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Effect of a Juvenoid on the Development of Compound Eyes of *Spodoptera litura* Fabr.

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An examination of the compound eyes of the different kinds of pupal-adult intermediates, produced by treating pupae of *Spodoptera litura* with a juvenoid 6, 7-epoxy-3-ethyl-1 (p-ethyl phenoxy)-7 methylnonane reveals that the facets and internal differentiation of ommatidial structures are variously inhibited in different regions. In the heavily pigmented region, cuticle is thick and densely laden with pigment and the degree of inhibition of differentiation of various components is proportionate to the amount of pigmentation. The treatments affect the division and differentiation of retinular cells, primary iris cells, secondary iris cells, crystalline cone cells and also the secretion of crystalline cone, depending upon the stage of treatment. The differentiation of lamina and nerve supply is also severely affected.

Key Words: *Spodoptera litura* **Juvenoid effect, Compound eye development**

Introduction

While studying the effects of treating insect pupae with juvenoids, several workers have mentioned externally visible effects on the development of compound eyes (Bhaskaran 1972 in *Sarcophaga bullata;* Riddiford & Ajami 1973 in *Manduca sexta;* Metwally & Sehnal 1973 in *Trogoderma granarium* and *Caryedon gonagra*). In almost all these studies, the only parameter for eye development taken into account was pigmentation, and the degree of pigmentation has been regarded to indicate the degree of development of the eyes. In *Sarcophaga bullata,* however, Srivastava and Gilbert (1969), for the first time, observed in sections through the head region of the pupal-adult intermediates, that while the brain had well developed optic lobes, there was no trace of compound eye development. In the eye of deuteropupae of polyphemus, Willis (1969) noted differentiation of comparatively smaller eyes, each bordered by a 'growth zone' and having abnormal differentiation of individual elements of the ommatidium. Since development of different components of the compound eyes depends upon the division and differentiation of hypodermal cells which are known to be affected by juvenoid administration and also since it has been shown that juvenoid treatment does not always suppress pigment formation, but sometimes actually promotes it (Willis 1969, Abdallah et al. 1974, Gawaad et al. 1974 and Shukla 1980), it was considered worthwhile to study in some detail the effect of juvenoid treatment on the development of the compound eyes. The present paper describes this in the lepidopteran *Spodoptera litura*.

Materials and Methods

The juvenoid used was 6,7-epoxy-3 ethyl-1 (p-ethyl/phenoxy)-7 methylnonane, cis/trans mixture (RO–10–3108/018, R Maag Ltd.).

S. litura has a normal pupal period of 6–7 days at 28 ± 2°C. 2, 14, 20, 26 and 38 hr old pupae of *S. litura* were selected from the laboratory stock, reared under controlled conditions, and treated with 2, 5, 10, 15, and 25 μg of the juvenoid dissolved in acetone with the help of a microapplicator. One of the effects of juvenoid treatment was production of pupal-adult intermediates with varying degrees of pupal or adult characters. On the basis of the combinations of these characters, the intermediates were divided into the following grades:

Grade I: Adultoid with bifurcated proboscis and unstretched wings; external genitalia developed but deformed. In males, socii and gnathos not developed; in females, substitutional ovipositor fused. Eye pigmentation resembling that of the normal compound eye.

Grade II: Anterior abdomen pupal; wings developed but unstretched; proboscis bifurcated. In males, tegumen, uncus, gnathos and socii not developed while the remaining parts are deformed; in females substitutional ovipositor fused and reduced. Eyes showing a strip-like, heavily pigmented zone towards the proboscis, the rest of the eye remaining weakly pigmented. Ratio of pigmented and weakly pigmented zones is about 1 : 6.

Grade III: Anterior and middle abdomen pupal; wings developed but unstretched; proboscis bifurcated. External genitalia, eye pigmentation and ratio between heavily pigmented and weakly pigmented zones similar to those of Grade II.

Grade IV: Anterior and middle abdomen pupal; wings fused and pigmented; proboscis bifurcated. External genitalia, eye pigmentation and ratio between heavily pigmented and weakly pigmented zones similar to those of category II.

Grade V: Abdomen pupal except the tip which is adult-like; wings non-pigmented; proboscis bifurcated. Male genitalia very small and deformed. Tegumen, uncus, gnathos and socci not differentiated. Substitutional ovipositor absent. Eye pigmentation similar to that of Grade II, but the pigmented area is relatively larger. Ratio between heavily pigmented and weakly pigmented zones about 1 : 2.

Grade VI: Whole body pupal. Each eye can be differentiated into a strip-like pigmented zone lying between two unpigmented zones. Ratio of heavily pigmented and unpigmented areas about 1 : 2.

For histological examination, specimens of different categories were fixed in aqueous Bouin's fluid, dehydrated in butanol, embeded, sectioned and stained with haematoxylin and eosin.

Observations

Normal Development

External appearance and normal development of compound eye

The normal compound eye of adult *S. litura* is uniformly dark brown in colour. Ommatidial facets are of nearly equal size and evenly distributed throughout. The number of facets is about 3330/mm^2.

In the 36 hr old pupa, the eyes appear unpigmented externally and the facets cannot be differentiated from outside. In a vertical section (figures 1, 5, 9) clusters of undifferentiated hypodermal cells are observed immediately beneath the cuticular layer. Each cluster is destined to give rise to an ommatidium. The basement membrane lies below and closely attached to these clusters.

At this stage groups of compactly arranged nerve cells are noted internally to the basement membrane.

In the 60 hr old pupa, the eyes appear weakly pigmented but externally the facets still cannot be distinguished. However, in sections (figures 2, 6, 10) four groups of cells are distinguishable in the region of each ommatidium, viz. (1) A group of crystalline cone cells, which secrete the crystalline cone. Indeed, the developing crystalline cone can be seen at this stage; (2) Primary iris cells surrounding the developing crystalline cone; (3) A group of secondary iris cells surrounding the primary iris cells; and (4) A group of retinular cells which continue into an axon or the postretinal fibre each. At this stage, each ommatidium bulges out somewhat superficially. The compactly arranged groups of nerve cells below the basement membrane, seen in the 36 hr old pupal stage, now take the form of an oval group of nerve cells, one below each developing ommatidium.

In a 4-day old pupa, in comparison to adult, externally the eyes are still weakly pigmented. In sections (figures 3, 7, 11), the different parts of the ommatidium are found further differentiated. The corneal lens and crystalline cone have now been formed. The retinula is differentiated and nuclei of the retinular cells are clearly observed in the swollen part lying below the crystalline cone. The secondary iris cells have proliferated and these surround the primary iris cells and retinula, and are full of pigments. At this stage, the post-retinal fibres are formed from certain nerve cells, the cell bodies of some of which can be seen among the axons.

In a 5-day old pupa, the eyes are fully pigmented externally and the development of ommatidia is almost complete (figures 4, 8, 12). The retinula is further differentiated and 5–7 groups of retinular cell nuclei are found located in the middle due to which this area is swollen. The secondary iris cells are further proliferated and a few nerve fibres are also supplied to the secondary iris cells. In adult, the ommatidia are compactly arranged.

