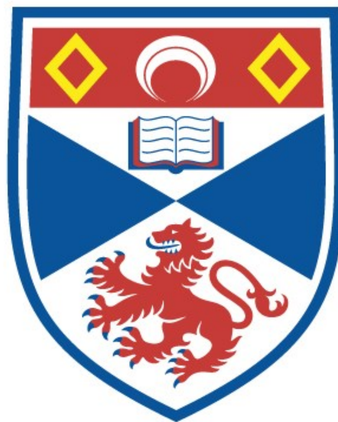
THE FIRST AND SECOND NEURAL PROJECTIONS OF THE INSECT EYE

Ian A. Meinertzhagen

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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by

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The Gatty Marine Laboratory

and

Department of Natural History

University of St. Andrews

1971

A thesis submitted for the Degree of Doctor of Philosophy

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SUPERVISOR'S CERTIFICATE

I certify that Ian Meinertzhagen has fulfilled the conditions laid down under Ordinance No. 16 of the University Court, St. Andrews, and is accordingly qualified to submit this thesis for the Degree of Doctor of Philosophy.

DECLARATION

I declare that the work reported in this thesis is my own and has not previously been submitted for any other degree.

CURRICULUM VITAE

I graduated in Physiology from the University of Aberdeen in 1966. During the years 1966 to 1969 I was in receipt of a St. Andrews Studentship awarded by the University. The work reported in the thesis was commenced early in 1968 and was continued in the Department of Neurobiology, Australian National University, Canberra.

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CONTENTS

ACKNOWLEDGEMENTS iii

CONTENTS iv

INDEX OF FIGURES ix

INDEX OF TABLES xii

SUMMARY 1

INTRODUCTION

A. The general features of insect retinae and optic lobes 3

The retina 7

The first projection and lamina 9

The second projection and medulla 12

B. The insects studied and the reasons for their selection 13

C. The methods available for the analysis of connectivity patterns in insect optic lobe 15

Light microscopical methods 16

1. Hodological methods 16

2. Serial sectioning methods 20

Electron microscopical methods 23

Methods combining electron and light microscopy 26

D. The retina and optic lobes of the insects studied 28

The fly 28

1. The retina of the fly 28

2. The neurones of the fly lamina 30

3. The lamina-medulla projection of fly 34

The water-bug and *Notonecta* 37

1. The retinae 37

2. The optic lobes 38

The locust 39

1. The retina and lamina of the locust 39

The honey-bee 40

1. The retina of the bee 40

2. The neurones of the bee lamina 42

3. The lamina-medulla projection 45

Pieris 45

1. The retina of *Pieris* 45

2. The neurones of the lamina of *Pieris* 46

3. The lamina-medulla projection of *Pieris* 48

The skipper 48

1. The retina of the skipper 48

E. The physiological and behavioural analysis of compound eye function 49

Evidence from the fly 50

Evidence from the bee 52

Evidence from the locust 53

Evidence from the skipper 55

Evidence from *Pieris* 56

Evidence from water-bug and *Notonecta* 56

MATERIALS AND METHODS

A. Animals 58

B. Fixation 59

C. Dissection 60

The fly 60

The locust 60

The bee and dragonfly 61

The water-bug and *Notonecta* 61

Pieris 61

D. Microtomy 63

E. Microscopy, photomicrography and tracing techniques 64

F. Electron microscopy 66

G. Reconstruction of axon pathways 66

RESULTS

A. The fly *Calliphora* 69

The short retinula axons 73

The long retinula axons 79

The spatial relationships of the axon lattice 79

The composition of the lamina cartridges 85

The neurones of the lamina 87

The lamina-medulla projection 90

B. The water-bug Lethocerus 101

C. Notonecta: the backswimmer 106

D. The locust 108

E. The drone bee Apis 115

The retina-lamina projection 119

The lamina 122

The lamina-medulla projection 131

F. Pieris: the small white butterfly 139

G. The skipper Trapezites 150

The retina-lamina projection 150

The lamina-medulla projection 155

H. The dragonfly Aeschna 157

DISCUSSION

A. The projection of the retina upon the medulla 160

The retina-lamina projection 161

1. The retinula cell composition of the ommatidium 165

2. The long visual fibres 167

3. The short retinula axons 173

The lamina-medulla projection 175

B. Functional consequences of the projection patterns 182

Visual information available at the medulla cartridges 182

The retinula cell input to the cartridges of the water-bug lamina 190

The significance of the open-rhabdomere retina 193

The significance of neurone-glia synapses in the fly lamina 195

Lateral interaction and movement perception 197

1. Behavioural performance of movement perception systems in arthropods 198

2. Models derived from behavioural performance 200

3. Components of the models and their possible anatomical substrate 202

C. The problem of connectivity 206

D. Some developmental aspects of connectivity in the optic lobe 210

The study of errors in the fly first projection 210

1. The occurrence of errors 211

2. Variability in the composition of cartridges 214

3. The origin of the equator in the fly eye 215

The development of optic lobes and the significance of chiasmata 218

E. The prospects for future work 224

REFERENCES 228

INDEX OF FIGURES

Figure

1. *Apis* HLS of retina and optic lobe 4

2. Diagram illustrating the three main planes of section of a compound eye 6

3. Serial sectioning method 62

4. *C. stygia* wholemount of cornea 67

5. *C. vomitoria* plan of retina 68

6. *C. vomitoria* projection of individual ommatidia 70

7. Projection plans converted to an hexagonal array facing 71

8. *C. vomitoria* plan of lamina facing 74

9. Projection patterns of axons that terminate in error 76

10. Block diagram of lamina facing 78

11. *C. vomitoria* micrograph of lamina 78

12. *C. stygia* HLS of first projection and lamina facing 79

13. Reconstruction of axon lattice 80

14. *C. vomitoria* pull-out series of micrographs 81

15. *C. vomitoria* organization of lamina cartridges 84

16. *C. stygia* cartridge array at the edge of the lamina 86

17, 18. *C. vicina* whole field of lamina-medulla projection 88, facing 89

19, 20. *C. vicina* one row of lamina-medulla projection 93, 94

21. Reconstruction of chiasmal pathways 97

22. *C. vicina* tangential process of medulla 98

23. *Benacus* random sections through retina and optic lobe 102

24. *Lethocerus* and *Notonecta* 104

25. *Notonecta* lamina-medulla projection 105

26. *Locusta* retina, Feulgen stain 107

27. *Schistocerca* random sections through retina and optic lobe 109

28. *Schistocerca* whole field of retina-lamina projection 110

29. *Schistocerca* retina-lamina projection of individual ommatidia 112

30. *Apis* section through cornea 114

31. *Apis* micrographs of retina 116

32. *Apis* plan of retina 117

33. *Apis* plan of lamina 118

34. *Apis* micrograph of lamina 120

35. *Apis* retina-lamina projection of individual ommatidia 121

36. *Apis* whole field of lamina cartridges 123

37. *Apis* the axons of individual lamina cartridges 125

38. Diagrams of the axons of a lamina cartridge 126

39. Reconstruction of the first projection and lamina 128

40, 41 *Apis* whole field of lamina-medulla projection 129, 130

42. *Apis* the lamina 132

43. *Apis* the medulla 133

44. *Apis* lamina-medulla projection of individual ommatidia 135

45. *Pieris* the arrangement of retinula cells 137

46. *Pieris* electron micrographs of retina 138

47. *Pieris* plan of retina 140

48, 49. *Pieris* whole field of retina-lamina projection 142, 143

50. *Pieris* plan of lamina 145

51. *Pieris* retina-lamina projection of individual ommatidia 146

52. *Trapezites* random electron micrographs of retina and optic lobe 148

53. *Trapezites* whole field of retina-lamina projection 149

54. *Trapezites* retina-lamina projection of individual ommatidia 151

55. *Trapezites* the projection of cartridges through the lamina 153

56. *Trapezites* the lamina-medulla projection 154

57. *Trapezites* tangential processes of the medulla 156

58. *Libellula* random micrographs 158

INDEX OF TABLES

Table

1. The first and second neural projections of insect eyes 27

2. The seventeen short retinula axons that terminate in incorrect cartridges 76

3. The long visual fibres and their cells of origin 166

4. The short retinula axons and their cells of origin 172

SUMMARY

1. The patterns of projection of some of the perpendicular neurones between the retina and medulla of the optic lobes of various insects have been studied. Axon paths have been studied from consecutive semi-thin plastic sections cut transversely and stained with toluidine blue. The termination positions and the paths of axons are both highly ordered and predictable.

2. In all insects with fused-rhabdomere eyes the axons of one ommatidium project to one cartridge of the lamina and the array of cartridges duplicates the array of ommatidia. In insects with open-rhabdomere eyes visual information is distributed amongst a number of lamina cartridges so that each cartridge receives information originating from one visual axis.

3. In both open- and fused-rhabdomere types the cartridge array of the lamina is exactly duplicated in the medulla but by the intervention of the chiasma is reversed about a dorso-ventral plane. The axons of one lamina cartridge contribute to one medulla cartridge. Thus, in all insects studied, the visual field is projected exactly from retina to medulla.

4. Most of the retinula axons from one ommatidium terminate in the lamina but usually one pair passes directly to the medulla. These are from the central retinula cells (open-rhabdomere eyes) or from the small retinula cells (apposition type fused-rhabdomere eyes). Retinal responses are known mainly only for the short retinula axons so that visual information delivered to the medulla cartridge is still largely unresolved.

