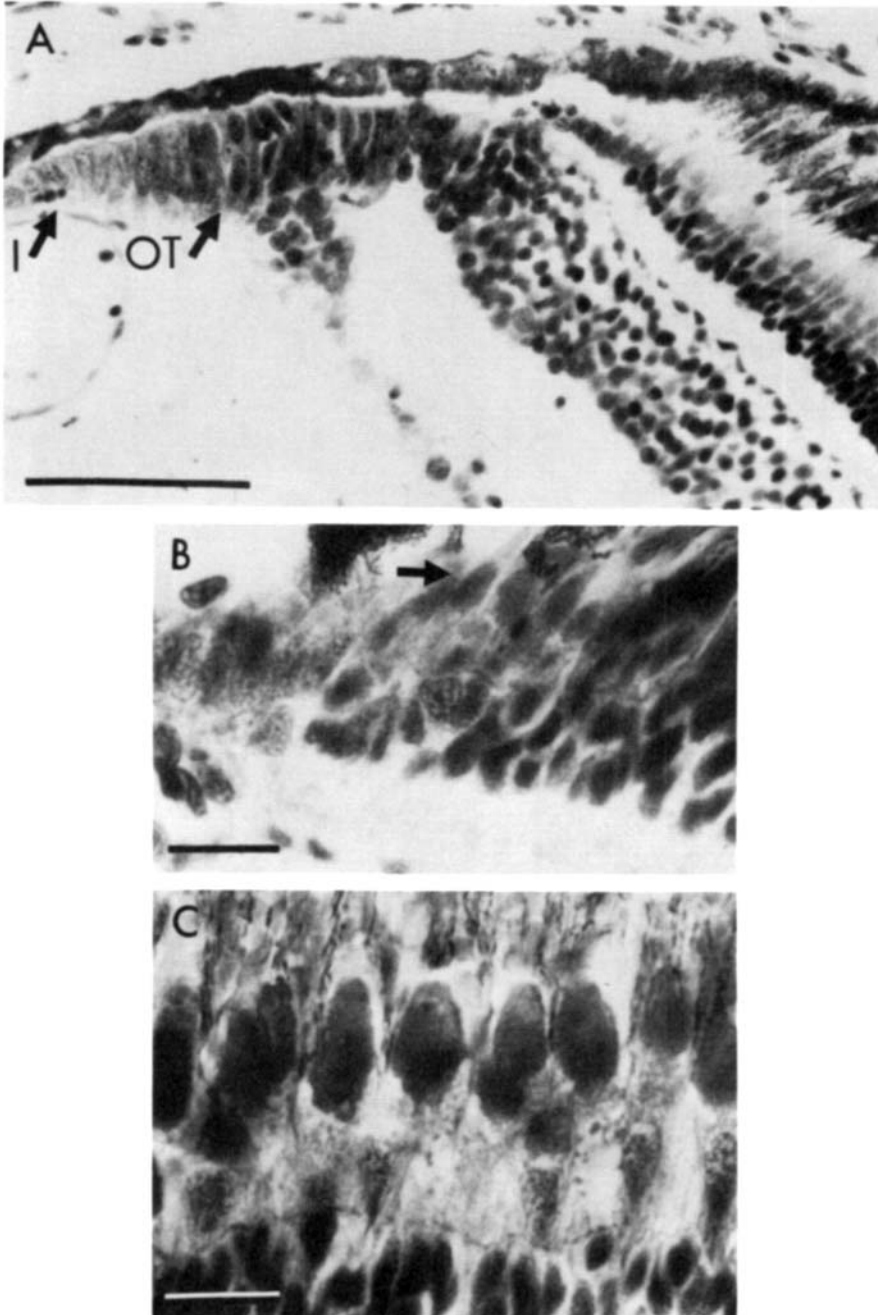
Fig. 5   The germinal zone. Paraffin sections cut at 5 μm and stained with Foot-Masson trichrome.
  A   The edge of the retina at the *ora terminalis* (OT) as it merges with the iris epithelium (I). Calibration bar: 50 μm.
  B   The distal processes of the cones (arrow) at the retinal margin. Calibration bar: 10 μm.
  C   Cones in the same retina as in B, but from a more central region. Calibration bar: 10 μm.
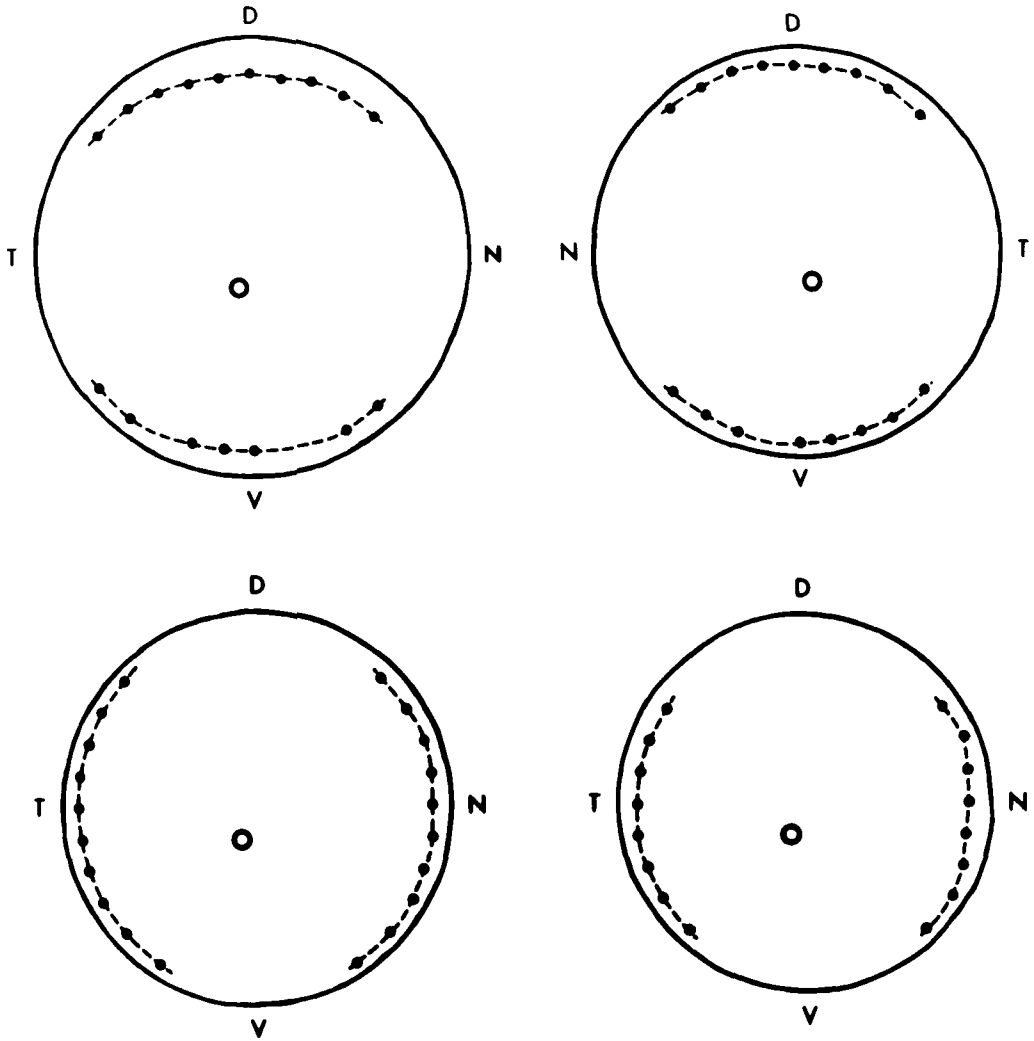
Fig. 6 Reconstruction of the pattern of cell labeling in the retinas of four fish killed at 336 days post-injection. The retinas are represented by circles. The distance from the *ora terminalis* to the central edge of the zone of labeled cells in the inner nuclear layer (the retinal increment) was measured in sections at 400-μm intervals; the labeled zone is indicated by the dots connected by dashed lines. D, dorsal; V, ventral; N, nasal; T, temporal. The small circle represents the optic disc. Calibration bar: 2 mm.