Differentiation of optic lobe and nerve supply

In the 36 hr old pupa (figures 1, 31), three optic centres, viz., medulla externa, medulla interna and lamina are found differentiated in each optic lobe. Fibres from the medulla externa and medulla interna cross each other on their way to lamina to form the chiasma externum. The lamina is in the form of a thin and curved medullary band, the outer side of which is surrounded by several layer thick cortical cells. The lamina remains separated from the neuropile by a group of cortical cells, the imaginal cortex of lamina. The internal surface of the medullary band has groups of numerous fibres which originate from the neurons or cortical cells of the imaginal cortex of the lamina. These fibres run through the medullary band and continue up to the compactly arranged nerve cells, lying below the basement membrane. Groups of glial cells scattered in between the nerve fibres and more frequently near the compactly arranged nerve cells. Several groups of nerve cells are observed among the nerve fibres.

In the 60 hr old pupa, nerve fibres from each optic lobe run into the post-retinal fibres of its side through the serially arranged oval groups of nerve cells lying below the basement membrane. Among the nerve fibres groups of nerve cells are also observed.

In the 4 and 5 day old pupa, groups of glial cells are relatively fewer in number, while in adults these are no longer observed.

Effect of JHA Treatment

Effect on the development of compound eye

External differentiation: Externally the compound eyes of the pupal-adult intermediates show three abnormalities in varying degrees : (i) In the whole or part of the eye, ommatidial facets cannot be differentiated, (ii) part(s) of the eye may show abnormally heavy pigmentation while the rest does not; (iii) The eye may be smaller than the normal compound eye.

On the basis of these characters, the JHA-affected eyes may be grossly classified into the following four types:

Type I: The whole eye surface is smooth and facets are not externally differentiated. The eye is differentiated into a vertical strip-like, about 0.39mm wide, pigmented zone, lying between two unpigmented zones. The ratio between the pigmented zones is about 1 : 2 (figure 13).

Such eyes were produced by treating 2 hr old pupae with 15 and 25μg of the compound.

Type II: The facets can be differentiated externally in a part of the eye which shows weak pigmentation and is situated towards the proboscis. The ratio between the pigmented and weakly pigmented zones is about 1 : 2 (figure 14). In the part(s) in which the facets have differentiated, they are similar to those of the normal adult eye in size and density.

Such eyes also resulted from the treatment of 2 hrs old pupae but with relatively smaller quantities, viz., 2, 5 and 10μg of the compound.

Type III: It resembles the eye of the second type, except that the pigmented area is relatively smaller and the ratio between the pigmented and weakly pigmented zones is about 1 : 6 (figure 15). Facets are externally well differentiated in the weakly pigmented area and their density and size are similar to those of the normal adult eye.

This type is produced by treating relatively older (14–26 hr old) pupae with any of the JHA doses tried (2–25 μg).

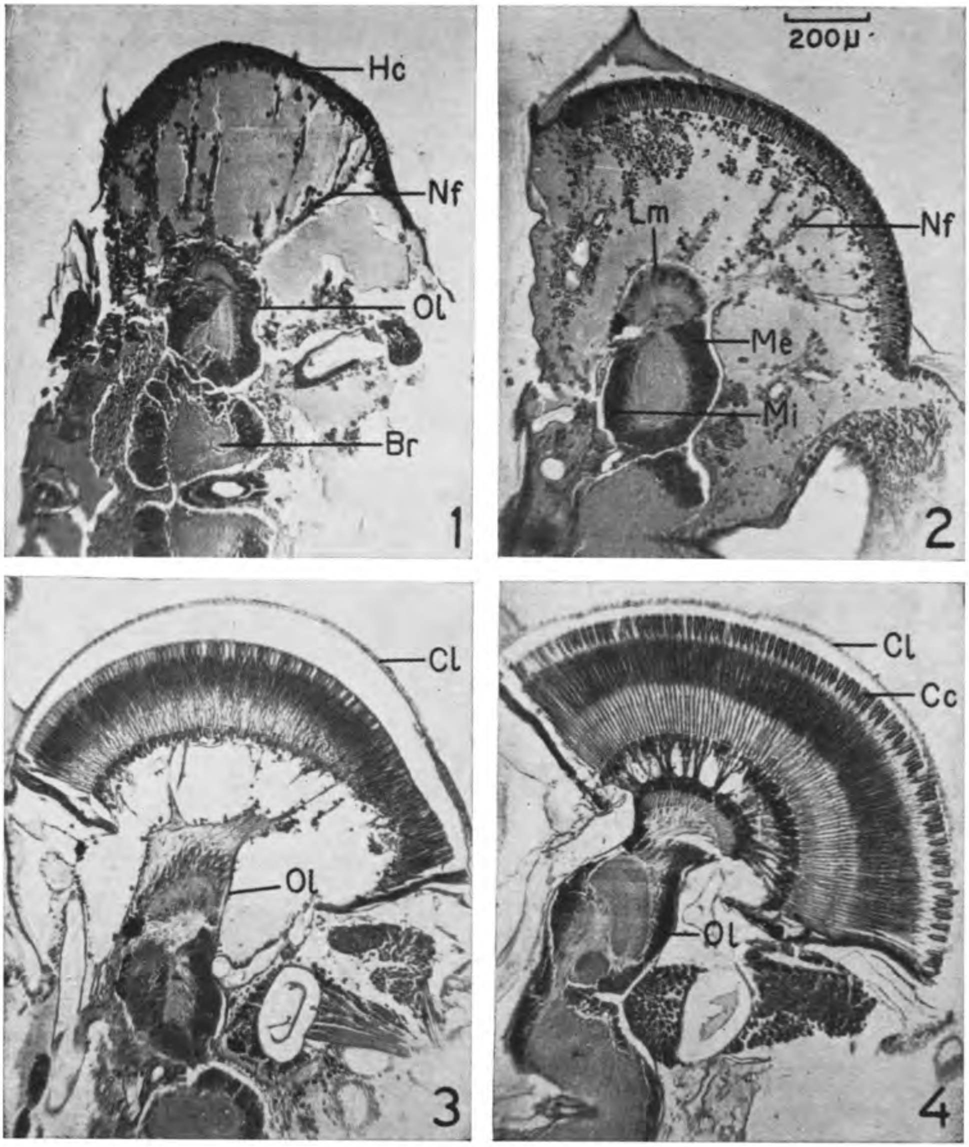
Type IV: Externally there is no effect on the compound eyes. The size and density of facets and pigmentation resemble those of the normal adult compound eye (figure 16).

These are produced by treating still older (38 hr old) pupae with any of the doses (2–25μg) of the compound tried.

