Postembryonic development of the visual system of Periplaneta americana

I. Patterns of growth and differentiation

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SUMMARY

The compound eyes of Periplaneta americana are connected by optic fibre tracts to an optic lobe composed of three sequential ganglia, the lamina, the medulla and the lobula respectively. The eyes and optic ganglia are organized into repeating sub-units arranged in a regular pattern. During postembryonic development, the number of subunits in the eye (ommatidia) increases from between 50 and 60 to over 2000, with a concomitant increase in the size of the optic lobe ganglia. The patterns of cell growth and proliferation were examined in serial section autoradiographs prepared following long and short exposures to [3H]thymidine during each developmental stage. Aspects of structural differentiation were examined in reduced silver-stained sections of nymphs at each developmental stage. Growth of the eye and optic ganglia resulted from the continuous proliferation of new cells throughout postembryonic development. Unlike other body tissues, growth of this system was independent of the moulting cycle. The pattern of growth observed in the optic ganglia directly reflected the growth of the eye. Growth of the compound eye occurs from a special zone of proliferation and differentiation located along all but its posterior margin. The lamina and medulla both grow by cell proliferation from a single neuroblast region located at the apex of the angle subtended by them. Cells which proliferate distally from this region differentiate into lamina neurons, while those that proliferate proximally differentiate into medulla neurons. Axons growing from these two adjacent regions meet at and add new new fibres to the distal end of the medulla neuropil. Specificity of the interneuronal connexions appears to result from a precise temporospatial sequencing of growth with the formation of the optic ganglia dependent on retinal development.

INTRODUCTION

A significant problem in neurobiology is understanding the principles which govern the establishment of specific connexions between differentiating neurons in the developing nervous system. Although the visual systems of certain vertebrate species have proved to be profitable objects of study for this problem,
equivalent systems of invertebrates, particularly arthropods, have received much less attention. This is in spite of their suitability as model systems for the study of neural integration in vision.

Detailed descriptions of the development of arthropod visual systems have been provided for crustaceans (Eloffson & Dahl, 1970; LoPresti, Macagno & Levinthal, 1973, 1974), holometabolic insects (Pflugfelder, 1937, Power, 1943; White, 1961; Nordlander & Edwards, 1968, 1969a; Meinertzhagen, 1972, 1973, 1975; Hanson, 1972; Hanson, Jiang & Lee, 1972), and hemimetabolous insects (Pflugfelder, 1947; Bodenstein, 1953; Panov, 1960; Edwards, 1969; Heller & Edwards, 1968; Wolbarsht, Wagner & Bodenstein, 1966; Horridge, 1968; Mouze, 1972, 1974; Shelton, Anderson & Eley, 1977; Anderson, 1978a). Studies in the latter group have been primarily restricted to the compound eye, and descriptions of area enlargement and the location of mitotic figures. A major distinction between the holometabolous insects is that in the former the adult visual system is produced in a cataclysmic event at metamorphosis, while in the latter this structure is formed gradually over a series of instars. A detailed description of the formation of the visual system in a hemimetabolous insect, such as the cockroach, would provide a system more readily accessible to experimental manipulation and therefore to the determination of controlling mechanisms.

In this paper we describe the postembryonic development of the visual system of the cockroach *Periplaneta americana*, by examining the pattern of cell proliferation and differentiation in the compound eye and ganglia of the optic lobe. The results of this study lead us to suggest that: (a) growth of the compound eye and ganglia of the optic lobe occurs asymmetrically, in concert, and in a precise temporospatial order; (b) the asymmetry of growth results in the formation of the optic chiasmata; (c) presumptive interneurons of the peripheral optic ganglia are produced in an orderly fashion leading to the establishment of specific interconnexions; and (d) the basic features of visual development in this hemimetabolous insect are essentially the same as those described for other arthropods. Possible modes of axonal guidance are also discussed.