peripherally, the retinal cells thinned to a single layer and merged with the cells of the iris epithelium, which were also sometimes labeled in the short-term radioautograms.

All of the above characteristics of the germinal zone were found not only in the small fish but in larger fish as well. Since the number of retinal neurons continues to increase

(Johns and Easter, '77), we can assume that this germinal zone remains active in these larger fish.

Geometry of the retinal growth. In the retina as a whole, the labeled cells formed a complete annulus. This was demonstrated by reconstructing the position of the labeled cells in radioautograms of spaced serial, radial sec-

TABLE 1

Geometry of the retinal growth

Time post-injection (days)	Area of new retina (mm²)	Ave. retinal length (mm)	Area of entire retina (mm²)	Area of "old" retina (mm²)	% increase in area of "old" retina	% of total growth due to hypertrophy
0	0	5.2	17.8	17.8	—	—
84	1.1	5.8	22.2	21.1	18.7	75.0
168	3.4	7.3	35.2	31.9	79.2	81.0
336	5.0	8.0	42.2	37.2	109.0	79.5

These data are average values based on the results from three or four retinas at each time point. The area of new retina was calculated from the overall mean retinal increment (see text). This value was taken as the height of a trapezoid whose parallel sides were the circumference (C) of the retina at the *ora* and at the edge of the zone of labeled cells, respectively (see METHODS for the calculation of C). The curvature of the retinal surface was ignored. The retinal length and the area of the retina were determined as described in the METHODS. The area of "old" retina is the difference between the entire area and the area of new retina.
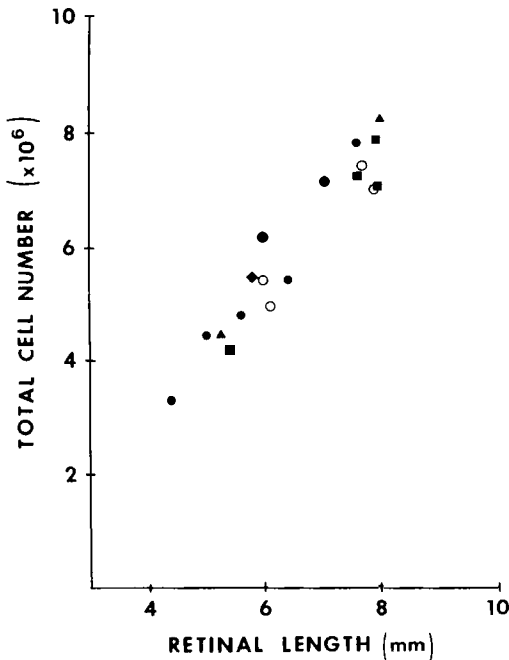

Fig. 7 The relation between cell number and retinal length. The total number of cells in the retina is plotted as a function of retinal length. Each point is one retina. The symbols are as in figure 1.

tions. The distance from the central edge of the zone of labeled cells to the point where the inner plexiform layer ended at the *ora* was measured at each retinal edge in 8 to 11 sections spaced at regular intervals of 400 μm through the central 90-110° of ten retinas using an ocular micrometer and a magnification of × 450. Sections farther toward the edge of the eye could not be used because they cut nearly tangentially through the retinal margin. The data for four retinas from fish killed at 336 days are shown in the recon-

structions in figure 6. The upper two eyes were sectioned through the dorsoventral axis and the lower two through the nasotemporal axis. Although in any one eye the entire boundary of the annulus of labeled cells could not be traced, these reconstructions, taken together, demonstrate that new retina is added around the entire circumference in concentric circles.

The region of retina peripheral to the zone of labeled cells is new retina that was added after the label injection. The distance from the labeled zone to the *ora*, the retinal increment, is a measure of this growth. The average area of new retina added at each post-injection time is given in table 1, column 2. The increase in the size of the entire retina was also calculated from the average retinal lengths at each time (table 1, column 3) and is given in column 4. The size of the "old" retina, that part originally present and central to the labeled annulus at later times, was determined by subtracting the area of new retina from the area of the entire retina. The size of the "old" retina increased with time, as shown in columns 5 and 6. This hypertrophic component accounted for about 80% of the total growth (column 7).

The reconstructions in figure 6 suggest that new retina was not added symmetrically. In many cases, the amount of retina added dorsally was greater than that added ventrally. Likewise, the amount added nasally was greater than that added temporally. This asymmetry in retinal growth resulted in a slight dorsonasal shift in the retinal axis, defined as the line connecting the center of the lens to the center of the nearly hemispherical retina. The magnitude of this shift was at the most 1.7°, and was directed away from the optic disk which is located in the

opposite, ventrotemporal quadrant (Easter et al., '77).