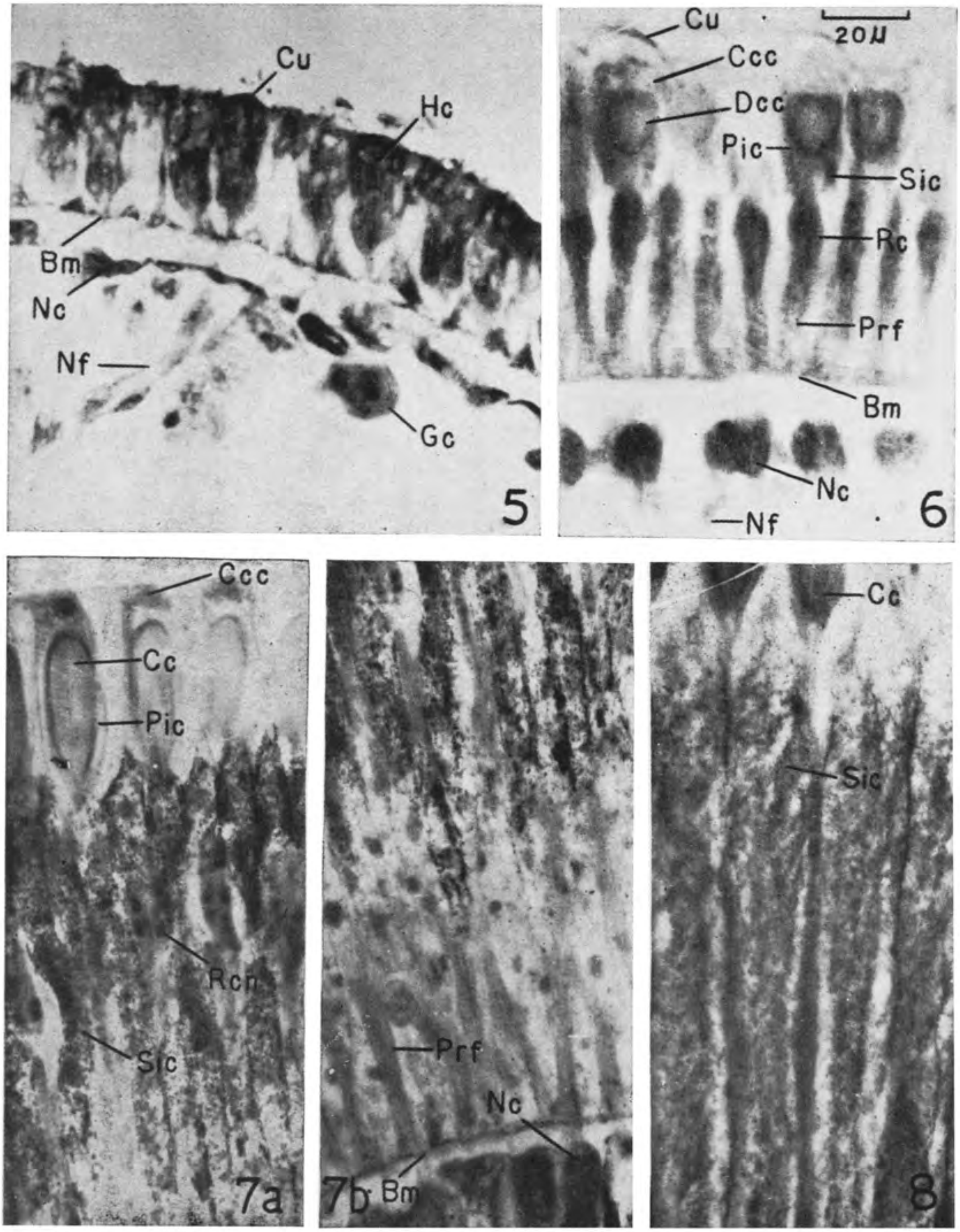
Histological study of the treated eye (table 1) : Vertical sections of the aforesaid different types of eyes reveal the effect of the juvenoid treatment on the differentiation of ommatidial components.

Type I: The nature of differentiation in the pigmented and unpigmented zones is conspicuously different (figure 17). In the pigmented zone (figures 17, 22, 27), the cuticular layer is thick with the pigment deposited in the epicuticular region. Below the cuticular layer, clusters of undifferentiated hypodermal cells are observed. Basement membrane is not clearly seen. Other structures are not developed. In contrast, in the unpigmented zone (figures 21, 26), the cuticular layer is thin, clear and without pigment. The cuticle is followed by clusters of hypodermal cells which have become differentiated into crystalline cone cells and retinal cells. Basement membrane is clearly seen.

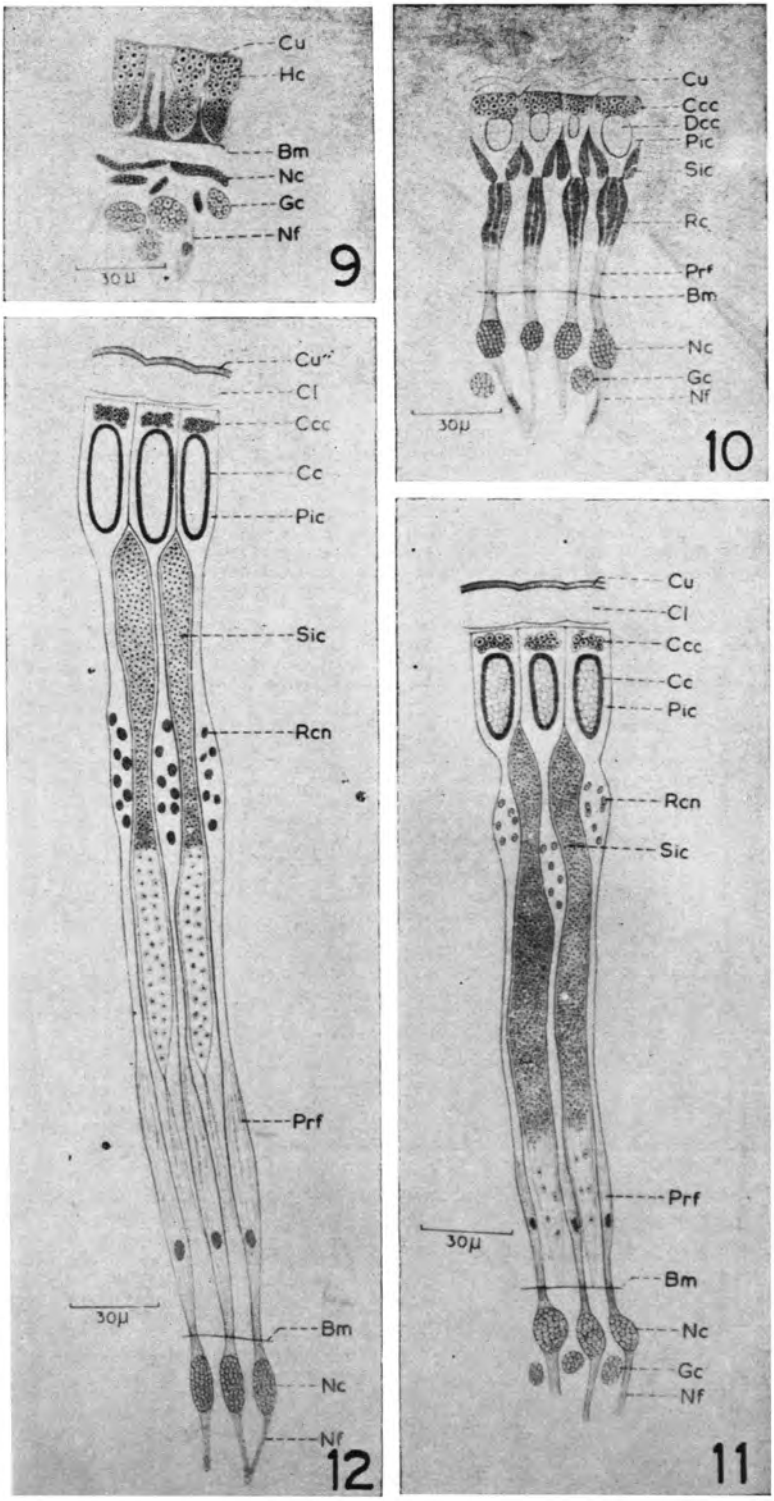
Types II & III: The histological structure of the eyes of types II and III is generally similar (figures 18, 23, 29). In the pigmented zone the circular layer is thick, with the epicuticle pigmented but less densely than in type I. In type III, the pigmented zone is comparatively smaller than in types I and II. The hypodermis shows clusters of undifferentiated cells which have not undergone further differentiation. Basement membrane is distinct. In the weakly pigmented zone (figures 24, 28), the cuticle is thin and completely free of pigments which are deposited under the influence of the juvenoid in the strongly pigmented zone, and it cannot be distinguished from the corneal layer. The mildly pigmented external appearance is due to the deposition of pigment in the iris zone but this is poor