**Materials and Methods**

Two strains of *Periplaneta americana*, maintained at 37 °C in a 12 h light/12 h dark cycle, were used in these experiments. One group was a white-eyed mutant which lacks the dense screening pigments of the ommatidium, but is otherwise normal. These animals were employed to obviate the difficulties of interpretation of autoradiographic experiments due to the similarity in size between the pigment granules and developed silver grains. Control experiments on the second strain of normal animals indicated that the two were similar in structure and developmental patterns. Postembryonic growth was examined in a population of 164 animals at various stages from hatching to adulthood.
Nymphs were obtained from 11 egg cases and raised with individuals from the same case. They were examined daily to detect molting and weekly to measure weight and length. These data permitted an assessment of growth rate, the relation between growth rate and molting, and the degree of synchronization of these parameters under experimental conditions.

**Autoradiography**

The location and fate of dividing cells in the developing visual system was examined with three types of autoradiographic experiments.

**Short-term labelling**

Five groups of animals, each from two or three egg cases hatched within a 6 h period, were isolated and raised as described above. Each group was used to examine a different instar. One or two animals from each group were removed daily, beginning one or two days before the expected moult to the stage of interest, and injected intra-abdominally with 10 μCi/g body weight of [3H]-thymidine (New England Nuclear) which had been diluted to 100 μCi/ml in sterile insect Ringer. They were sacrificed 24 h after injection and prepared for autoradiography (Type I). The stages examined in this manner were the 2nd to the 7th instars.

**Long-term labelling**

Experimental groups representing each of the 8 instars were obtained by collecting newly moulted animals (all moultng within an 8 h period) to a particular stage. Each group contained between 17 and 45 animals, and each animal received an injection of [3H]thymidine (10 μCi/g body weight) when collected and every two days thereafter until the next moult occurred. A portion of each group was immediately prepared for autoradiography (Type II) and the remainder were allowed to develop to the adult stage before being examined (Type III). Type II experiments produced continuous bands of label in which all nuclei had been marked. We take this to mean that a sufficient amount of [3H]thymidine was present during the intermoult period to label all cells produced.

**Autoradiography and reconstruction**

Animals to be prepared for autoradiography were decapitated, the head fixed in alcoholic Bouin's solution, embedded in paraffin, and serially sectioned at a thickness of 5–10 μm. The tissue was rehydrated and dipped while wet in Kodak NTB-3 photographic emulsion and stored in the dark over Drierite for 4–8 weeks at 4 °C. The emulsion was developed with Kodak D-19 and the sections were stained with haematin using a double bath technique (Searles, 1967). Serial reconstructions of this material were prepared by methods similar to that described by Stark & Searles (1973) and were compared to reconstructions.
of unlabelled material similarly prepared but stained with a modification of the Holmes-Blest reduced-silver technique (Weiss, 1972). Camera-lucida drawings were made of all intact heads to be used in reconstructions. Following sectioning this device was used to make drawings, of similar magnification, of each section. The relative positions of all regions of the optic lobe were oriented relative to the eye, whose reconstruction could then be compared to the drawings of the intact head to ensure proper orientation. Comparative reconstructions of different stages of development used superficial head structures (antenna, ocelli, compound eyes, etc.) in determining the relative position of the region in question (lamina or medulla) reducing the possibility of misalignment. In all, 374 animals were prepared for autoradiography and 145 animals for normal histology.

RESULTS

Figures 1C and 1D demonstrate the general morphology of the retina and optic lobe of *P. americana*. They represent histological sections of adult animals cut in perpendicular planes (see caption). Figures 1E and 1F compare these structures in the newly hatched nymph and the adult. During the interval between these two stages the number of retinal units (ommatidia) increases from about 50 to something over 2000. There is a concomitant increase in the size of the optic lobe. The experiments to be described examine the processes of cell proliferation contributing to this 40-fold increase in cell number.

The nymphs of *P. americana* undergo 8 moults during postembryonic development under our laboratory conditions. They approximately double in weight and length during each moult period even though the duration of this period varies with age (8–14 days for the first instar, 30–45 days for the last, and 20–22 days for instars 2–7). The regularity in the duration of the intermoult period suggests a synchrony which was indeed observed. Ninety percent of the animals hatched from a single egg case repeatedly moult within a 24 h period, and animals from several egg cases which hatched within 6 h continually moult within 48 h of each other. This synchrony permitted maintenance of colonies of animals at the same stage of development. A sexual difference was noted in the duration of the last instar, in that males moult to the adult stage 6–15 days later than females and are proportionately larger.