5. The lamina neuropile probably contains the elements responsible for the lateral correlation between parallel receptor inputs which is necessary for movement perception, but units with long lasting responses which could act as the delay circuit of movement perception are unknown.

6. The occurrence of errors in termination of the first projection of the optic lobe of the fly, which are reported for the first time in this work, provide no direct clues to the developmental processes by which such a morphologically complex system arises. Nevertheless errors may arise within a sequence of growth processes which are fundamentally quite simple and not obvious from knowledge of the generalized perfect pattern of connections.

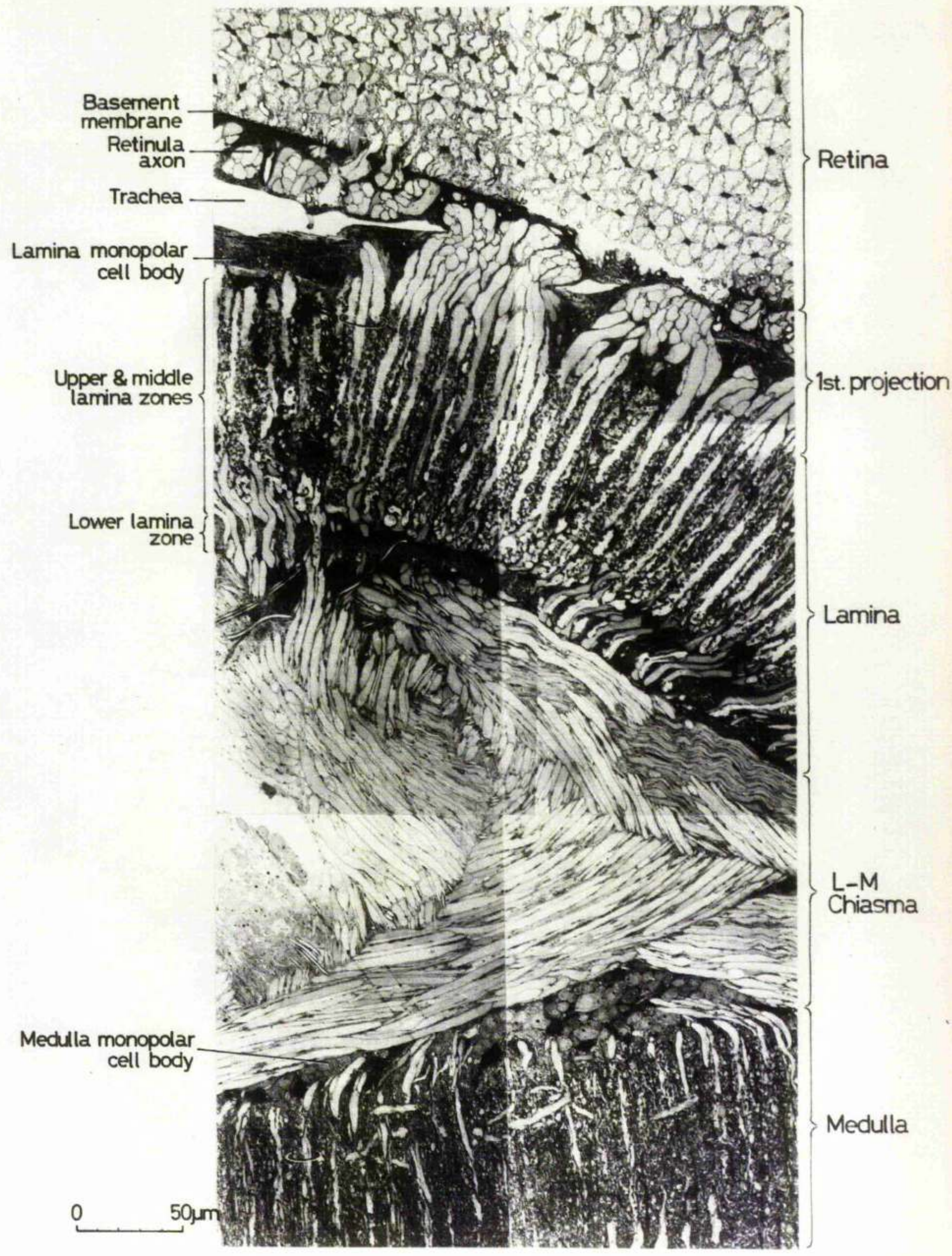
INTRODUCTION

A. The general features of insect retinae and optic lobes

The insect visual system consists of the external compound eyes and the internal optic lobes. The eye consists of the cornea and associated dioptric apparatus which overlie a layer of photoreceptors. These photoreceptors are collectively called the retina and they generate visual responses that are processed in the optic lobe. The optic lobe is composed of three main regions which, from distal to proximal, are the lamina, medulla and lobula. In both Diptera and Lepidoptera the most central region, the lobula, is subdivided into two regions, the lobula and lobula plate (Bullock and Horridge, 1965). Each region is composed of a band of neuropile surrounded by the cell bodies of that neuropile. The retina and lamina are connected by the axons of the first projection, while the lamina, medulla and lobula are connected in sequence by two chiasmata. The plane of decussation of these is horizontal in the lamina-medulla chiasma and vertical in the medulla-lobula chiasma. The main burden of this work has been to establish some of the neural connections contained within these tracts between the retina, lamina and medulla because knowledge of these connections is essential to interpret electrophysiological responses recorded from these regions. The results will be presented

Figure 1

Apis Horizontal longitudinal section of retina and optic lobe to illustrate the general features of the insect compound eye. From the periphery to the centre are seen the retina, with the ommatidia obliquely sectioned, basement membrane, retinula axon bundles, lamina, horizontal chiasma and medulla. Both lamina and medulla neuropile layers underlie a ganglion cell layer and each is subdivided longitudinally into an array of cartridges, one of which is circled in each.



Basement membrane
Retinula axon
Trachea
Lamina monopolar cell body
Upper & middle lamina zones
Lower lamina zone
Medulla monopolar cell body
0
50µm
Retina
1st. projection
Lamina
L-M Chiasma
Medulla

primarily in the light of their functional significance.

A concept of general interest that has been emphasized by the work is the high level of predictability and order of the connections within optic lobe. This emphasis raises some challenging developmental questions concerning the generation of such a precise organization. The classic study of Cajal and Sánchez (1915) did much to reveal the organization of the optic lobe into many pathways in parallel, each highly ordered. These features make the tissue favourable for tracing connections between neurones. Together with the contemporary trend towards the analysis of neuronal networks these factors have resulted in the renewed impetus with which the morphology of insect optic lobe has been studied in recent years, notably in the work of Trujillo-Cenóz, Braitenberg and Strausfeld on the fly.

An obvious feature of the eye and optic lobe is their organization into perpendicular and tangential components (fig. 1). The retina is composed entirely of a perpendicular array of cylindrical ommatidia while the lamina and medulla are divisible perpendicularly into arrays of cartridges which correspond numerically with the retinal array of ommatidia.

The neurones of the optic lobe are similarly categorized according to the orientation of their long axes. Perpendicular cells have axons that proceed in a direction normal to the

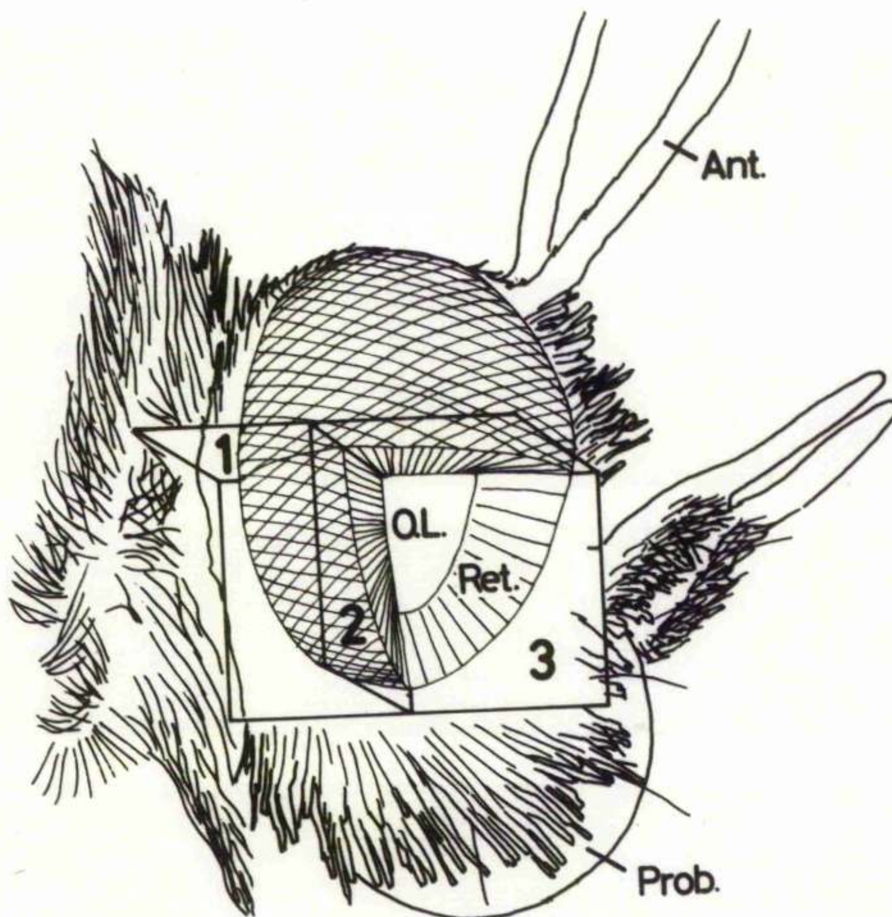


Figure 2

Diagram illustrating the three main planes of section (1 - 3) of one quadrant of the insect compound eye. 1 = horizontal (antero-posterior); 2 = vertical (dorso-ventral); 3 = tangential. Drawn from a photograph of the right eye of P. rapae. Ant. = antenna; Prob. = proboscis; O.L. = optic lobe; Ret. = retina.

retina or neuropile that contains their cell bodies; they convey information centripetally towards a more central neuropile or centrifugally away from it. Tangential cells have processes that spread across the visual field and collect visual information from many perpendicular cells in parallel which is then conveyed perpendicularly. Amacrine cells are intrinsic to one neuropile only and have both perpendicular and tangential processes but do not convey information between ganglia.