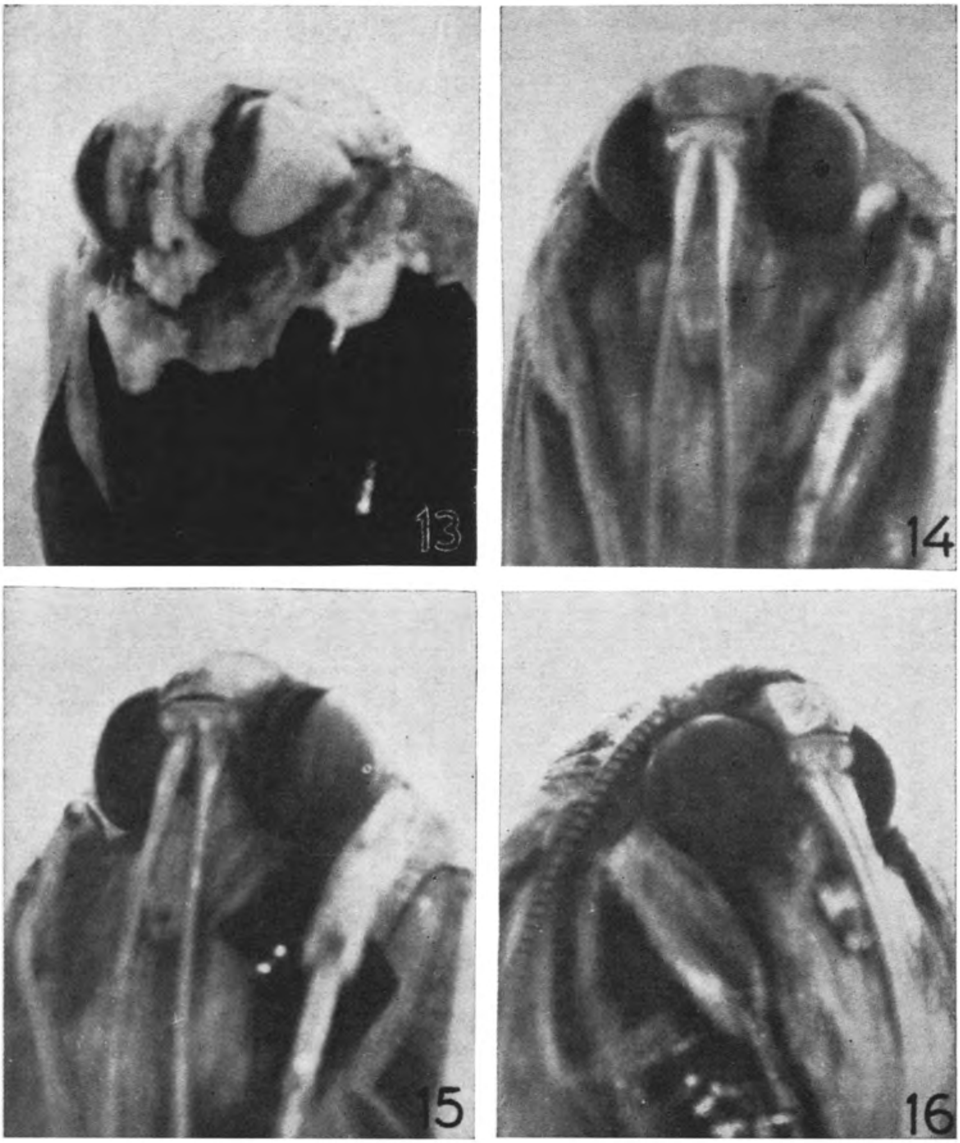
Figures 1–4 Photomicrographs showing normal development of compound eye of *S. litura*. *1*, V S of 36 hr old pupa through eye region showing hypodermal cells (Hc) and optic lobe (Ol); *2*, V S of 60 hr old pupa through same region showing beginning of the differentiation of ommatidial structures; *3*, V S of 4 day old pupa through same region showing further differentiation of ommatidial structures; *4*, V S of 5 day old pupa through same region showing near complete differentiation of ommatidial structures