Fig. 1. Photomicrographs of the compound eye and optic lobes of *Periplaneta americana*. (A) Diagram illustrating the plane of a typical frontal section. (B) Diagram illustrating the plane of a typical cross-section. (C) Frontal section of an adult male. (D) Cross-section of an adult male. (E) Frontal section of a 1st instar nymph (3rd-day post-hatching). (F) Enlargement of the optic lobe region in Fig. 1C, which can be compared to that of structure in Fig. 1E to illustrate the extent of growth occurring during postembryonic development (note the difference in scales). Symbols, a, anterior; p, posterior; d, dorsal; v, ventral, E, eye (retina); Ot, optic tract; L, lamina; M, medulla; Lo, lobula. Magnifications: C, ×78; D, ×40; E, ×320; F, ×200.
Fig. 2. A series of frontal sections through the eye and optic lobe of *Periplaneta americana* for three 6th-instar nymphs which had been injected with [3H]thymidine and prepared for autoradiographs (Type I). The left column was photographed with dark-field illumination and the right column is the same view in transmitted light. (A) Injection on the fifth day of the instar (intermoult period 20 to 22 days) and sacrificed after 24 h. Left eye (B) Injected on the 18th day of the instar and sacrificed after 24 h. Right eye. (C) Injected every 2 days for the entire instar (10 injections in total). The animal was sacrificed upon moulting to the 7th instar. The label indicated all cells produced during the instar. Left eye. Symbols: e, eye (retina); l, lamina; m, medulla; lo, lobula.

**Short-term labelling (Type I)**

Results of this type of experiment suggest that there are three phases of cell division during all instars examined.

1. No cell division. No labelled cells were observed when [3H]thymidine was injected during the first 2 or 3 days of the moult period.
(2) Restricted cell division. When $[^3H]$thymidine was injected later in the moulting period than Phase I but before Phase III (below) labelled cells appeared only along the anterior, dorsal, and ventral margins of the compound eye; between the lamina and medulla cortices; between the base of the medulla and the posterior lobula regions; and along the anterior edge of the lobula region (Fig. 2A).

(3) Unrestricted cell division. Examination of cells labelled when $[^3H]$thymidine was injected during the last 3–6 days of the intermoult period revealed dividing nuclei in the areas described above as well as in the tracts connecting them, in the epidermis, muscles, and connective tissue (Fig. 2B).

No further patterns or sequences of cell division could be observed, so that there appears to be a continuous generative process acting throughout the intermoult period and not a sequence of temporally isolated events producing new cells in different regions of the visual system.

**Long-term labelling (Types II and III)**

Figure 2C illustrates a typical result of the Type II labelling experiment performed at any instar. Four distinct clusters of labelled cells appear in each of the four regions of the visual system described above. In the case where animals were injected with $[^3H]$thymidine during a particular instar and then examined as adults (Type III), the cells produced during that instar can be found in distinct bands of the retina and in the cortices of the lamina and medulla; (Figs. 3 and 4). Furthermore, there is an equivalent number of rows of ommatidia and lamina units labelled. This relationship was observed in all cases and was independent of the total number of labelled cells during a given instar. The lamina unit depicted in Fig. 3 appears to consist of a group of five cells in a stack located just distal to the lamina neuropil and along the bundles of entering retinular cell axons. A second consistent result was that labelled glial elements in the optic tract were located in a band between the labelled ommatidia in the eye and the labelled cells in the lamina.