Retinal growth by cell addition. The total number of retinal cells (excluding glia in the optic fiber layer) is shown in figure 7 as a function of retinal length. The points cluster, indicating that the experimental procedures (injections of ^{3}H-thymidine or non-radioactive thymidine or enucleation) had no effect on retinal growth by cell addition.

The rate of retinal cell addition was estimated by comparing the number of cells in the right and left eyes of the IO-X fish. The left eyes of these fish were removed at the beginning of the experiment and the right eyes when the fish were killed 84, 168, or 336 days later. Table 2 gives the number of rods, INL cells, cones, and ganglion cells in the two eyes. The mean rates are given in the bottom row, and the mean total rate of cell addition in the last column.

DISCUSSION

Cell labeling not associated with retinal cell addition. A few cells scattered throughout the retina were labeled at short times following ^{3}H-thymidine injection and were identified as glial or vascular elements on the basis of their retinal position and lack of persistence. Such scattered labeled cells were also found in larval *Xenopus* (Jacobson, '76) and newly-hatched chick retinas (Morris et al., '76) and were similarly identified.

Other labeled cells were found throughout the PRE in the fish injected intraperitoneally. Since PRE cells are capable of regenerating neural retina in adult newts (Keefe, '73a,b) it was thought that these cells might be responsible for the increase in the number of rods in central retinal regions. This proved incompatible with other observations, however, since in the radioautograms from longer survival times the label in these cells had been diluted by further cell divisions with no evidence of cell migration into the neural retina. Despite the difference in labeling patterns in the PRE (between the IO and IP fish) at short survival times, at longer intervals, the radioautograms from the two groups were indistinguishable. This provides further evidence that the PRE made no cellular contribution to the neural retina during normal growth.

Implications of peripheral cell addition. The restriction of retinal cell labeling to the *ora* confirms all previous studies on postembryonic retinal cell proliferation, and indi-

TABLE 2

Rate of cell addition

Fish		Days	No. Rods ($\times 10^6$)	Rate (rods) ($\times 10^3$/day)	No. INL ($\times 10^6$)	Rate (INL) ($\times 10^3$/day)	No. Cones ($\times 10^5$)	Rate (cones) ($\times 10^3$/day)	No. GC ($\times 10^5$)	Rate (GC) ($\times 10^3$/day)	All Neurons	Rate (neurons) ($\times 10^3$/day)
1A LE	4		1.57		1.38		2.74		0.60		3.28	
1A RE	84		2.45	11.00	1.94	7.00	4.63	2.36	0.85	0.31	4.94	20.67
1D LE	4		2.10		1.90		3.36		0.87		4.42	
1D RE	168		2.68	3.54	2.01	0.67	6.03	1.63	0.995	0.08	5.39	5.93
1F LE	4		2.14		2.10		4.59		0.84		4.78	
1F RE	336		4.60	7.41	2.38	0.84	6.27	0.51	1.98	0.34	7.81	9.11
Mean rates		($\times 10^3$/day)		7.32		2.84		1.50		0.24		11.90

The fish (group IO-X) and the time post-injection at which the eye (left, LE; right, RE) was removed are given in the first column. The total number of each type of retinal cell and the rate of cell addition are given in the following columns, the total of all retinal cells in the last column, and the mean rates in the last row. INL, inner nuclear layer cells; GC, ganglion cells.

cates that a germinal zone persists and is active in the adult goldfish retina. In morphology this region is similar to the germinal zones described by Stone ('59) and Müller ('52) in the retinas of adult newts and guppies, respectively.

The radioautograms revealed that new retinal tissue was added appositionally in the goldfish, as it is in other species (Hollyfield, '68, '72; Straznicky and Gaze, '71; Jacobson, '76). This mode of growth has a specific effect on the spatial and topographical properties of the cells already present in the retina. The receptive field of a cell that in the small eye reported on information in the periphery of the visual field, would be gradually displaced centrally with growth. This means that the spatial information relayed to the brain by an individual ganglion cell changes with growth. How the brain accomplishes the required reinterpretation of the retinal input is not known.