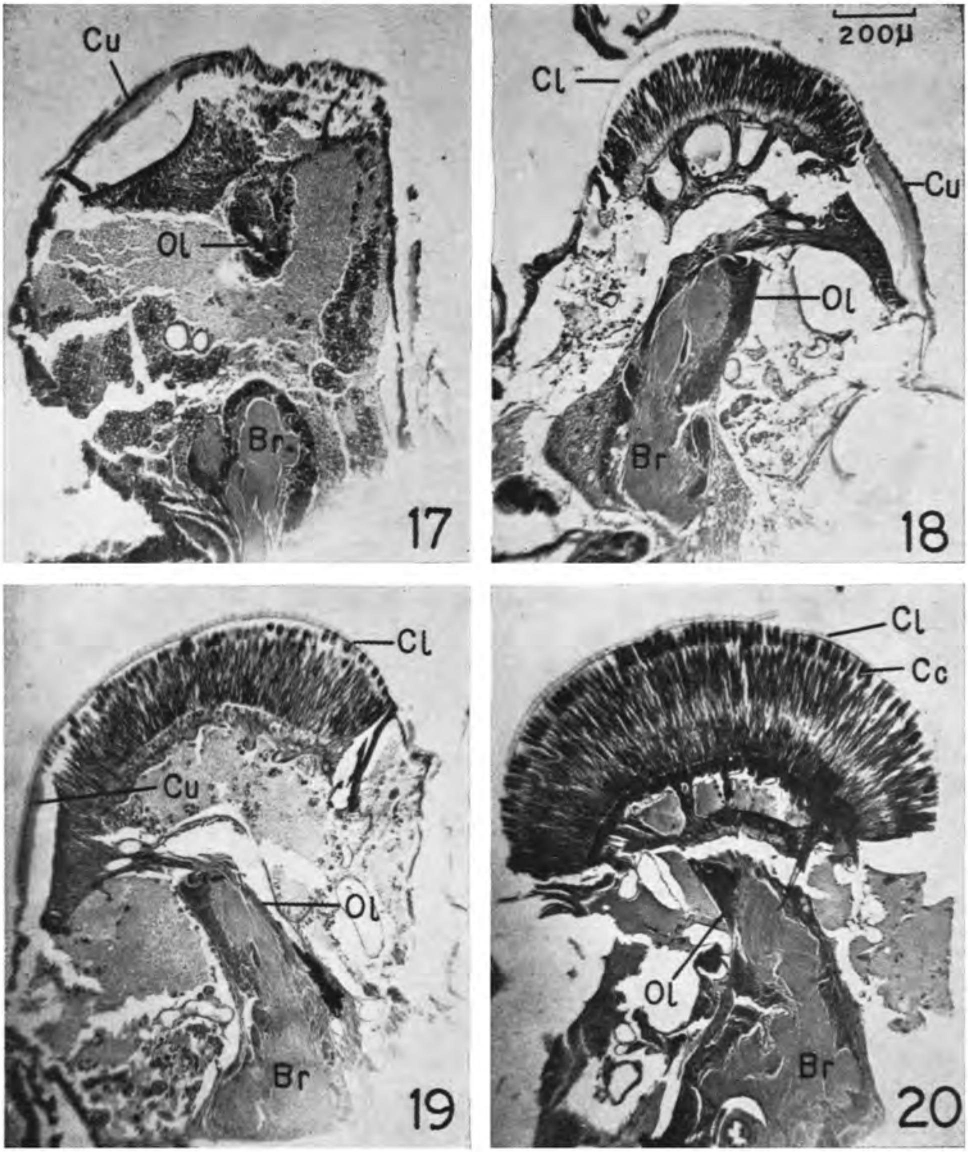
Figures 5–8 Photomicrographs showing normal development of compound eye of *S. litura*. 5, V S of 36 hr old pupa through eye region, highly magnified, showing hypodermal cells (**Hc**), cuticle (**Cu**), nerve cells (**Nc**) and glial cells (**Gc**); *6*, V S of 60 hr old pupa through eye region, highly magnified, showing crystalline cone cells (**Ccc**), primary iris cells (**Pic**), secondary iris cells (**Sic**), retinular cells (**Rc**), and nerve cells (**Nc**); *7a,b*, V S of 4 day old pupa through eye region, highly magnified, showing the same structures further differentiated and well developed corneal lens (**Cl**); *8*, V S of 5 day old pupa, highly magnified, showing almost complete differentiation of



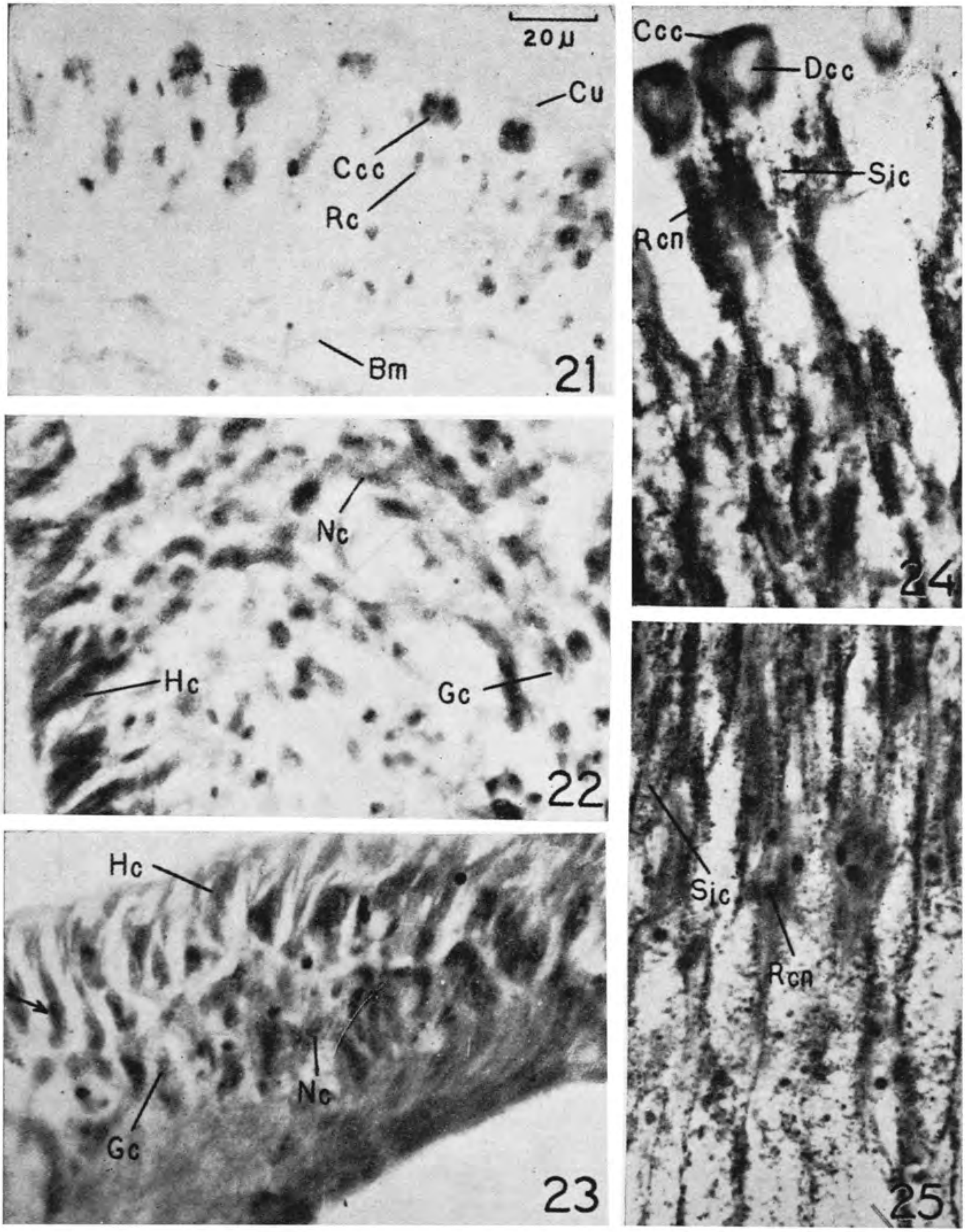
Figures 9–12 Cameralucida drawngs showing normal development of compound eye of *S. litura*. *9*, V S of 36-hr old pupa through eye region showing hypodermal cells (**Hc**), cuticle (**Cu**), nerve cells (**Nc**) and glial cells (**Gc**), *10*, V S of 60-hr-old pupa through eye region showing crystalline cone cells (**Ccc**), primary iris cells (**Pic**), secondary iris cells (**Sic**), retinular cells (**Rc**), and nerve cells (**Nc**); *11*, V S of 4-day-old pupa through eye region showing the same structures further differentiated and well developed corneal lens (**Cl**); *12*, V S of 5-day-old pupa showing almost complete differentiation of ommatidial structures