The general growth pattern and fate of cells being produced during each instar was reconstructed from serial sections of both labelled and unlabelled material. Figure 4 is a composite diagram summarizing the results. The boundaries of the retina, lamina, and medulla shown for any instar represent average dimensions determined from at least ten reconstructions of that stage. The range in the dimensions between animals of the same stage is less than 3% of the difference between animals of consecutive stages. Figure 4 demonstrates a common asymmetry in the growth of the three regions. Serial reconstructions of the Type III group of animals demonstrated that the location of cells labelled during a given instar corresponds to the growth region for that instar (Fig. 4). This suggests that growth was mainly by addition of new cells along the periphery of each structure.
Fig. 3. A frontal section through the eye and lamina of an adult male *Periplaneta americana* that had been injected with \([^3]H\)-thymidine every 2 days during the 3rd instar (8 injections in total). The injections were terminated at the moult to the 4th instar and the animal was allowed to develop into an adult when it was fixed and prepared for autoradiography. (A) Dark-field illumination. (B) Same view as 3(A) in transmitted light. (C) Enlargement of the lamina region of 3(B) showing that the labelled portion of the lamina consists of three columns of five neurons each. Note that there are three labelled ommatidia (e) and three labelled columns of lamina neurons (1) with the labelled glial cells (g) located along the interconnecting fibre tracts.
Fig. 4. A summary of the growth of the compound eye and peripheral ganglia of the optic lobe in *Periplaneta americana* based on camera-lucida drawings and serial section reconstructions of at least ten animals at each stage. The growth of the retina of a male and female are depicted in the upper diagrams. Growth of the lamina is depicted in the middle diagram. The lamina is normally canoe shaped but has been flattened into a two-dimensional diagram here. To the right is an actual dorsal view and to the left is a transverse section in the plane of the dotted line. The lower diagram shows the growth of the medulla, first in a view of the distal end (toward the lamina) and then in an anterior view. The labelled portion of each region in Type II experiments when reconstructed corresponded to the growth rings seen in these drawings.

**Growth zones**

It appears, therefore, that the peripheral regions of the visual system, including the retina, lamina, and medulla, arise from two regions of dividing cells. The first is located in the retina, and the second between the lamina and medulla.
Fig. 5. Photomicrographs of the retinal growth zone in juvenile *Periplaneta americana*. (A) The retinal growth zone in the left retina of a 7th-instar nymph. (B) The same region in the right eye of a 5th-instar nymph. (C) A composite enlargement of the region shown in 5(A). (D) A composite enlargement of the retinal growth zone of a 2nd-instar nymph shown in low magnification in Fig. 5(E). (F and G) A section through the marginal growth zone of a 6th-instar nymph from a Type II labelling experiment. Note that all of the marginal retinal cells are covered by dense clusters of exposed silver grains while none of the cells in the adjacent epidermal cluster was labelled. Symbols: GZ growth zone; ec, epidermal cells.
Retinal growth zone

The zone of retinal growth and differentiation is easily recognized along all but the posterior margin of the eye. When viewed in frontal section (Fig. 5), there is a distinct progression which is evident in all of the specimens we have examined. There is always a thickened cluster of epidermal cells at the junction of the head epidermis and eye (Fig. 5). It is of significance to note that in none of the labelling experiments did any of the cells in this region show label (Fig. 5G, F). In the Type II labelling experiments these cell clusters were conspicuous in this regard when compared with adjacent epithelium and the growth zone of the compound eye. In the eye, and immediately adjacent to the cluster of epithelial cells, appear several unitary rounded cells. In some cases those located more centrally appear to be elongating toward the basement lamina (Figs. 5C, D). There is a progressive thickening of the retina, indicated by the increasing distance from the cornea to the basement lamina, as it extends inward from the margin of the eye. As the retina begins to thicken these cells appear to aggregate and establish separate columns of increasing length and ommatidial differentiation. The number of these differentiating columns increased from two or three in the early nymphal stages up to five in the last instar. Fibre bundles were observed emerging from even the earliest groups of cells and progressing towards the basement lamina. The differentiating clusters give rise to fully differentiated ommatidia which continue to grow, by cell enlargement, throughout the remainder of postembryonic development. This results in a size gradient with the oldest and largest elements being located along the posterior margin of the eye.