This work is concerned almost entirely with the perpendicular cells between the retina and medulla analysed from series of tangential sections.

The three main planes of section are illustrated by Strausfeld and Blest (1970), and given by them the names in common usage viz. tangential, vertical (i.e. dorso-ventral) and horizontal (i.e. antero-posterior) (see fig. 2).

The retina

Light reaches the photoreceptive rhabdome through the dioptric apparatus which is composed of the cornea, overlying the whole eye, and the cone and ancillary structures of each ommatidium. The surface of the cornea is an array of facets the arrangement of which varies between species. Commonly the facets are regular hexagons and the array is defined by three axes z (horizontal) and two oblique dorso-ventral axes

x and y either at 30° to the horizontal e.g. Drosophila or at 60° to the horizontal e.g. bee, locust and white butterfly Pieris. The flies Musca and Calliphora have a corneal array that has both types of pattern (in the anterior and lateral regions of the eye respectively) separated by a mid-lateral transitional zone of a rhomboidal array (Braitenberg, 1967, 1970). The axes x, y and z are useful because they also define the array of ommatidia underlying the corneal facets.

Each ommatidium in tangential section has the appearance of a rosette or retinula composed of approximately eight retinula cells each bearing many microvilli containing visual pigment along the length of its inner margin. Categories distinguished within the functional morphology of different insect retinae include the arrangement of rhabdomeres and the pattern, arrangement and number of retinula cells within ommatidia (Table 1).

The relationship between the distal end of the rhabdomeres and the central end of the cone has been the basis for the longstanding and possibly erroneous distinction between retinae of different insect groups into apposition and superposition types, first proposed by Exner (1891). Superposition eyes have a refractile crystalline thread interposed between cone and rhabdome whereas in apposition eyes the rhabdome abutts onto the cone.

The arrangement of rhabdomeres within an ommatidium may

be fused (the rhabdomeres are united into a single axial rod: locust, bee etc.) or open (the rhabdomeres are separated from each other: fly, water-bug).

The arrangement of retinula cells within an ommatidium also varies; usually the cells are of two sizes, the larger long retinula cells (often six) and the small basal or central retinula cells (one or two). The ommatidial complement of retinula cells is eight for fly, locust, dragonfly, water-bug and the backswimmer Notonecta; and nine for drone bee, the small white butterfly Pieris and the skipper Trapezites. The long retinula cells may contribute rhabdomeric microvilli for the entire length of their inner margin or the rhabdome may be divided along its length so that the rhabdomere of one cell is in front of that of another along the optical path, in which case the retina is said to be tiered.

The ommatidium is limited proximally by a basement membrane pierced at intervals by bundles of retinula axons which proceed centrally to the lamina.

The first projection and lamina

The retinula axons are of two types; the long visual fibres which proceed uninterrupted to the medulla and the short retinula cell fibres which end in the lamina. This terminology, derived from the original accounts of Cajal and Sánchez (1915) is confusing because, where it has been

established, it is the long retinula cells that have short retinula axons. This situation is known for fly, bee and _Pieris_ and is probably true for water-bug too; it is perhaps a general principle for all eyes. The existing terminology will however be retained to avoid further confusion.

The ommatidial bundles of retinula axons travel proximally, passing in turn the basement membrane, the fenestration zone containing parallel rows of horizontal tracheae, the cell body layer which is composed of columns of cell bodies mainly of lamina monopolar neurones and finally reaching the neuropile of the external plexiform layer. Commonly retinula axon bundles twist during their passage to the lamina although the rotational sequence of axons within the bundle remains constant, at least for the short retinula axons. This sequence is the relative positions of the axons established at the base of the ommatidium.

In the external plexiform layer of the lamina the short retinula axons terminate within the unit structure of the lamina neuropile, the cartridge, where they make synaptic contact with various lamina neurones. The long retinula axons go straight through the lamina without synaptic contact, at least in fly and worker bee. The distribution of the retinula axon terminals from one ommatidium amongst the cartridges of the lamina is of two types: either each terminal is distributed to a different cartridge (as in fly and probably

also water-bug), or the terminals end in a single cartridge (as in all fused-rhabdomere eyes so far examined).

The neurone types of the lamina are known from Golgi and methylene blue studies on various insects (notably Cajal and Sánchez, 1915 on various species, Zawarzin, 1913 on the dragonfly Aeschna, Strausfeld and Blest, 1970 on some Lepidoptera and Strausfeld, 1970 on some Diptera). The perpendicular neurones comprise monopolar neurones with cell bodies overlying the lamina neuropile and centrifugal neurones with cell bodies to one side of the chiasma. Centrifugal neurones are usually T-shaped with a termination in the medulla and a second termination in the lamina of which one type is the basket ending. The term centrifugal, now in common usage, will be used throughout this work. It is used entirely as a morphological description and should not be taken to imply polarity of conduction. The monopolar neurones which were further classified by Cajal and Sanchez (1915) as large (having lateral dendrites for a large vertical depth of the lamina) and small (all other forms with lateral dendrites localized to only one or a few positions in the lamina) have been reclassified by Strausfeld and Blest (1970) into large (those with overlapping fields of lateral dendrites spreading through more than one cartridge) and small (those with lateral processes restricted to one cartridge). These two schemes are a source of confusion because very often

they conflict; in this work both schemes are used after the two original accounts and acknowledgement is made to whichever account is used. Tangential neurones are known from several species, one type with a cell body above the lamina (fly), another with a cell body at the anterior edge of the lamina (Pieris), a third with a cell body beneath the medulla (Aeschna) but the cell bodies of other tangential neurones in these insects are not known nor are they known for other insects. Amacrine neurones have cell bodies proximal to the lamina and peripherally directed processes (fly and Aeschna).

The organization of the different lamina neurones and incoming retinula axons within the cartridge is variable. The cartridges may be separate (fly, Pieris) or indistinct (locust, Trapezites, bee, water-bug, and Notonecta) depending largely on the extent of lateral arborization of the component dendrites. In the fly most synaptic connections of the lamina occur between inwardly directed dendrites. The long retinula axons may occupy a central position in the cartridge (locust, bee, Pieris, Trapezites and probably water-bug) or a satellite position to the cartridge (fly).

The second projection and medulla

Axons from horizontal rows of lamina cartridges enter the first optic chiasma (which will be called here more simply the chiasma) and become disposed in horizontal strata within which the paths of axons cross so that their horizontal order

becomes inverted about a vertical plane. Axons from near the anterior or posterior margins of the lamina move more obliquely than those at the centre and the axons are segregated into alternate horizontal rows. Consequently tangential sections have a striated appearance with axons cut longitudinally (those undergoing large horizontal displacements) and axons cut transversely (those making only a small lateral shift) occupying alternate layers. The uncrossed projection of some lamina neurones upon the medulla has also been depicted by Cajal and Sánchez (1915) but apparently does not apply to the perpendicular neurones with which this work is primarily concerned.

Chiasma axons again group into bundles at the central end of the chiasma, pass through the ganglion cell body layer of the medulla and enter the neuropile of that region. They pass through columns of medulla cell bodies in the medulla ganglion cell layer and each bundle eventually forms a single cartridge of the medulla. Each cartridge also receives the axons from a number of monopolar neurones from the columns of medulla cell bodies. No medulla neurones have been studied in this present work.

B. The insects studied and the reasons for their selection

The selection of insect species used in this work was based in the first instance on previous work on visual

behaviour and physiology. The results have been supplemented by studies on other insects with large axons that are easy to trace.

Since the early anatomical work on compound eye some insect species have been preferred, notably fly, bee and locust while other whole insect groups have been relatively neglected. Bee and fly have attracted most attention because of their interesting behavioural patterns whilst locust has been particularly favourable for electrophysiological analysis of its optic lobes. Of the less well-studied species the water-bug Lethocerus is potentially interesting because it is a second example of an open-rhabdomere eye (but with an apparently more complex organization of the lamina than that of the fly). Two lepidopterans have also been studied in this work, members of two specialised superfamilies the Papilionoidae and Hesperoidae respectively; they are the white butterfly, Pieris and the skipper Trapezites. Though lepidopteran vision has been little studied physiologically and behaviourally these insects represent an important group with a variety of retinal organizations including both superposition (e.g. the skipper) and apposition types (e.g. Pieris). An important recent anatomical account of Pieris and the hawkmoth Sphinx (Strausfeld and Blest, 1970) was the immediate stimulus for the inclusion of Pieris in this work. The Lepidoptera together with the Diptera have

divided lobulae, an arrangement not found in any other group.

In the next sections are given the anatomical methods of study currently available while in the last two sections follow for each species firstly, a more detailed account of the present state of knowledge of anatomy of the compound eye and secondly, a brief review of the physiological and behavioural evidence of compound eye function already available.