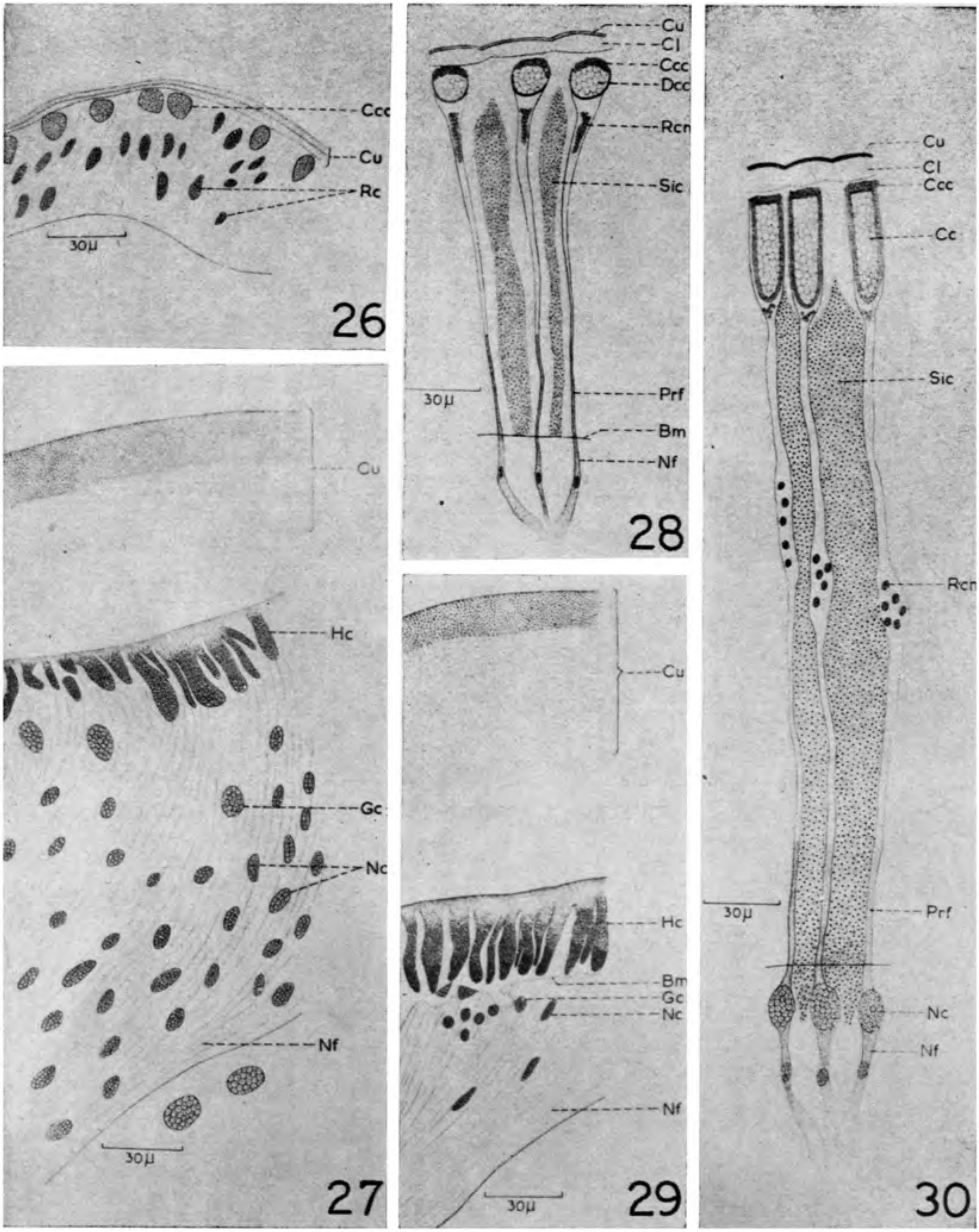
Figures 13–16 Photographs showing pigmented, unpigmented and weakly pigmented regions in the compound eyes in p–a intermediates produced by juvenoid treatment and normal eye of *S. litura*; *13*, eye of type I; *14*, eye of type II; *15*, eye of type III; *16*, normal eye



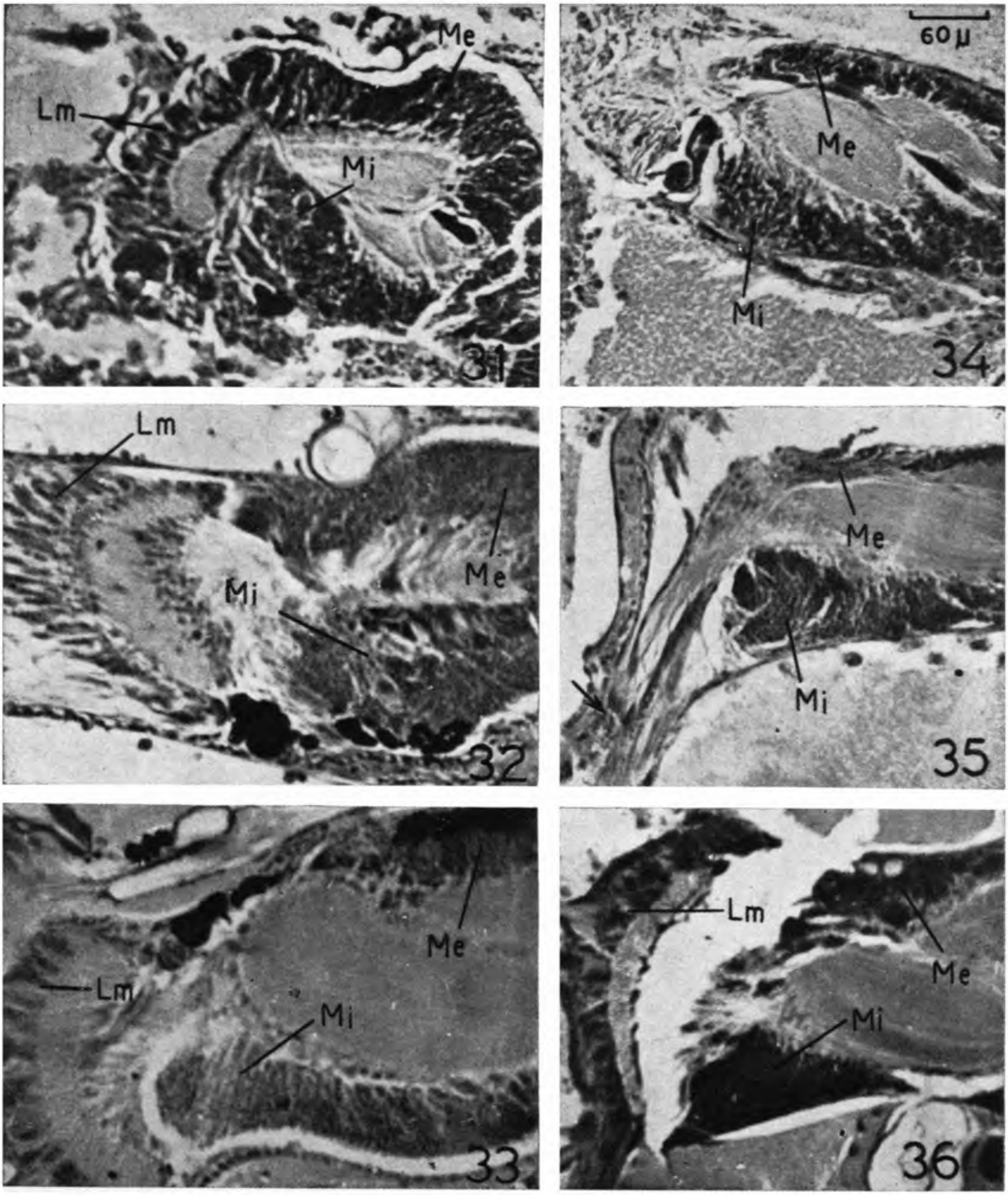
Figures 17–20 Photomicrographs showing histological structures of pigmented, unpigmented and weakly pigmented regions in the compound eye in p–a intermediate produced by juvenoid treatment, *17*, V S of eye of type I, *18*, V S of eye of type II; *19*, V S of eye of type III; *20*, V S of eye of type IV