Short-term labelling (Type I) experiments indicated that only cells in the first one or two differentiating clusters and the single cells on the margin incorporated thymidine until just prior to the moult. In the period immediately preceding the moult, no additional eye regions incorporated the label, but the cells of the marginal cluster and epidermis did incorporate the label. In the long term labelling experiments (Type II), all of the marginal cells were labelled, as were a few lens and accessory pigments cells at the distal ends of the ommatidia immediately adjacent to the fully labelled marginal groups (Fig. 2C). It appears that cell division ceases first in the proximal cells, namely the prospective photoreceptors, and later in the distal components which will form the lens and accessory structures. Axonal fibres can be observed emerging from the differentiating ommatidia while cells are still incorporating thymidine and therefore dividing. The degree of resolution of these preparations does not allow us to determine if the fibres actually came from the labelled cells, nor does it allow us to rule out this possibility. Our data do not permit a precise description of the sequence of ommatidial differentiation or of cell lineages.
Growth of the outer optic anlage

The zone of cell proliferation leading to the formation of the lamina and medulla regions of the optic lobe is illustrated in Fig. 6. Figures 6a–c depict the finer detail of the area of cell proliferation. Figure 6B is a reconstruction of the zone shown in Fig. 6A. The reconstruction compensates for differences in planes of focus and section at the high magnification required to visualize this area. This region consists of a large neuroblast (NB) cell, double columns of ganglion mother cells (GMC), and groups of small differentiating neurons located between the GMC and the medulla cortex. Mitotic figures were often observed in the neuroblasts (Fig. 6C) and among the ganglion mother cells (Figs. 6A, B). The location of the [3H]thymidine-labelled cells in the short-term (Type 1) experiments was always restricted to this region (except in the immediate premoult period, when glial cells throughout the visual system incorporated the label). We never observed more than one neuroblast in association with each pair of GMC columns. When mitotic figures were present in the neuroblasts: (1) the axis of the spindle was oriented perpendicular to the axis of the GMC columns (Fig. 6C) and the division was equal, or (2) the axis of the spindle was oriented towards one of the columns and division appeared unequal. The spindles of the dividing GMC were always perpendicular to the column axes and equal (Fig. 6A–C). Each neuroblast, therefore, appears to divide to produce two columns of ganglion mother cells, one of which divides to produce prospective lamina neurons and the other to produce prospective medulla cells.

Ganglionic differentiation and fibre paths in this region are best visualized in nymphs of the first few instars because of the spatial proximity of the retina and optic lobe (Fig. 6D) as well as the small number of elements present. Although these features are best illustrated in the early stages, they can be observed in all stages of development except adults, which lack the growth zone. Figures 6D–F show fibre tracts in a 1st instar nymph for a case where a fortuitous sectioning artifact has partially separated the optic lobe from the overlying tissues without destroying the axonal connexions. This can be seen at low magnification in 6D and the region of laminar growth is enlarged in 6E.
Fig. 7. A diagrammatic section through the head of *Periplaneta americana* at approximately the level indicated in Fig. 1A, representing the pattern of growth of the compound eye and the optic lobe ganglia. The arrows indicate the direction of growth by the addition of new cells. Symbols: Br, brain; CE, compound eye; FT, fibre tracts; LC, lamina cortex; LN, lamina neuropil; LOC, lobula cortex; LON, lobula neuropil; MC, medulla cortex; MN, medulla neuropil; O, ommatidia; OT, optic tract; regions with single hatching represent the growth zones of the eye, lamina, medulla, and lobula; the lamina-medulla proliferation centre is represented by the darkened area between the respective growth zones; lamina neurons are depicted with filled somata while medulla neurons are open. The hatched line depicts the fibre path for the lamina cell in the older established region of the ganglion. It is interrupted at the growth region for clarity.

Fibres entering the lamina growth zone appear to branch from the anterior edge of the optic tract, pass over an established lamina unit, and enter the area of differentiating lamina neurons. This is shown diagrammatically in Fig. 6F. This sketch is a reconstruction of a series of sections that includes the one depicted in 6D and E. It reveals a sequence of distinguishable cell morphologies spanning the region from the neuroblast to the zone of contact with
retinular cell fibres. Presumably this represents a temporal developmental sequence as well.