C. The methods available for the analysis of connectivity patterns in insect optic lobe

Available methods may be divided into those utilizing light microscopy, those utilizing electron microscopy and those which rely upon a combination of both levels of resolution. Each method has its own advantages but none is wholly adequate, and only by a correlation between methods will a complete analysis of connectivity be possible. However a problem is that individual workers specializing in different techniques prefer particular animals e.g. flies are impregnated well by the Golgi method; diverging pathways are shown well in reduced silver preparations; small animals have smaller volumes of neuropile to reconstruct by the laborious methods of electron microscopy; only some animals are suitable for degeneration methods; the methods used here require axon diameters in excess of 0.5μm etc; consequently

use of different methods results in diversification of the morphological descriptions as well as consolidation. In general dipteran flies have received most attention and are most favourable for analysis by both anatomists and physiologists alike.

Light microscopy provides gross details within a large volume of tissue of cell types, axon pathways, termination positions etc., but the resolution of electron microscopy is needed for identification of synaptic contacts between cells. Electron microscopy however can only be applied to reconstruct small volumes of tissue.

The small size of the optic lobe and its arrangement into many parallel pathways have facilitated the analysis of a single neuronal circuit because the important features are emphasized by their repetition in numerous essentially identical circuits. The favourability of the tissue is indicated by the definitive studies that have appeared recently (Trujillo-Cenoz and Melamed, 1963; Trujillo-Cenoz, 1965; Trujillo-Cenoz and Melamed, 1966; Braitenberg, 1967; Melamed and Trujillo-Cenoz, 1968; Trujillo-Cenoz, 1969; Boschek, 1970; Horridge and Meinertzhagen, 1970 a, b; Strausfeld and Blest, 1970; Strausfeld, 1970; Strausfeld and Braitenberg, 1970; Trujillo-Cenoz, 1970; Varela, 1970).

<u>Light microscopical methods 1. Hodological methods</u>

The framework upon which all morphological analyses of

insect optic lobe rest is provided by the so-called hodological methods (i.e. Golgi impregnation and methylene blue staining), in which the entire extent of only a few neurones in either whole mounts or thick slabs of tissue are selectively stained. These methods provide information on neuronal profiles, perikarya positions etc. and from them the classification of morphological cell types. Completely impregnated, the individual processes of the dendritic arborization of a particular neurone are visible with light microscopy; the lower limit of the diameters of the finest processes found in the neuropiles of the optic lobes and of many other insect ganglia range between 0.1 and 0.15μm (Trujillo-Cenóz, 1959; Trujillo-Cenóz and Melamed, 1962; Smith and Treherne, 1963; Smith, 1965; Trujillo-Cenóz, 1965; Steiger, 1967; Trujillo-Cenóz and Melamed, 1970). Such fine processes however are only just visible with the largest numerical aperture objectives, which are those least suitable for photomicrography because of their shallow depth of focus.

Selectivity is the essential quality of these methods which gives them their great clarity. However selectivity also imposes limitations. Two such important limitations are that no reliable information on the relative numbers of different cell types is provided and that little information is available on the connections between processes of different neurones. In addition, errors of both omission and commission

are inherent in the methods; the former, that some neurones are consistently left unstained in every preparation, the latter, that the technique may produce artifactual staining either of non-neuronal structures or of part of a neurone only. These limitations have always to be borne in mind when assessing results obtained by these methods (Blest, 1966), though the general agreement between the accounts from methylene blue preparations by Zawarzin (1913) and from Golgi preparations by Zawarzin (1913) and Cajal and Sanchez (1915) is good evidence of the relative reliability of both methods. In the case of Golgi impregnations these limitations have been fully discussed by Strausfeld and Blest (1970) for the optic lobes of Lepidoptera and Diptera. Strausfeld (1970) and Strausfeld and Blest (1970) found every cell type previously described by the Spanish authors which is evidence against random artifactual impregnation. In addition they found new types not described by Cajal or described only from partially impregnated preparations. In his work Strausfeld used a greater variety of impregnations from a larger number of animals than Cajal and paid particular attention to the reconstruction of wide-field elements stained in their entirety only infrequently. Lastly, no major disagreement has emerged between results from hodological staining methods and those of other techniques (though the methylene blue and Golgi preparations of Zawarzin were probably slightly less

reliable than the Golgi impregnations of Cajal and Strausfeld). Even so it is still not clear to what extent the large number of cell types and subtypes which may be compiled with relative ease using the Golgi method are a meaningful classification in terms of the connectivity of the neurones. A recent modification of a reduced silver staining method (Strausfeld, personal communication) introduces a degree of selectivity into the impregnation and permits the staining of entire single neurone classes preferentially, thus allowing a degree of correlation between Golgi and reduced silver methods not previously attainable. The results are not yet available.

A third method of great potential value for the selective staining of entire neurones arises with the recent demonstration by Lund and Collett (1968) that various of the reduced silver techniques for demonstrating neuronal degeneration in vertebrates work satisfactorily in insect optic lobe. The profile of the entire neurone is visible by light microscopy while the terminal processes of degenerating neurones are visible by electron microscopy of the same section. The method has the limitation that discrete areas of degeneration have to be induced. Consequently not only has the insect species to be selected for postoperative recovery and the conspicuousness of its degenerative changes but the value of the method is also limited by the size of

experimental lesion except for those neurones where the soma is isolated from its neuropile e.g. for neurones with somata in the contralateral optic lobe.

Light microscopical methods 2. Serial sectioning methods

Serial sections examined by light microscopy provide in general the intermediate between Golgi impregnation and electron microscopy; they allow the description of the projection of axonal pathways between neuropile layers of neurone types known from Golgi impregnations, but without the final resolution of synaptic contact. These projections are especially difficult to study because they consist of relatively long axons (say 100μm -1mm) of fine calibre (say 0.25μm-5μm). The methods are most useful only when the staining of all the elements of a projection is possible. Two types of serial sectioning method have been used; the reduced silver staining methods of e.g. Bodian, 1936; Holmes, 1943 and its modifications by Blest, 1961; Rowell, 1963; etc. and the following of axon pathways through serial 1 μm sections of plastic embedded material stained non-selectively by toluidine blue (Horridge and Meinertzhagen, 1970 a, b).

Reduced silver staining methods have proved particularly successful for the study of anatomical pathways at single axon level in a few favourable cases where the axons move horizontally in a section and are separate from their neighbours. Thus Braitenberg (1967) was able to study the

first projection of the fly optic lobe because the pattern of divergence is the characteristic feature of this system. By identifying individual retinula axons within the separate ommatidial bundles (from a correlation with their cyclic order at the point of origin near the basement membrane) their pathways over the top of the lamina could be followed individually because they were cut longitudinally in that region. Similarly Strausfeld and Braitenberg (1970) were able to use reduced silver staining methods to study the extremely fine lateral prolongations from the L4 neurones at the base of the fly lamina after identifying the profile of the L4 axis fibre from its size and position within the cartridge. The specificity of reduced silver staining for neurones makes axons easy to visualize against a clear background so that the connections of very fine processes may be studied. However, the confusion resulting from the staining of a tract of axons viewed in a 10µm section is so great that it is difficult to identify individual axons even of 2µm diameter. Thus reduced silver methods are unsatisfactory for the study of projections where bundles of retinula axons unite into large axon tracts as they do in all insects examined except the fly.

In an effort to overcome this particular problem the method developed in this work and reported in Horridge and Meinertzhagen (1970 a, b) has been to study by light

microscopy 1µm plastic transverse sections cut consecutively. The number of sections is thus not too prohibitive although still very large, whereas the thinness of the section allows near maximal resolution available with light microscopy. This method is much more laborious than either of the silver methods and has much in common with the methods of serial sectioning electron microscopy though lacking their extreme resolution. The poorer resolution of light microscopy is of course compensated for by the larger field and most important the greater depth that can be studied. In common with all the light microscopic methods statements can only be made of neuronal proximity and not of synaptic contact which must be inferred from other electron microscopical studies or from physiology. Synaptic connections between adjacent neurones can be excluded for many classes of fibres if the fine processes of the neurones are known. These are best described from silver preparations since they are not so easily seen in toluidine blue stained sections. Although light microscopy of 1µm sections allows maximal resolution and eliminates the confusion resulting from the superimposition of the images of many parallel axons stained in a thick section, the visibility of individual, fine axonal processes travelling horizontally within a section is much greater in such a thick section either impregnated by the Golgi method or stained with reduced silver. The visibility is greater for two

reasons; firstly, it is easier to visualize a process stained as a black thread against a clear ground than as a hollow profile of the same diameter in a thin section, and secondly, because any one thick section contains a larger portion of the process.

Electron microscopical methods

The remaining methods, those of electron microscopy, provide the definitive morphological evidence of connectivity and refer usually to connections within a neuropile (as opposed to the preceding methods which are concerned mainly with connections between neuropiles).