Quantitative aspects of retinal cell addition. The slight asymmetry of growth, with more cells added dorsally and nasally, is both quantitatively and qualitatively different from that described by Hollyfield ('71) and Jacobson ('76) for larval *Xenopus,* in which many more cells are added ventrally than dorsally. No explanation for this difference is readily apparent, but the reasons for asymmetric growth may be different in the two species. In goldfish of the sizes studied here, the optic disk is displaced 5-11° in the ventrotemporal direction from the retinal axis (Easter et al., '77). If new retina were added symmetrically around the circumference, the optic disk would shift toward the retinal axis with growth. The amount of extra retina added to the dorsonasal quadrant (about 30% after 336 days) is approximately that required to maintain the position of the optic disk with respect to the retinal axis. In *Xenopus,* the density of cells is greater ventrally than dorsally, and the increased cell proliferation at the ventral pole may be associated with this fact.

The rate of ganglion cell addition in the adult goldfish retina is comparable to that found by Jacobson ('76) for larval and post-metamorphic *Xenopus.* Because only one size of goldfish was studied here, nothing can be inferred about changes in the rate with age, although both Müller ('52) and Lyall ('57a) found that the rate of cell addition decreased with age in other teleosts.

The hypertrophic component of retinal growth. In addition to providing evidence for a germinal zone in the adult goldfish retina, the radioautograms were useful for quantifying the hypertrophic component of the growth. The annulus of labeled cells provided a marker for the *ora* at the time of the injections and was used to estimate the size of the "old" retina after various intervals. Of course, each retina could be measured at only one time point, so conclusions are of necessity based on the assumption that all of the retinas were approximately the same size at the beginning. Since the fish were approximately the same length, their retinas were at least similar in size (Johns and Easter, '77: fig. 2). The area of the "old" retina more than doubled over 336 days, implying that the retina does indeed stretch as Weiss ('49), Coulombre ('55) and Ali ('64) suggested.

Both the radioautograms and the cell counts (Johns and Easter, '77) showed that the rods did not follow the same pattern as the other cells. The rods were the only ones whose density remained constant with growth, and they were the only cells with labeled nuclei not confined to the narrow annulus. Following is a discussion of several hypotheses which might explain the addition of rods to the central retina, and an evaluation of them in terms of their predictive value for the radioautographic results.

Central addition of rods—an evaluation of the hypotheses. There are four major ways in which rods might be added to the central retina.

(1) *Mitotic division throughout the retina*

The easiest way for the population of rods to increase would be by in situ mitotic division, as Hollyfield ('71) suggested occurs in the inner nuclear layer in larval *Xenopus* retina. To account for the differential addition of rods to the goldfish retina, such a mechanism would require that labeled cells be scattered *throughout* the layer of rods and persist during growth. This was not found in the radioautograms.

(2) *Transformation*

Rods might be added to central retina via transformation of other cells, and both the cones and the cells in the INL have been suggested as possible sources (Bernard, 1900; Lyall, '57a,b; Blaxter and Jones, '67). The de-

crease with growth in the relative proportions of INL cells and concomitant increase in the number of rods in both the goldfish (Johns and Easter, '77) and in the herring (Blaxter and Jones, '67) is consistent with this idea, but the numbers argue against their being the sole source of new rods, since not as many cells were lost from the INL as were added to the rod population in either species. Unfortunately, the radioautographic results have nothing to offer concerning this idea since changes taking place without an intervening cell division step cannot be detected with this method. We must therefore consider this hypothesis to be still tenable.

(3) Cell migration

New rods or their precursors might be born at the margins and migrate into the central regions. There is a precedent for this type of cell migration in the amphibian retina (Glücksmann, '40; Hollyfield, '68). The migration hypothesis predicts that labeled rods in the goldfish retina would move progressively from the margin to the center. This is inconsistent with the data since labeled rods were never found in the center of the retina. The cell migration hypothesis is therefore ruled out.