Cells immediately adjacent to the neuroblast are small and have darkly staining nuclei, while those further removed assume an oblate shape becoming distinctly elliptical at the point of fibre entry. In the final stage of the sequence the neurons appear to round up and migrate out along the tract of incoming fibres. Such cells are still distinguishable from established lamina elements in that their nuclei are more darkly stained. In the medulla the newly produced ganglion cells do not appear to differ morphologically from the mature ganglion cells. As they move away from the growth zone they tend to form columnar aggregates and develop a central axonal bundle which enters the medulla neuropil. Necrotic cells are frequently observed in the region of the newly formed medulla cortex (Figs. 6A, B). No necrotic cells were observed in the lamina cortex. The reconstructions also show fibres emerging from the regions of presumptive lamina and medulla cortex. These join and travel across the distal margin of the medulla neuropil (Fig. 6F) where they presumably form functional synapses.

The regions of actively dividing cells found in the proximal visual system, in the region of the lobula, have not been investigated in detail. It is worthy of note that this proliferation of neural elements during postembryonic development was almost exclusively restricted to the visual system. The only other regions of the central nervous system showing [3H]thymidine label during these experiments were corpora pedunculata in the protocerebrum. The significance of the extensive cell proliferation in this region is not clear. There is a large increase in the number of antennal segments and corresponding sensory afferents during postembryonic development, which could possibly result in this proliferation in the corpora. It appears that most components of the adult nervous system have differentiated at the time of hatching and that production and specification of additional neural elements is restricted to the visual system and the corpora pedunculata.

**DISCUSSION**

In these studies we have attempted to describe the dynamics of neuron proliferation and formation of neural structure in the peripheral visual system of a hemimetabolous insect. Figure 7 provides a diagrammatic summary of our interpretation of the results. It depicts four areas of cell proliferation in each compound eye system, and the direction of growth (by cell addition) for the eye and the two peripheral ganglia of the optic lobe. Examples of neural structures produced at different times during the developmental process are provided.
Asymmetrical growth and chiasma formation

Figures 4 and 7 illustrate the asymmetric growth of the compound eye and related optic ganglia from a fixed posterior margin. This results in an apparent movement of the L–M zone from posterior to anterior during postembryonic development (arrows of Fig. 7). As it moves, it lays down neurons of the lamina cortex which in turn contribute to the lamina neuropil by forming synapses with incoming receptor axons and presumably with centrifugal fibres from deeper centres. The effect of this morphogenetic process is that the oldest lamina neurons lie at the extreme posterior edge of the ganglion while the youngest lie at the other extreme.

A second consequence of this process is that the cell somata of the medulla cortex are pushed anteriorly and proximally such that the oldest neurons lie deeper in the visual system adjacent to the lobula. The newest elements of this ganglionic region are being produced distally. This means that neurons of the lamina and medulla, which were produced early in development and in close spatial proximity to each other, come to occupy extreme positions in the adult optic lobe. It also means that during development the axons of newly formed lamina neurons must cross older-established fibres to enter the newly forming medulla neuropil. The result is a chiasma in the anteroposterior plane of the visual system. Such a structure is therefore a consequence of the asymmetrical and temporal growth pattern of the eye and peripheral visual system. Since these structures grow symmetrically in the dorsoventral plane, a chiasma does not occur. A chiasma is observed in the anteroposterior plane of the fibre tract connecting the medulla and lobula. A similar process of asymmetric growth resulting in a medulla–lobula chiasma has been observed in other arthropods (Eloffson & Dahl, 1970; Meinertzhagen, 1973, 1975; Mouze, 1972; Anderson, 1978a). Our study did not provide any details, but a similar process could be involved as well.