The great increase in resolution available with electron microscopy, which is necessary for the identification of synaptic contacts between neurones, results in a very great increase in the limitations and difficulties of this technique compared with those of light microscopy. Only small volumes of tissue may be sampled because of the thinness of section required so that neuronal processes can rarely be traced from their cell of origin. Consequently cells of origin must be inferred from previous light microscopical work by the position and orientation of their axons at the region where these enter the neuropile. This has been possible in the case of the ordered projections of perpendicular elements in the fly lamina (Trujillo-Cenóz, 1965; Boschek, 1970) and less completely in the bee lamina

(Varela, 1970) and will probably also be possible for the medulla cartridges in some insects. Ultrastructural clues are also available in some cases by which the cell type of neuronal processes are recognizable without needing to trace the neurone through long distances, but it is doubtful if all cell types can be so labelled and impossible to identify each representative of that type uniquely (e.g. between fly long retinula axon terminals 7 and 8 in the medulla). Similarly the pattern of dendritic arborization can be used to identify a neurone in a reconstruction from serial electron microscopy by correlation with the same pattern seen by light microscopy from Golgi impregnations. A recent development (Shelton, Horridge and Meinertzhagen in press) in which thicker sections are cut for viewing with electron microscope accelerating voltages of 200kV may prove a partial answer to the problem of cell identification since larger volumes of tissue may be sampled and neural processes followed to their cell of origin, but there is still an upper limit to section thickness, imposed by the confusion in the micrograph where the collective images of many processes are superimposed. Synaptic contacts are still conspicuous in these thicker sections and could be further enhanced by the use of synapse specific stains e.g. the phosphotungstic acid method of Aghajanian and Bloom (1967) (Lamparter, Steiger, Sandri and Akert, 1969) or the zinc-iodide method of Akert

and Sandri (1968) (Lamparter et. al., 1969).

In general, arthropod central synapses have all the features of chemical junctions in vertebrate nervous tissues, with well defined presynaptic ribbons and two sizes of vesicle (Trujillo-Cenoz, 1965; Hamori and Horridge, 1966,a; Steiger, 1967; Smith, 1967; Lamparter et al., 1969; etc.) although Varela (1970) saw no conspicuous presynaptic ribbons in bee lamina. The occurrence of specialized synaptic regions between two neurones can, according to current ideas, be taken as evidence of synaptic interaction and has been used, in the fly (Trujillo-Cenóz, 1967; Boschek, 1970) to infer the polarity of transmission.

Most authors are at pains to qualify conclusions based on such analyses (for the most recent of many reviews see Szentagothai, 1970; Akert and Sandri, 1970). In view of the polarized nature of chemical transmission it seems justified to accept the asymmetrical organization of morphological synapses as an indication of polarity until proved wrong more times than is acceptable. One anomaly to have emerged is the active site specializations identified between tall epithelial glial cells of the fly lamina and either a photoreceptor terminal or a centrifugal process as the presynaptic element (Boschek, 1970). This finding presumably requires a modification of our ideas of the function of glia as non-neuronal elements or of the function

of an active site as a region of rapid chemical transmission (see Discussion p. 195).

Methods combining electron and light microscopy

A variety of combined methods may be devised in an effort to complement the resolution of electron microscopy with the section size and thickness available with light microscopy. Only one such method has so far been used (Trujillo-Cenoz and Melamed, 1970) the EM/Golgi combined method (Blackstadt, 1965; Stell, 1967; Hillman, 1969). This method allows the examination by electron microscopy of the distribution of the processes of an impregnated neurone identified by light microscopy. Because the ultrastructure of the impregnated processes is not visible, synapses in which they are the pre-fibre cannot be identified, but by comparison between electron micrographs of Golgi silhouettes and limited serial electron microscopic reconstructions, Trujillo-Cenóz and Melamed (1970) were able to study the synaptology of the lamina basket endings of one of the medulla centrifugal neurones of the fly.

Other combinations of methods are described, e.g. Höllander (1970), Tyrer (1971); others will doubtless be devised.

Table 1

The first and second neural projections of insect eyes.

Retina Type Open (O) Fused (F)	Genera	Order	Retinula Cell Number
O	Calliphora Musca Lucilia (Drosophila)	Diptera	8 (6 + 2) (Dietrich 1909 etc.)
O	Benacus Lethocerus	Hemiptera	8 (6 + 2) (Walcott, in press)
O	Notonecta	Hemiptera	8 (6 + 2) (Horridge, 1968)
F	Schistocerca	Orthoptera	8 (6 + 2) (Horridge and Barnard, 1965 Horridge, 1966)
F	Apis (drone)	Hymenoptera	9 (6 + 3) (Perrelet, 1970) Cells 1 and 4 larger
F	Pieris	Lepidoptera	9 (4 + 4 + 1) Cells 8 and 6 larger (Nowikoff, 1931)
F	Trapezites	Lepidoptera	9 (7 + 2)
F	Libellula	Odonata	8 (4 + 4) (Horridge, 1968; for Sympetrium) 8 (5 + 2 + 1) (Eguchi, 1971; for Aeschna

Retinula axon projection
2 central cells have long retinula axons to medulla 6 short retinula axons terminate in six different cartridges (*Musca*, Braitenberg, 1967; *Lucilia*, Trujillo-Cenóz and Melamed, 1966; *Calliphora*, Horridge and Meinertzhagen, 1970).
Retinula axons diverge on entry into lamina. Exact pattern of connections unknown.
not known. Long visual fibres seen in Golgi preparations (Horridge, unpublished).
8 axons go to one lamina cartridge. 2 basal cells and one other have small axons.
9 axons go to one lamina cartridge. Cells 1-6 have short retinula axons 2 cells of 7, 8, 9 have long retinula axons to medulla.
9 axons go to one lamina cartridge. Cells 1-4, 6 and 8 have short retinula axons. Cells 9, 7 and 5 probably have long retinula axons. Strausfeld (1970) incorrectly describes some axons diverging.
9 axons go to one lamina cartridge; 8 and 9 extremely fine, have central position and possibly are long retinula axons.
8 axons to one lamina cartridge one axon extremely fine. Zawarzin (1913) describes only short retinula axons for *Aeschna*.

Known perpendicular neurons of lamina cartridge
__entering:__ 6 short retinula terminals from different ommatidia; 2 long retinula axons __leaving:__ 2 large monopolars; 2 small monopolars; 2 centrifugal from medulla; 2 long retinula (Boschek, 1970; _Musca_)
__entering:__ 8 axons from an unknown number of ommatidia __leaving:__ at least six axons
__entering:__ not known __leaving:__ at least six axons including two long retinula axons
__entering:__ 8 axons from one ommatidium remainder unknown __leaving:__ at least six axons of unknown identity
__entering:__ 6 short retinula terminals; 3 fine axons of cells 7-9 __leaving:__ 2 long retinula axons (from cells 7-9); 3 monopolars probably 2 large, 1 small; 1 large axon, probably a fourth monopolar; 1 fine axon, probably a centrifugal
__entering:__ 6 short retinula axons and 3 axons of cells 7-9 __leaving:__ 2 long retinula axons probably and at least 4 more including one with a peripheral position in the cartridge
__entering:__ 9 retinula axons __leaving:__ at least 6 axons of unknown identity

Lamina-medulla projection
At least eight axons of one cartridge traverse the chiasma, of which six go between a single lamina and a single medulla cartridge. These are: 2 long retinula, monopolars L_1, L_2 and L_3 and one other probably centrifugal.
not known
At least six axons go between a single lamina and a single medulla cartridge.
not known
At least seven axons go between a single lamina and single medulla cartridge. These are: 2 long retinula, 3 monopolar and 2 other (one of which probably is centrifugal).
Possibly permutation of lateral relations between long retinula and lamina monopolar neurones (Strausfeld, 1970). Otherwise not known.
At least six axons go between a single lamina and a single medulla cartridge.

D. The retina and optic lobes of the insects studied

A summary is given of the current state of knowledge about the arrangement and organization of the retinae and optic lobes of the insect species that have been studied in this work. The account is condensed in table 1. Information is original where no reference is given.

The fly. 1. The retina of the fly

The ommatidium of the fly retina has an open-rhabdomere arrangement of eight retinula cells. Photoreceptive rhabdomeres are borme on the inner margin of six separate retinula cells (1-6), while the seventh rhabdome is composed of two rhabdomeres, those of the pair of central cells 7 and 8 (Dietrich, 1909; Trujillo-Cenóz, 1968). In transverse section the seven structures form a characteristic radially asymmetrical pattern best seen just beneath the dioptric apparatus. This pattern, first described by Dietrich (1909), is seen in a wide variety of dipterans of the sub-order Brachycera (the pattern in various nematoceran dipterans on the other hand is symmetrical). The retinal array of these rhabdomere patterns has a sharp discontinuity about a horizontal equator so that the pattern of an ommatidium of the ventral half is the mirror image of that in the dorsal (Dietrich, 1909). The retinula cells which were numbered 1-8 by Dietrich have opposite rotational sequences in the two halves (fig. 5, p. 68). The arrangement of the two central

cells 7 and 8 in the ring of photoreceptor cells is as follows: cell 7 (the superior central cell of Melamed and Trujillo-Cenóz, 1968) is situated between cells 1 and 6 and contributes the distal portion of the central rhabdome; cell 8 (the inferior central cell) is situated between cells 1 and 2 and contributes only 75 µm of the central rhabdome. The rhabdomere of cell 8 has microvilli arranged orthogonally to those of the superior rhabdomere overlying it and throughout the basal 75 µm in which it is situated, cell 7 is represented by an axon (Trujillo-Cenóz and Melamed, 1966; Melamed and Trujillo-Cenóz, 1968).