Figures 21–25 Photomicrographs showing histological structures of pigmented, unpigmented and weakly pigmented regions in the compound eyes in p–a intermediates produced by juvenoid treatment; *21*, V S of type I eye through unpigmented zone, highly magnified; showing crystalline cone cells (Ccc), and retinular cells (Rc); *22*, V S of type I eye through pigmented zone, highly magnified, showing undifferentiated hypodermal cells (Hc), glial cells (Gc) and nerve cells (Nc), 23, V S of type II eye through pigmented zone, highly magnified, showing undifferentiated hypodermal cells (Hc) with deposit of pigments in lower region (see arrow); *24*, V S of type II eye through weakly pigmented zone, highly magnified, showing crystalline cone cell (Ccc), developing crystalline cone (Dcc) and secondary iris cells (Sic); *25*, V S of type IV eye, highly magnified, showing almost complete differentiation of secondary iris cells (Sic) and retinal cell nuclei (Rcn)



Figures 26–30 Camera lucida drawing, showing histological structures of pigmented, unpigmented and weakly pigmented regions in the compound eyes in p–a intermediates produced by juvenoid treatment. *26*, V S of type I eye through unpigmented zone showing crystalline cone cells (**Ccc**) and retinular cells (**Rc**); *27*, V S of type I eye through pigmented zone, showing undifferentiated hypodermal cells (**Hc**) and thick cuticular layer with deposit of pigments (**Cu**); *28*, V S of type II eye through weakly pigmented zone showing cuticle (**Cu**), corneal lens (**Cl**), crystalline cone cells (**Ccc**), developing crystalline cone (**Dcc**), retinal cell nuclei (**Rcn**) and secondary iris cells (**Sic**): *29*, V S of type II eye through pigmented zone showing undifferentiated hypodermal cells (**Hc**) with deposit of pigments in lower region (see arrow) and thick cuticular layer with deposits of pigments (**Cu**); *30*, V S of type IV eye, showing almost complete differentiation



Figures 31–36 Photomicrographs showing normal differentiation of optic lobes of *S. litura* and effect of a juvenoid on the same. *31, 32 & 33*, V S of 36 hr, 4 day and 5 day old pupa through optic lobe showing complete differentiation of medulla externa (Me), medulla interna (Mi) and lamina (Lm); *34*, V S of type I eye through optic lobe showing differentiation of medulla externa (Me) and medulla interna (Mi) only; *35*, V S of type II eye through optic lobe showing differentiation of medulla externa (Me), medulla interna (Mi) and only a few cortical cells of lamina (see arrow); *36*, V S of type IV eye through optic lobe showing complete differentiation of medulla externa, medulla interna and lamina (Me, Mi, Lm)

Table 1

Components of compound eye	Differentiation of various components of compound eye			
	Normal pigmentation (type IV)	Unpigmented zone of type I	Weakly pigmented zone of type II, III	Deeply pigmented zone of type I, II, III
1. Cuticle	Thin	Thin	Thin	Thick with deposit of pigments.
2. Corneal lens	Developed	Not developed	Developed	Not developed
3. Crystalline cone cells & crystalline cone	Crystalline cone developed but small	Crystalline cone cells differentiated	Crystalline cone cells & developing crystalline cone	Crystalline cone cells not differentiated
4. Primary iris cells	Differentiated	Not differentiated	Not differentiated	Not differentiated
5. Secondary iris cells	Differentiated	Not differentiated	Differentiated	Not differentiated
6. Retinal zone	Retinal zone differentiated	Retinular cells differentiated	Retinular cells differentiated	Retinular cells not differentiated

Table 2

Components of optic lobe & nerve supply	Differentiation of various components of optic lobe and nerve supply		
	Type IV	Type I	Type II, III
1. Medulla externa	Differentiated	Differentiated	Differentiated
2. Medulla interna	Differentiated	Differentiated	Differentiated
3. Chiasma externum	Formed	Formed	Formed
4. Lamina	Differentiated	Not differentiated	Incompletely differentiated with a few cortical cells
5. Never supply	Normal nerve supply	In the form of dense bundle in pigmented zone and poor in unpigmented zone	In the form of dense bundle in pigmented zone and normal in weakly pigmented zone

as compared to the normal iris. Corneal lenses have been formed. Crystalline cone cells have become differentiated and crystalline cones have developed as in the normal 60 hrs old pupa. Groups of retinal cells are also differentiated below the crystalline cone cells. Post-retinal fibres arising from the retinal cells are seen. Secondary iris cells have become differentiated and are full of pigments and resemble those of the 4 days old pupa.

Type IV: Corneal lenses are developed. Crystalline cones are formed but are small in size and the retinal zone is differentiated; both resemble the structures of the 5 days old pupa. Primary iris cells are distinct and surround the crystalline cones. Secondary iris cells surround the retinal zone and are full of pigments (figures 20, 25, 30).

Effect of JHA treatment on differentiation of optic lobes and nerve supply (*table 2*): In the optic lobe of the 1st type (figure 34), the medulla externa and medulla interna are differentiated and nerve fibres run from these to the clusters of hypodermal cells through the chiasma but the lamina is not developed. Several groups of nerve cells are observed among the nerve fibres. Groups of glial cells are also seen.

In the optic lobes of types II and III (fibres 35), the medulla externa and medulla interna are fully differentiated. The lamina starts differentiating but as yet it has only a few cortical cells. A few groups of glial cells are also scattered below the hypodermal layer and near the bundle of nerve fibres.

In type IV (figure 36), the optic lobe is fully developed and optic nerves are differentiated as in the normal compound eye. Groups of glial cells are also not observed as in case of normal adult compound eye.