The developmental sequence we have described is similar to that described for other arthropods, with the exception that the growth zones for the eye and peripheral ganglia are fused in crustacea (Eloffson & Dahl, 1970). Hyde (1972) has indicated that the compound eyes of *P. americana* grow symmetrically, with new ommatidia added to all points on the margin. However, this description was primarily based on external observations of eye growth. Our labelling data clearly indicated that this is not the case and that no new cells were produced along the posterior margin of the eyes. In all other experiments described the eye grows asymmetrically from a fixed posterior margin. One possible exception is in *Daphnia* (LoPresti et al. 1973) where a single eye grows symmetrically around a median group of cells. Even this case would fit the generalization if the single eye represented a phylogenetic fusion of paired eyes at their posterior margins. Other variations include elimination of the first chiasma in some crustacea by forming medulla neuropil on the proximal rather
than distal edge of the ganglion (Eloffson & Dahl, 1970), and species- or group-
specific rotational rearrangement of the medulla (Eloffson & Dahl, 1970;
Nordlander & Edwards, 1969a,b; Edwards, 1969; Meinertzhagen, 1973; Hanson,
1972; Mouze, 1972). This does not appear to occur in Periplaneta.

We found the process of cell division and differentiation in the visual system
to be rather independent of the particular phase of the moulting cycle in con-
trast to such activity in other tissues. Similar relationships have been observed
in Danius (Nordlander & Edwards, 1969a, b), Drosophila (Power, 1952),
Gryllus (Panov, 1962), the Antheraea (Panov, 1963), the Aeschnidae (Mouze,
1972) and Schistocerca (Anderson, 1978a).

Retinal development and axonal guidance

The compound eye develops through the addition of new ommatidial units
along all but the posterior margin. There is a progressive sequence of ommatidial
differentiation extending medially from the margin. Differentiation is initiated
from a pool of actively dividing marginal cells which begin to form cellular
aggregates prior to the cessation of cell division. During the initial phases of
retinal differentiation we have repeatedly observed thin filamentous processes
extending from the early cell aggregates to the basement lamina and in many
cases from single cells located near by (Fig. 5). While our techniques do not
allow a more detailed description of these processes, or their fate, we point
out that their location relative to the clustered cells is similar to that occupied
by developing axons relative to nearby differentiating ommatidia. These proces-
ses may represent early stages of retinular cell differentiation and axon growth.
The separation of the aggregates into ommatidial bundles is first observed in
the proximal portions (photoreceptor portion) and then later in the distal
portion (lens and pigment cells). Evidence from experiments on Periplaneta
(Shelton et al. 1977) and Drosophila (Ready, Hanson & Benzer, 1976) and
Schistocerca (Anderson, 1978a) indicate that the ommatidia develop from a
random aggregation of undifferentiated cells rather than from a group of
clonally derived cells, and that differentiation of specific components results
from their position in the aggregate. The results of the present study suggest
that photoreceptor differentiation may be initiated during the aggregation phase
and prior to the cessation of cell division. Shelton and coworkers (1977) in their
studies on Periplaneta demonstrated that the prospective retinal cells could
be derived from the adjacent head epidermis (prospective eye tissue) only. The
cluster of epidermal cells located at the margin of the eye (see Fig. 5) would
at first be a likely candidate for such a population of progenitor cells. However,
the observation that these cells do not actively divide would argue against this
hypothesis, unless these cells were to migrate into the eye margin and then
resume division. Such a sequence of events would be expected to gradually
deplete the epithelial pool, but a decrease in the size of this marginal epithelium
with age was not observed. Therefore we must conclude that new eye elements
are constantly being generated from a progenitor stem cell line located along the dorsal, anterior, and ventral margins of the eye. While it was not possible for us to follow cell lineages, the description of retinal growth and differentiation presented here does not conflict with the concept of random aggregation and provides no evidence to support a clonal process in the formation of the ommatidia.

The axons of new receptors would appear to be led to the region of differentiating lamina neurons by the optic tract formed by older and established retina–lamina connexions. The temporal sequence of formation coupled with some form of ‘tracking’ or contact guidance would adequately account for the orderly array of these projections. Such a tracking mechanism has been demonstrated in *Daphnia* (LoPresti et al. 1973) and in *Schistocerca* (Anderson, 1978b) in guiding retinal axons to their laminar connexions. Similar considerations would provide an attractive explanation for the orderly projection of newly produced medulla units into the forming neuropil of that ganglion. Similarly, the temporal and spatial proximity of the differentiating lamina and medulla units suggest a mechanism by which emerging lamina and medulla axons could recognize each other and establish orderly lamina–medulla connexions. It would also explain how the deep retinal fibres, which also terminate in the medulla, could continue to grow with differentiating optic lobe neurons if their processes arrived in the lamina at the appropriate time. This might allow them to establish their appropriate connexions in the medulla neuropil. The temporospatial sequencing of development provides an attractive mechanism for establishing the highly specific organization typical of these regions of the arthropod visual system.