The optical consequence of the distribution of the intraommatidial rhabdomeres at the bottom of the pseudocone is that each rhabdomere accepts light from a different portion of the visual field to its intraommatidial neighbours (Autrum and Wiedemann, 1962; Kuiper, 1962; Wiedemann, 1965) the exact portion being determined by the lateral position of the rhabdomere (Kirschfeld, 1967). The spatial relationships between the rhabdomere optical axes within ommatidia and the angles between neighbouring ommatidia is such that seven rhabdomeres (each of a different cell number, one within each of seven neighbouring ommatidia) have the same optical axis (Kirschfeld, 1967). This pattern is the consequence of the rhabdomere divergence angles being equal to the interommatidial angle. Gemperlein (1969) has

described the patterns of facet rows of the cornea in terms of two sets of concentric arcs, the centres of which are located in the dorsal and ventral halves of the retina. The patterns of intersection of these arcs can be used to predict, with respect to the external coordinates of the animal, the exact arrangement of the rhabdomeres of any ommatidium and the exact arrangement within any group of ommatidia of those rhabdomeres with coincident optical axes.

2. The neurones of the fly lamina

In this work results from various genera of brachyceran flies will be used interchangeably except where specific differences are known to exist. Cajal and Sánchez (1915) used *Calliphora vomitoria* and the horse-fly *Tabanus bovinus*; Kirschfeld, Braitenberg, Boschek and the Tubingen group use the house-fly *Musca domestica*; Trujillo-Cenoz uses blowflies mainly *Lucilia* spp. but also *Calliphora* and the flesh fly *Sarcophaga*; Strausfeld (1970) used *Calliphora vomitoria* and *C. vicina* (= *erythrocephala*), and the hover flies *Eristalis tenax*, *Syrphus elegans* and *S. nitidicollis* while Horridge and Meinertzhagen (1970 a) used *Calliphora vomitoria*.

Six short retinula axons from six different ommatidia converge upon a single cartridge of the lamina (Trujillo-Cenoz and Melamed, 1966; Braitenberg, 1967; Horridge and Meinertzhagen, 1970 a) where they have simple cylindrical terminals (Cajal and Sanchez, 1915; Strausfeld, 1970).

They encompass and make synaptic connection with the axons of two monopolar neurones (Trujillo-Cenóz and Melamed, 1963), which were labelled L1 and L2 by Braitenberg (1967) but which are called g (=L1) and G (=L2) in this account. These two neurones are uniquely identifiable by subtle differences in their position and morphology. Two additional smaller monopolar neurones L3 and L4 with cell bodies situated immediately above those of L1 and L2 have axons that proceed as a pair down the posterior-equatorial quadrant of the periphery of the cartridge, usually between retinula terminals 5 and 6 (Braitenberg, 1967; Boschek, 1970). The axon of L3 is larger than that of L4 and receives synaptic input from at least one retinula terminal, but the axon of a fifth monopolar neurone described by Trujillo-Cenóz and Melamed (1970) for Lucilia (and also for Calliphora and Sarcophaga) is not known. A variety of types of monopolar neurones is known from Golgi impregnations (Cajal and Sánchez, 1915; Strausfeld, 1970) but in Calliphora the lateral extents of their dendrites are never more than one cartridge width (Strausfeld, 1970). The correlation between the Golgi silhouettes of Strausfeld (1970) and the classification derived from reduced silver methods (Braitenberg, 1967; Strausfeld and Braitenberg, 1970) based on cell body position and axonal location within the cartridge, is not yet available (see Discussion, p.179). L4 has lateral connections to the L1 and L2 axons of

neighbouring cartridges (Strausfeld and Braitenberg, 1970), and probably these connections are presynaptic to either or both through an unidentified fibre fragment (Boschek, 1970). Both L3 and L4 receive synaptic inputs in *Musca* from the two members (α and β) of the remaining class of perpendicular neurone, the centrifugal neurones of the medulla (Boschek, 1970). Three types of centrifugal neurones are known from Golgi impregnations of several species (Cajal and Sánchez, 1915; Strausfeld, 1970). One of these has a basket ending which surrounds a single lamina cartridge and in *Lucilia*, *Calliphora* and *Sarcophaga* the processes of this ending are said to contain synaptic ribbons at sites *presynaptic* to retinula terminals (Trujillo-Cenoz, 1965; Trujillo-Cenóz and Melamed, 1970). In contrast, in *Musca* it is the two centrifugal neurones which are said to be *postsynaptic* to the retinula axon terminals (Boschek, 1970). In addition, the two centrifugal neurones make synaptic contacts with themselves and with additional unidentified fibre fragments (Boschek, 1970) but the lamina ending of the second (or possibly third) centrifugal neurone(s) is not yet identifiable with types known from Golgi impregnations, nor is it known if the same types are found in all lamina cartridges of one species. The remaining known synaptic connections, in *Musca*, are onto a tall epithelial glial cell first described by Cajal and Sánchez (1915) with a periodic distribution

between the cartridges throughout the lamina. These cells are post synaptic to the short retinula cells and to one of the centrifugal neurones of the cartridge (Boschek, 1970) but have not been described as presynaptic to any element. Identification of synaptic polarity in all cases is based upon the location of synaptic ribbons in presumed presynaptic neurones, with the exception of the main retinula terminal-monopolar synapses which are known from electrophysiological records of the responses of dye-injected postsynaptic cells (Autrum, Zettler and Järvilehto, 1970; Ioannides, personal communication). The long retinula axons are unique among the perpendicular neurones in having no synaptic contacts in the lamina (Trujillo-Cenóz, 1965; Boschek, 1970).

In addition to the perpendicular neurones of the lamina, representatives of tangential and amacrine cells are also known to occur in Golgi impregnated preparations. Three types of tangential neurone with aperiodic distributions are described by Strausfeld (1970), one type with a large cell body beneath the lamina; of the remaining pair one (Lam :tan2) has not been seen in Calliphora. Amacrine cells of one type only are known and are described both by Cajal and Sánchez (1915) and Strausfeld (1970); they have a periodic distribution with a small cell body immediately beneath each cartridge. Their synaptic connections are not known but their lateral extents as seen in Golgi impregnations do not exceed the

compass of the immediately adjacent cartridges. Absence of further information is an important omission in our knowledge of the functional connections of the cartridge.

3. The lamina-medulla projection of fly

Each lamina cartridge is represented by a bundle of axons in the chiasma that contains at least eight axons visible by electron microscopy (Boschek, 1970). These are: two large monopolar L1 and L2, two small monopolar L3 and L4, two long retinula and two centrifugal. Five of these were seen and traced through the medulla by Horridge and Meinertzhagen (1970 a, b) using light microscopical methods. The final number of axons is probably larger than eight since Trujillo-Cenóz and Melamed (1970) report a fifth monopolar neurone, and Strausfeld (personal communication) has counted at least ten and up to twelve axons in each chiasma bundle, although at least one of the twelve is of an aperiodic tangential neurone.

Some (and probably all) axons of these bundles project homotopically upon the medulla after crossing in the chiasma (Strausfeld, 1970; Horridge and Meinertzhagen, 1970 a,b).

In the medulla, axons from the chiasma form cartridges but the number of cartridges is not clear. Thus, Strausfeld (1970) describes from reduced silver stained sections two medulla columns for each lamina cartridge, with one long monopolar axon (either L1 or L2) entering each column,

whereas Horridge and Meinertzhagen (1970 a, b) describe a one-to-one correspondence between cartridges of the two neuropiles with all the axons of one entering the other.

The two large lamina monopolar neurones L1 and L2 stay together in the chiasma (Trujillo-Cenóz, 1969; Horridge and Meinertzhagen, 1970 a) but terminate at different levels in the medulla neuropile (in common with all monopolar neurones; Strausfeld, 1970) after establishing numerous synaptic connections along their path through the first synaptic stratum (Trujillo-Cenóz, 1969).

The two long visual fibres are described by Strausfeld (1970) with three types of medulla ending, two at the same level and one slightly more superficial (a fourth form is seen in E. tenax only). All forms terminate at a deeper level than either the lamina monopolar endings or the medulla component of centrifugal neurones (Cajal and Sánchez, 1915; Strausfeld, 1970) and their terminals have been identified from ultrastructural criteria in electron micrographs at this level (Melamed and Trujillo-Cenóz, 1968). They contain synaptic structures but the processes to which they are presynaptic remain unknown (Melamed and Trujillo-Cenóz, 1968).

The medulla component of the centrifugal neurone, which has a basket ending in the lamina, has a large terminal in the first synaptic stratum of the medulla. This is in close association with the large club ending of one of the lamina

monopolars (L1 or L2) (Trujillo-Cenóz and Melamed, 1970). Although synaptic connections between these two terminals is not known certainly, they are seen in the monopolar ending at the point of contact with a Golgi-impregnated centrifugal terminal, while presynaptic ribbons are not seen in the unidentified presumed centrifugal terminal in unstained material (Trujillo-Cenóz, 1970). Thus the evidence of Trujillo-Cenóz (1970) is consonant with the basket ending controlling the activity of the lamina cartridge through a feedback loop from the medulla, while in Musca the morphological polarity of the photoreceptor terminal-basket terminal synapses (Boschek, 1970) is inappropriate for such a circuit.

Tangential processes in the first synaptic layer, called by Cajal and Sanchez (1915) the first plexus of snaky fibres, establish synaptic contact with one of the pair of lamina monopolar terminals. Several monopolar terminals were observed to be presynaptic to a short segment of a tangential process (Trujillo-Cenóz, 1969). This process belongs to a superficial tangential neurone, apparently that classified by Strausfeld (1970) as the type 3 medullary tangential (Med: tan. 3).