Discussion

Effect of JHA on Development of Compound Eye

The only observation on the effect of JH/JHA on development of eyes in insects which seems to have received wide notice of several workers relates to lack of uniformity in pigmentation and it has been largely believed that the difference in the state of pigmentation indicates difference in the state of development in different parts of the eyes (Willis 1969 in *H. cecropia*, *S. cynthia* and *A. polyphemus*; Riddiford & Ajami 1973 in *M. sexta*; and Metwally & Sehnal 1973 in *T. granarium* and *C. gonagra*). Further, most of these workers have regarded that the pigmented part is more developed than the unpigmented part. This inference, however, is neither based on a careful study of the external appearance of the eye nor its histology. On the other hand, in *Sarcophaga bullata*, Bhaskaran (1972) noted complete suppression of pigmentation as a result of juvenoid treatment of the pupae and Sehnal and Zdarek (1976) also observed inhibition of pigment development in the compound eyes of certain species of cyclorrhaphous Diptera. Similarly, Madhavan (1973) noted retardation of synthesis of brown pigment in the eyes of *Drosophila* resulting from juvenoid treatment. Somehow, all observations showing the reduction of normal pigmentation of compound eyes by juvenoid treatment relate to the Diptera. That JH/JHA inhibits compound eye development has, to the best of the present workers information, been categorically proved only by Willis (1969) who made a histological examination of the eyes of polyphemus pupae injected with JH and observed that the eyes were smaller, the development of rhabdoms was inhibited and the crystalline cones were also smaller. In *S. litura*, we find clear evidence of the fact that in the pupal-adult intermediates, developed from JHA-treated pupae, development of compound eye is adversely affected in certain regions and in varying degrees and that often the inhibition of eye development is related to the nature of the intermediates.

We have also clearly noted that in parts of the compound eyes which externally show pigmentation, development is considerably inhibited and not relatively more advanced, as believed by several earlier workers (Willis 1969, Riddiford & Ajami 1973 and Metwally & Sehnal 1973). Further the extent of pigmentation appears proportionate to the degree of inhibition of development of the ommatidia and its components. Histological observations in fact, reveal that the pigmentation seen externally is due to pigment deposition in the cuticle and not in the iris region, and the latter has not developed at all and also that very often in the pigmented zone, even the facets have not differentiated. Finally in eyes of type I, it is seen that although the hypodermal cells in the pigmented zone have divided to form clusters, no further differentiation of these cells has occurred. Apparently wherever JHA treatment has induced pigment deposition in the cuticle, the development of other components has also been inhibited. There are other observations to show that JHA treatment induces pigment secretion. Willis (1969) had noted that when polyphemus larvae and pupae were treated with large quantities of JH, a highly pigmented larval cuticle was seen at the larval-pupal moult and a heavily tanned pupal cuticle at the pupal-adult moult. Abdallah et al. (1974) and Gawaad et al. (1974) had observed that newly ecdysed larvae of all instars of *S. littoralis* acquired a reddish colour when they were treated with a juvenoid and that the intensity of colour was greater when the quantity of juvenoid was larger. Recently in *E. fabia*, Shukla (1980) has observed deposition of heavy pigments in certain parts of the body of developing larvae as a result of juvenoid treatment of eggs. It seems that in the Lepidoptera, excess of JH induces sporadic and excessive development of pigmentation in the cuticle of different parts and that the prospective compound eye region is no exception to this response; perhaps it is more sensitive to this specific reactivity. Further the hypodermal cells which show this response are also the ones in which further activity towards the development of ommatidial components stops. At this stage it is not possible to speculate whether the two processes, viz., secretion of pigment and further differentiation of the cells are independently affected or the former is the cause of the latter.

It may be noted that in the pigmented zones of the eyes of type I, II and III, only clusters of undifferentiated hypodermal cells are observed. Willis (1969) had designated such clusters of hypodermal cells in the eyes of deuteropupa of polyphemus as 'growth zones'. He compared the eyes of deuteropupa to those of hemimetabolous insects, such as *Notonecta* (Ludtke 1940), which have a growth zone around the periphery of the nymphal eye and which contributes to the addition of new facets in every instar. Our observation in *S. litura* contradicts those of Willis. We conclude that far from representing zones of growth, the clusters of undifferentiated hypodermal cells in the pigmented zone of *S. litura* eyes represent cells in which further development and differentiation has been arrested. The fact that treatment with JH/JHA arrests cell division and differentiation has also been shown earlier. Srivastava and Singh (1979) noted that in *S. ruficornis* treatment of early pupa with a juvenoid suppressed division of hind gut epithelial cells and their differentiation into imaginal epithelium. Highnam and Hill (1969) have postulated that cell division and differentiation are intrinsic properties of the cell, residing in the genes, and that the particular expression at any time is controlled by juvenile hormone.

Willis (1974) has also noted that JH blocks DNA synthesis and cell division. Meola and Mayer (1980) in *Stomoxys calcitrans* have observed-nonproliferation of imaginal epidermal-cells when 24 hrs old pupae were treated with diflubenzuron, a chitin synthesis inhibitor. Diflubenzuron also acts as a juvenoid in *Achaea janata* (Subrahmanyam et al. 1980). Recently, Deb and Chakravorty (1981) have shown that an excess of the juvenoid could interfere directly with cell division in the germarium. In the present work, we have noted suppression of the process of division of the hypodermal cells in the pigmented region of the eyes when very early pupae were treated with the juvenoid and also the treatment inhibited the differentiation of several elements of the ommatidia in the pigmented zone. We thus find that the differentiation of primary iris cells and retinal zone cells may be inhibited and secretion of crystalline cone may be blocked by juvenoid treatment.

Our study also shows that clusters of undifferentiated hypodermal cells of the pupal eyes are most sensitive to JHA in the very young pupa and their sensitivity decreases as the pupa advances. In the 14–26 hrs old pupa, the differentiation of hypodermal cells may be inhibited only in the pigmented part. The importance of the quantity of the juvenoid administered is also clearly shown in this study.

Effect of JHA on Differentiation of Optic Lobes and Nerve Supply

There is no earlier account of the effect of JH treatment on the development of the optic lobe and optic nerve. In our study we find that JHA treatment completely inhibits the differentiation of lamina when very young pupae are treated with relatively large doses of the juvenoid. Even with smaller doses, its differentiation is severely affected and only few cortical cells are formed when a treatment is given at this stage. When the hormone is administered at a later stage, lamina differentiation may still be arrested at this stage. Apparently lamina cells are as sensitive to the hormone as other cells in respect of division and differentiation. However, when the age of treatment is advanced to about 38 hrs lamina differentiation is not affected.

A rich nerve supply comprising densely packed fibres to the undifferentiated hypodermal cells in the pigmented zones of the treated eyes (type I) is noted in the pigmented zones of the compound eye while the unpigmented zone shows very poor nerve supply and the weakly pigmented zone (types II and III), comprising relatively better differentiated region of compound eye and characterised by distinct ommatidial facets shows normal nerve supply. This indicates that the nerve supply is also affected by the juvenoid and the phenomenon is possibly associated with the differentiation of the other parts of the ommatidia specially the iris region. However, it appears from the present study that juvenoid treatment does not affect the differentiation of medulla externa and medulla interna nor does it affect chiasma formation.

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