(4) Retinal shearing

The density of rods could be maintained if they resisted the retinal expansion occurring in the other layers. This hypothesis was first suggested by Müller ('52) based on his analysis of cell numbers in the growing larval and adult guppy retina. His results, including the increase in the proportion of rods, were qualitatively similar to ours for the goldfish (Johns and Easter, '77). The hypothesis predicts that all of the retinal cells would be added exclusively at the margin and differentiate in situ, but with time, as a result of the hypertrophic expansion of the rest of the retina, the labeled rods would be displaced en masse to a more central position relative to the other labeled cells. Although it might be argued that this is merely a modification of the cell migration hypothesis, it is conceptually different in at least two ways. The first difference concerns which cells are moving. Cell migration as it was presented above applies to individual rods or their precursors moving actively toward the center of the retina through a network of other cells and their processes. The shearing
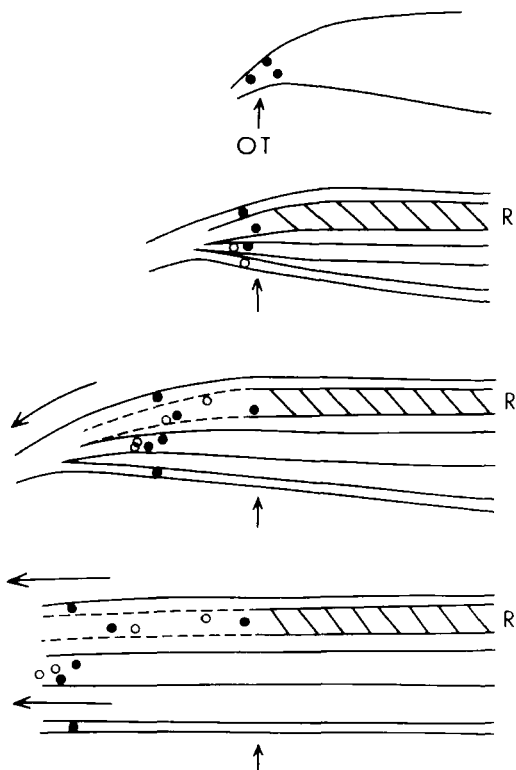

Fig. 8 Schematic of the modified shearing hypothesis. The upper diagram represents the radioautographic results at short intervals following injection of ³H-thymidine. Labeled cells (filled and open circles) are found only at the ora terminalis (OT). Later, these cells differentiate into retinal neurons which are at first together, but as the retina expands (large arrows) many of the labeled rods (R) are left behind while the other labeled cells move peripherally. The stable, central part of the rod nuclear layer is indicated by the diagonal hatching. The small arrows show the original position of the ora.

hypothesis, on the other hand, implies that it is the rest of the retinal cells which are moving peripherally in association with the retinal expansion, whereas the rods are the stable elements. The second difference lies in the intercellular relations among the rods. The migration hypothesis requires that nearest neighbors change, whereas the shearing hypothesis predicts that the rods adjacent to one another in the small eye will be the same as those in the large.

The shearing hypothesis predicts most closely what was actually found in the goldfish radioautograms, but requires a slight modification to be completely consistent with the data. If we imagine that the rods were not entirely

unaffected by the centrifugal force created by the retinal expansion, and that some were influenced more than others, then the spreading apart of the labeled rods is explained. If this is the correct interpretation, (1) none of the rods should have been peripheral to the labeled zone, which they were not, and (2) none should have been located farther centrally than the position of the *ora* at the time of labeling. This second condition is difficult to evaluate. The extent of the annulus of labeled rods could not be easily measured since they were so diffuse, and the former position of the *ora* could not be predicted exactly in any one retina. In all cases, however, there was a central region of retina that contained no labeled rods. This observation is consistent with the idea that the labeled rods were spread apart by their varying degrees of susceptibility to the shearing forces, and that no new rods were added to the retina central to the *ora.*

The modified shearing hypothesis allows for movement within the layer of rods, so that neighboring rods are not necessarily the same ones before and after growth. Although this negates one of the distinctions between migration and shearing, the other remains: the active components are the retinal cells participating in the expansion, and not the rods. Figure 8 is a schematic diagram showing the essential features of the modified shearing hypothesis.

In summary, rods are relatively more dense in the central retina of big fish *not* because rods have been added there, but because non-rod cells have left, pulled peripherally in the retinal expansion. The rods' resistance to this movement would lead to a shearing strain between the retinal layers resulting in a shift in the relative positions of the rods with respect to the other cells.