**Lamina–medulla growth zone**

The lamina–medulla growth zone is visualized as having a central proliferative area which consists of a large neuroblast cell at the end of two adjacent columns of cells; one column will eventually give rise to neurons of the lamina (2nd order cells) and the other will give rise to the medulla neurons (2nd or 3rd order cells). One striking feature is the orderly appearance of cells in this proliferative zone (Fig. 6). Our description of this region is limited by the resolution of our techniques; however, our observations suggest that neuroblasts each produce two columns of ganglion mother cells which in turn generate the lamina and medulla ganglion cells. A similar organization has been observed in a number of other insects (Panov, 1962; Nordlander & Edwards, 1969a, b; Mouze, 1972; Meinertzhagen, 1973, 1975; LoPresti et al. 1973; Anderson, 1978a).

After being produced from the GMC, the lamina ganglion cells undergo a temporal and spatial series of morphological changes before becoming mature neurons in contact with incoming receptor axons (see Fig. 6 D–F). A detailed description of lamina differentiation, resulting from electron microscopic studies, has been reported for *Daphnia* (Lopresti et al. 1973) and the fly (Hanson,
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1972; Meinertzhagen, 1973, 1975; Trujillo-Cenoz & Melamed, 1973). These studies showed that as the new retinal cells grow into the pool of prospective lamina neurons the first cell contacted begins to grow out along the bundle of retinal fibres, forms an axon, and differentiates into a lamina neuron. This process is repeated until there is a stack of five lamina neurons located along the bundle. The order of contact and therefore the position in the stack appears to determine the type of cell produced and therefore its connexions. Once formed, the neurons then collapse toward the base of the retinular bundle, losing their stacked arrangement. We believe that a similar series of events occurs in the cockroach. In Fig. 6D–F, differentiating neurons appear to be migrating out from the pool of lamina ganglion cells along the incoming retinal fibres. Eventually five lamina neurons will form along the retinal bundles and contribute to the lamina unit or cartridge. It is of interest to note that in all of these organisms five lamina neurons are generated. The only difference is that in the cockroach they remain stacked along the retinal bundle while in the others they do not. In none of the previous studies has the fate of the cells which do not form lamina neurons been discussed. In the long-term labelling experiments (Type III), labelled glial cells were always restricted to the fibre tracts connecting the retinal and laminal cells produced during the same period. These results suggest that the cells which do not form lamina neurons may migrate out along the fibre tracts and form glial elements. No necrotic cells were observed in the lamina but were frequently found in the distal portion of the medulla cortex, adjacent to the growth zone (Fig. 6A, B). This would indicate that the excess cells in the medulla may degenerate, while in the lamina they contribute to the glial cell population.

We believe that invasion of the region of the lamina ganglion cells by retinular cell axons is necessary for the process of lamina differentiation to occur. The evidence is at best indirect, but such a notion is supported by the fact that these fibres normally arrive prior to differentiation and manipulations which prevent these axons from reaching the growth regions (Stark, unpublished observations; Mouze, 1974; Anderson, 1978a) result in cessation of lamina growth.

The great similarity between the results presented here and descriptions of similar processes in other arthropods indicates that the postembryonic development of the cockroach visual system is essentially the same. The most significant difference is that in this case the process occurs gradually over a period of months rather than in a single metamorphic event. It is of interest to note that several features of the developmental pattern observed in *P. americana*, for example the five stacked lamina neurons and the unrotated medulla, appear in other arthropods as transient structures that are then modified later in development. This would suggest that this insect could represent a primitive and therefore model system for the study of arthropod development. The visual system of *P. americana*, being readily accessible to experimental perturbation during development, provides an ideal example for studying the nature
of those interactions essential for the proper formation of the arthropod visual system.

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