Up to nine types of medulla monopolar neurones are described by Strausfeld (1970), which leave the medulla and convey information to the deeper neuropiles, but the cells have no described synaptic input, nor is the number of such

neurones in each medulla cartridge known.

The water-bug and *Notonecta* 1. The retinae

The retina of Lethocerus is described by Walcott (1971 a, in press). There are a total of eight retinula cells, six arranged around the periphery of the ommatidium and two at the centre. The arrangement of the rhabdomeres of these eight cells and the morphological changes on light/dark adaptation show structural characteristics of both fused- and open-rhabdomere eyes. Each cell contributes a rhabdomere to the box-shaped rhabdome; each of these rhabdomeres tapers proximally and does not extend to the basement membrane. As a consequence of the tapering, the rhabdome alters in cross sectional appearance from the distal end, where the rhabdomeres are packed tightly together, to the proximal end where they are arranged in an open box configuration surrounding the rhabdomeres of the two central cells 7 and 8. The rhabdomere microvilli are orientated in orthogonal planes with four of the outer retinula cells and the large central cell 7 having microvilli in one plane, at right angles to those of the remaining two peripheral cells and cell 8. Striking structural changes occur between the dark- and light-adapted ommatidium (Walcott, 1969, 1971 a). In the dark-adapted retina the rhabdome is closely applied to the underside of the cone, while in the light-adapted retina

these two structures become separated by a crystalline tract 40 µm long which elongates from the four cone cells.

The retina of Notonecta has a similar retinula cell composition to that of Lethocerus (Horridge, 1968 a) but differs in the following respects. The arrangement of the six peripheral rhabdomeres is more open, each rhabdomere being quite separate. The two central cells have adjacent rhabdomeres but these do not lie entirely at the same level. Where they overlap they are closely applied and electron micrographs show that the microvilli are parallel, not orthogonal, both sets orientated along the antero-posterior axis of the animal.

2. The optic lobes

There is one published account of Golgi studies on heteropteran optic lobes (Pflugfelder, 1937). The preparations are mainly from the bug Pyrrhocoris and are incomplete. Two observations are relevant to the work reported here. First, no long visual fibres were impregnated and second, the short retinula axons apparently do not interweave in the lamina but are disposed radially. An unpublished observation on Golgi preparations of the optic lobes of Notonecta is that long visual fibres are found in this animal and that they consist of at least one stout axon running perpendicularly through the lamina.

The locust 1. The retina and lamina of the locust

The retina of locust is described in accounts, now known to be inadequate, by Horridge and Barnard (1965) and Horridge (1966 a). Each ommatidium consists of a total of eight retinula cells with either six or seven long retinula cells and either two or one eccentric cells (Horridge and Barnard, 1965). In this and a subsequent account (Horridge, 1966 a) the eccentric cell has not been properly distinguished from a pair of basal cells in each ommatidium (see Discussion, p.170). The rhabdomeres of each of the long retinula cells are fused into a single triangular rhabdome with microvilli orientated along three axes separated by 120°. The (one or two) eccentric retinula cells contribute very few rhabdomeric microvilli.

Some cell types of the optic lobe are known from fragmentary observations of Cajal and Sánchez (1915) on three species of orthopterans including Locusta. From reduced silver preparations it has proved impossible to trace the projection of the eight axons of one ommatidium to the lamina (Burtt and Catton, 1962; Shaw, 1968 a) although from Golgi impregnations, Horridge (1968 b) shows four of eight retinula axons terminating on a pair of monopolar neurones in a single cartridge, while there is at least one long retinula axon from each ommatidium. It was further observed from Golgi impregnation that at least one short

retinula axon diverged laterally to a different cartridge from that receiving other retinula axons from the same ommatidium (Horridge, personal communication),

The neurones of the lamina described by Cajal and Sánchez (1915) (in an acridid orthopteran) are two types of monopolar, small and large, both with lateral dendrites of not very wide extent and some with an extremely long isthmus between cell body and axis fibre. In addition, Strausfeld has observed large monopolars with lateral wide dendrites at the base of the cartridges only (Strausfeld and Blest, 1970). The lamina terminations of centrifugal neurones are also shown; they have very wide-field basket endings.

The honey-bee 1. The retina of the bee

The retina of the honey-bee is known definitively from an account by Varela (Varela and Porter, 1969; Varela, 1970) on worker bee, and the work of Perrelet (Perrelet and Baumann, 1969; Perrelet, 1970) on drone; the two differ in some respects, and drone has larger ommatidia and a wider retina than worker.

Both worker and drone ommatidia have six long retinula cells (Perrelet and Baumann, 1969; Perrelet, 1970; Varela and Porter, 1969), two of which are larger in diameter and have fatter axons than the others, at least in some ommatidia under some conditions of fixation (Gribakin, 1969).

In addition, in the drone there are three small retinula cells each giving rise to an axon (Perrelet and Baumann, 1969; Horridge and Meinertzhagen, 1970 b), each bearing a few microvilli and therefore of presumed sensory function (Perrelet and Baumann, 1969). These three cells are SR1 (labelled cell 8 in this work), SR2 (cell 7 in this work) and SR3 (cell 9 in this work). Cell SR3 (9) is smaller than the other two and occurs only in the basal portion of the ommatidium, whereas SR1 and SR2 (8 and 7) occupy the whole length of the ommatidium. Other accounts of both worker and drone ommatidia have probably misinterpreted or missed the presence of SR3. Naka and Eguchi (1962) reported eight cells in drone and Goldsmith (1962) a similar number in worker (in both cases because sections were examined at a level too distal to include the basal cell), whereas a similar account of Varela and Porter (1969) on worker was later modified (Varela, 1970) to account for the ninth cell by the observation that the ninth axon arose from a bifurcation of SR2. Only Perrelet and Baumann (1969 a) have observed the nucleus, the most decisive evidence of the ninth cell's independant existence.

The hairs covering the cornea give rise to the remaining type of neurone in the retina. These hairs, of presumed mechanoreceptive function, possess a dendrite but the cell body is not known from electron microscopy (Perrelet, 1970).

The cell body underneath the sensillum is described by Sánchez (1920) from Golgi impregnations together with a fine axon passing through the retina and piercing the basement membrane. This axon is not however one of the nine axons of the ommatidial bundle, indeed Strausfeld (1970) describes the first-order hair receptor fibres as bypassing the lamina and deep optic lobe entirely.

2. The neurones of the bee lamina

Neurones of the bee lamina are known from two studies on workers, the Golgi study by Cajal and Sánchez (1915), an electron microscopic study by Varela (1970) and a third study on drone (Horridge and Meinertzhagen, 1970 b). The neuropile of the lamina is conspicuously divisible into three zones (Cajal and Sánchez, 1915), narrow upper and lower zones and a wide middle zone (Varela, 1970; see Fig. 1).

The axons of one ommatidium are distributed within a single cartridge of the lamina (Horridge and Meinertzhagen, 1970 b; Varela, 1970). Six short retinula axons terminate in that cartridge (Horridge and Meinertzhagen, 1970 b; Varela, 1970) forming coarse tufted terminals composed of a few parallel processes. These are found at two different levels of the middle zone of lamina neuropile (Cajal and Sánchez, 1915), short terminals ending in the middle of the middle zone, and long terminals ending at the bottom of this layer. Two of the axons of the three small retinula

cells pass through the lamina at the centre of their cartridge (Horridge and Meinertzhagen, 1970 b; Varela, 1970) without making synaptic contact (Varela, 1970) and enter the chiasma (Horridge and Meinertzhagen, 1970 b). The axon of the third cell in worker terminates in the lamina (Varela, 1970), probably in the bottom of the middle zone of that neuropile (see Discussion, p.173).

The short retinula axons are presynaptic to the lateral processes of the two types of monopolar neurone in the lamina (Varela, 1970). These two types are the large monopolars of Cajal and Sánchez (1915) with lateral narrow field processes throughout the middle zone of the lamina and often with further processes in the lower zone, and the small monopolars with lateral processes of considerably wider spread in only the upper and lower lamina zones. Cajal and Sánchez (1915) have reported that for the short visual fibres, those with short terminals are in close association with monopolar axons having small-field dendrites in only a small vertical depth of the lamina. Those with long terminals are close to monopolar axons with lateral dendrites extending down to the very bottom of the middle zone of lamina neuropile. Additional monopolars have been seen by Strausfeld with a wide-field dendritic spread in the lower zone of the lamina only (Strausfeld, unpublished; in Strausfeld and Blest, 1970). Because of the redefined

terminology of these latter authors, they are described as large monopolars although they are clearly similar to the small monopolar neurones of Cajal and Sánchez (1915). The Spanish authors have pointed out that because of the rather well developed stratification of the lamina in bee the wide-field dendrites of monopolar neurones cannot directly have a retinula input because the short retinula terminals do not penetrate the lower zone of the lamina. Consequently the monopolar dendrites of the lower lamina zone must receive activity exclusively from centrifugal and/or amacrine neurones (Cajal and Sánchez, 1915). Varela (1970) described one cartridge as containing usually two large and one small monopolar neurones together with an unspecified number of the other class of perpendicular element, the medulla centrifugal neurone, first described by the Spanish authors.

Two types of centrifugal neurone are distinguished, both with cell bodies in the external zone of the chiasma (Cajal and Sánchez, 1915). The first type has a termination in the lamina only, and might functionally therefore be a type of amacrine. The second is T-shaped with terminations in both lamina and medulla. The processes of both types are especially dense in the lower zone of the lamina and receive synaptic input from short retinula axons and also from other centrifugal processes (Varela, 1970). The centrifugal

processes are themselves presynaptic to retinula terminals and to monopolars of unspecified type (Varela, 1970).