Implications of shearing between retinal layers. Although some of the rods are pulled peripherally by the retinal expansion, others remain behind, and the post-synaptic cells (bipolars and horizontals) adjacent to them and with which they form synapses may change during growth. If they were to maintain contacts with the same horizontal and bipolar cells, the rod axons would have to be pulled peripherally along with the post-synaptic cells, while their nuclei remained behind. The only examples of vertebrate photoreceptors with slanting axons are both cones: (1) the oblique or diagonal cones found in non-

mammalian retinas (Ramón y Cajal, 1892) and (2) the cones surrounding the fovea (Ramón y Cajal, '01-'11; Polyak, '57; Kolb, '70; Missotten, '74). In contrast, rod axons in the fish retina are short and radially oriented (Ramón y Cajal, 1892; Stell, '67; Scholes, '75; Stell and Hárosi, '76). Alternatively, if the rod axons were not pulled peripherally, the dendrites of the post-synaptic cells would have to be displaced centrally. This has not been reported in the fish retina, either. Both mechanisms, central elongation of INL cell dendrites or peripheral elongation of rod axons, lead to the prediction that rod-dominated receptive fields of ganglion cells ought to be displaced centrally with respect to the cone-dominated fields of the same cells. This has not been found; rather the two fields are roughly concentric (Raynauld, '72; Beauchamp and Daw, '72). For these reasons, the shearing hypothesis implies that the rods change synaptic partners throughout growth, forming contacts with bipolars and horizontals which the process of retinal hypertrophy brings near them, losing connections with other bipolars and horizontals which move peripherally. Although the present study has provided no direct evidence for synaptic promiscuity among retinal neurons in growing adult goldfish, such a process would best explain the radioautographical observations.

LITERATURE CITED

Ali, M. A. 1964 Stretching of the retina during growth of salmon *(Salmo salar)* Growth, *28:* 83-89.

Attardi, D. G., and R. W. Sperry 1963 Preferential selection of central pathways by regenerating optic fibers. Exp. Neurol., *7:* 46-64.

Beauchamp, R. D., and N. W. Daw 1972 Rod and cone input to single goldfish optic nerve fibers. Vision Res., *12:* 1201-1212.

Blaxter, J. H. S., and M. P. Jones 1967 The development of the retina and retinomotor response in the herring. J. Mar. Biol. Ass. U. K., *47:* 677-697.

Bernard, H. M. 1900 Studies in the retina: rods and cones in the frog and in some amphibia. Quart. J. Micros. Sci., *43:* 23-47.

Cohen, A. I. 1963 Vertebrate retinal cells and their organization. Biol. Rev., *38:* 427-459.

Coulombre, A. J. 1955 Correlations of structural and biochemical changes in the developing retina of the chick. Am. J. Anat., *96:* 153-189.

Coulombre, J. L., and A. J. Coulombre 1965 Regeneration of neural retina from the pigmented epithelium in the chick embryo. Devel. Biol., *12:* 79-92.

Denham, S. 1967 A cell proliferation study in the neural retina in the two-day rat. J. Embryol. Exp. Morph., *18:* 53-66.

Easter, S. S., P. R. Johns and L. Baumann 1977 Growth of the adult goldfish eye. I. Optics. Vision Res., *16:* 469-476.

Gaze, R. M., and W. E. Watson 1968 Cell division and migration in the brain after optic nerve lesion. In: Growth of the Nervous System. G. E. W. Wolstenholme and M. O'Connor, eds. Ciba Found. Symp., Churchill Ltd., London, pp. 53-67.

Glücksmann, A. 1940 Development and differentiation of the tadpole eye. Brit. J. Ophthal., *24:* 153-178.

Hollyfield, J. G. 1968 Differential addition of cells to the retina in *Rana pipiens* tadpoles. Devel. Biol., *18:* 163-179.
———— 1971 Differential growth of the neural retina in *Xenopus laevis* larvae. Devel. Biol., *24:* 264-286.
———— 1972 Histogenesis of the retina in the killifish *Fundulus heteroclitus.* J. Comp. Neur., *144:* 373-380.

Jacobson, M. 1958 Cessation of DNA synthesis in retinal ganglion cells correlated with the time of specification of their central connections. Devel. Biol., *17:* 219-232.
———— 1976 Histogenesis of retina in the clawed frog with implications for the pattern of development of retinotectal connections. Brain Res., *103:* 541-545.

Johns, P. R. 1976 Growth of the Adult Goldfish Retina. Ph.D. thesis, University of Michigan.

Johns, P. R., and S. S. Easter 1977 Growth of the adult goldfish eye. II. Increase in retinal cell number. J. Comp. Neur., *176:* 331-342.

Keefe, J. R. 1973a An analysis of urodelian retinal regeneration. I. Studies of the cellular source of retinal regeneration in *Notophthalmus viridescens* utilizing ³H-thymidine and colchicine. J. Exp. Zool., *184:* 185-206.
———— 1973b An analysis of urodelian retinal regeneration. IV. Studies of the cellular source of retinal regeneration in *Triturus cristatus carnifex* using ³H-thymidine. J. Exp. Zool., *184:* 239-258.

Kolb, H. 1970 Organization of the outer plexiform layer of the primate retina: electron microscopy of Golgi-impregnated cells. Bull. Biol. Sci., *258:* 261-283.

Kopriwa, R. M., and C. P. Leblond 1962 Improvements in the coating technique of radioautography. J. Histochem. Cytochem., *10:* 269-284.

LaVelle, A., and F. W. LaVelle 1970 Cytodifferentiation in the neuron. In: Developmental Neurobiology. W. A. Himwich, ed. Charles C. Thomas, Springfield, Illinois, pp. 117-164.

Levine, R. 1975 Regeneration of the retina in the adult newt, *Triturus cristatus,* following surgical division of the eye by a limbal incision. J. Exp. Zool., *192:* 363-380.

Lyall, A. H. 1957a The growth of the trout retina. Quart. J. Micros. Sci., *98:* 101-110.
———— 1957b Cone arrangements in teleost retinae. Quart. J. Micros. Sci., *98:* 189-201.

Mann, I. 1969 The Development of the Human Eye. Grune and Stratton, New York.

Müller, H. 1952 Bau und Wachstum der Netzhaut des Guppy *(Lebistes reticulatus).* Zool. Jb., *63:* 275-324.

Missotten, L. 1974 Estimation of the ratio of cones to neurons in the fovea of the human retina. Invest. Ophthal., *13:* 1045-1049.

Morris, V. B., C. L. Wylie and V. J. Miles 1976 The growth of the chick retina after hatching. Anat. Rec., *184:* 111-113.

Ramón y Cajal, S. 1892 The Structure of the Retina. S. A. Thorpe and M. Geidestein, translators. Charles C. Thomas, Springfield, Illinois, 1972.
———— 1909-1911 Histologie du Système Nerveux de l'Homme et des Vertèbres. L. Azoulay, translator. Reprinted by Institute Ramón y Cajal del C.S.I.C., Madrid, 1952-1955.

Raynaud, J.-P. 1972 Goldfish retina: sign of rod input in opponent ganglion cells. Sci., *177:* 84-85.

Schmidt, J. T. 1976 Expansion of the Half Retinal Projection to the Tectum of Goldfish. Ph.D. thesis, University of Michigan.

Scholes, J. H. 1975 Colour receptors and their synaptic connexions in the retina of a cyprinid fish. Phil. Trans. Roy. Soc. Lond., B *270:* 61-118.

Stell, W. K. 1967 The structure and relationships of horizontal cells and photoreceptor—bipolar synaptic complexes in goldfish retina. Am. J. Anat., *121:* 401-424.

Stell, W. K., and F. I. Hárosi 1976 Cone structure and visual pigment content in the retina of the goldfish. Vision Res., *16:* 647-657.

Sidman, R. L. 1960 Histogenesis of mouse retina studied with thymidine-³H. In: The Structure of the Eye. G. K. Smelser, ed. Acad. Press, New York, pp. 487-506.

Stone, L. S. 1959 Experiments testing the capacity of iris to regenerate neural retina in eyes of adult newts. J. Exp. Zool., *142:* 285-308.

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

Weiss, P. 1949 Differential growth. In: The Chemistry and Physiology of Growth. A. K. Parpart, ed. Princeton Univ. Press, New Jersey, pp. 135-186.