3. The lamina-medulla projection

Long retinula, monopolar and centrifugal axons are grouped together in one bundle beneath each lamina cartridge. Horridge and Meinertzhagen (1970, b) have reported the existence of at least five axons in this bundle, though the number now traced is seven (see Results). Each bundle of five axons projects to a single cartridge of the medulla (Horridge and Meinertzhagen, 1970, b).

Pieris 1. The retina of *Pieris*

The retina of *Pieris* is known from an accurate light microscopic study by Nowikoff (1931) on *P. napi*. The arrangement of nine retinula cells within each ommatidium is summarised as follows. Eight long retinula cells are divisible into two groups of four by the positions of their nuclei. The four cells with distal nuclei have a prominent connection with the central fused rhabdome in the distal part of the ommatidium while the proximal four cells with nuclei about half way down the ommatidium are only conspicuously in contact with the rhabdome in the proximal half of the ommatidium (Nowikoff, 1931). In other words, the ommatidium has the appearance of being tiered. The two quartets of cells form crosses in transverse sections

of ommatidia with the arms of one cross at 45° to those of the other so that cells from the two groups occupy alternate circumferential positions in the ommatidium. A ninth cell with a nucleus at the base of the ommatidium is depicted by Nowikoff (1931) in only a few transverse sections of ommatidia. Another receptor cell body described by Strausfeld and Blest (1970) from methylene blue staining of P. brassicae is situated just beneath the cone in some ommatidia and corresponds to minute hair receptors scattered sparsely over the cornea.

At its proximal end the fused rhabdome contacts a cuticular elaboration (the "tracheenblase") of the junction of a prominent bifurcate tracheole (Nowikoff, 1931). This structure represents the reflecting body found in many lepidopterans which is responsible for the phenomenon of eye-glow in these animals (Miller and Bernard, 1968), first seen in Pieris by Exner (1891).

2. The neurones of the lamina of Pieris

The neurones of the lamina (and medulla) in Pieris are known from one study only from Golgi impregnations by Strausfeld and Blest (1970).

Short retinula axons are described with three types of simple ending and groups of these terminals, apparently within one cartridge, are seen in both Pieris and the hawkmoth Sphinx. The groups comprise one type 2 (a slender

terminal), two type 3 and three to four type 1 terminals (types 1 and 3 are of similar calibre). Although four of these are said to project from one ommatidium, Strausfeld (1970) claims two more are derived from nearby ommatidia, an observation at variance with this work (see Results).

Three types of long retinula axons are also described, but each cartridge apparently has only one pair consisting of one of some of the combinations of the three types. This pair has a satellite position to its cartridge (a finding also at variance with this work, see Results).

There are four types of small monopolar neurone (by the criteria of Strausfeld and Blest, 1970) i.e. all have a narrow-field simple dendritic spread like those of the fly. There is at least one pair of monopolar neurones at the centre of each cartridge. Two centrifugal neurones are also known both with lamina basket endings, one with a lateral spread of a single cartridge width, the other with a width of two or three cartridges.

One type of tangential neurone only is described in Pieris, with arborizations at the peripheral and central margins of the lamina and linking fibres which project distally to the level of the receptor cells derived from the interommatidial hairs and centrally to the medulla. (This system does not appear to have been confused with the system of more numerous retinula axons). Each neurone has a strip

field across the whole vertical extent of the lamina and for a quarter of the horizontal width, and there are probably three or four such neurones in each eye.

Amacrine neurones are not described but neurones similar to the amacrines of the fly, with perikarya underlying the lamina, probably exist.

3. The lamina-medulla projection of *Pieris*

From reduced silver-stained preparations Strausfeld (1970) has tentatively described the projection of groups of four axons ("quads") through the chiasma upon the medulla. Each quad, composed of a pair of monopolar axons from the centre of each cartridge, and a pair of long visual fibres satellite to each cartridge, projects upon two neighbouring medulla cartridges so that each cartridge receives four fibres, two long retinula fibres of one lamina cartridge and two monopolar axons from an adjacent or subadjacent cartridge. This observation is at variance with the chiasmal projection described for the perpendicular neurones of other fused-rhabdomere eyes (Horridge and Meinertzhagen, 1970 b).

The skipper 1. The retina of the skipper

The ommatidia of various hesperioid lepidopterans are known from rather inadequate accounts by Yagi and Koyama (1963)(on various species), Miller, Bernard and Allen (1968) and Swihart, 1969 (on *Epargyreus*), while some early electron micrographs of the eight long retinula cells and their

rhabdomeres in _Epargyreus_, are available from Fernández-Morán (1958). Unpublished observations by Giddings and Horridge are also available (see Results).

Skippers are diurnal lepidopterans with ommatidia containing a crystalline thread i.e. a superposition eye. Beneath the crystalline thread lie the eight long retinula cells (Fernández-Morán, 1958; Giddings and Horridge, unpublished observations) with distal nuclei (Yagi and Koyama, 1963; Swihart, 1969). The cells are surrounded by a ring of tracheoles (Fernandez-Moran, 1958; Bernard and Miller, 1970) which functions as a tapetum and is responsible for the eye-shine of this animal (Miller et al., 1968).

At the base of the ommatidium are the nuclei of two cells, the ninth basal retinula cell and a basal pigment cell beneath it at the level of the basement membrane (Giddings and Horridge, unpublished observations). The existence of the basal pigment cell only has been reported previously (Swihart, 1969).

No previous study has been made of the skipper optic lobe.

E. The physiological and behavioural analysis of compound eye function

Three main approaches to compound eye function have been followed. In ascending order of complexity of the system they analyse, these are:

First, single cell analysis from intracellular recording and marking techniques mainly of the retinal receptors and also latterly in the lamina. These have now largely superceded the more doubtful analysis of ERG mass responses from the entire eye.

Second, single unit analysis, usually extracellular often from spiking, movement-sensitive neurones of unknown origin and wide lateral spread within the deep neuropiles of the optic lobe.

Third, the analysis of perception in intact animals either by optomotor responses of stationary animals or by the orientation or discriminatory behavour of freely moving animals.

The four stimulus parameters that have received most attention are spectral composition, orientation of E-vector component, angle subtended at the cornea and movement. The first three of these are qualities that characterize the retinal responses while movement sensitivity necessarily involves temporal correlation at a more central level between parallel receptor inputs.

<u>Evidence from the fly</u>

Most complete information about compound eye function is available from a wide range of experimental approaches for the fly eye. The angular sensitivity of the large retinula cells (Kirschfeld, 1965; Vowles, 1967; Scholes, 1969).

the polarization sensitivity of long retinula cells (Scholes, 1969), the spectral sensitivity of both long retinula cells and central retinula cells (Burkhardt, 1962) and the spectral absorption of their rhabdomeres (Langer and Thorell, 1967) have been studied. Responses (depolarizations) from the convergent ring of short retinula terminals were described by Scholes (1969) and their corresponding second-order responses by Autrum et al., (1970). Responses from the deep optic lobe have been recorded from wide-field units with sensitivity to motion, form and intensity (McCann and Dill, 1969) but the identity of these units is not known nor is the anatomical substrate of the integrative mechanism by which their activity is derived. Behavioural evidence on the flight control system in Drosophila and Musca (Götz, 1968) indicates the existence of two subsystems independently controlling flight torque and thrust which are maximally sensitive to movement in horizontal and vertical planes respectively. Units which have sensitivities corresponding to those predicted for one component of the torque subsystem of flight control have been recorded by Bishop and Keehn (1967) and their spectral sensitivity indicates that their receptor input involves the six short retinula terminals. In addition some anatomical information is available on the various neurone types present in the optic lobe and on some of their interconnections (see p. 30).

Evidence from the bee

Bees have also been a favourite insect for the analysis of compound eye function. Outstanding is the extraordinary wealth of information available on navigation, pattern recognition and discriminatory behavour (von Frisch, 1967; Lindauer, 1969 a, b). This provides striking examples of visual memory which in combination with the large repertoire of instinctive behaviour shown by bees makes the experimental analysis of visual function in this animal particularly desirable. An elementary distinction will be made at this point viz. that the behavioural evidence is derived entirely from worker bees whilst for technical reasons most information about receptor physiology has been derived from drone bees and that there are probably significant differences between the two castes. Information is available for drones on angular sensitivity and polarization sensitivity (Shaw, 1969 a), on spectral sensitivity (Shaw, 1969 a; Autrum and von Zwehl, 1964) and on interreceptor coupling of retinula cells (Shaw, 1969 a) while in workers only angular sensitivity (Laughlin and Horridge, in preparation) and spectral sensitivity (Autrum and von Zwehl, 1964) are known. One account is available on the responses of direction sensitive movement detector units from the deep optic lobe and of their spectral sensitivity (Bishop, 1970). Some relevant information from behavioural work is that worker bees

have both a well developed polarization plane vision (von Frisch, 1950) and colour vision (Daumer, 1956) upon which much of their visual behaviour is based. The polarization plane sensitivity is itself maximally sensitive in the UV wavelengths suggesting that the receptor inputs to the polarization analyser are maximally sensitive in the UV (von Frisch, 1967). This test has been made for the dorsal half of the eye.

There are several accounts of the anatomy of bee retina and optic lobe, from electron microscopy (Perrelet and Baumann, 1969 a, b; Perrelet, 1970; Varela and Porter, 1969; Varela, 1970) and from Golgi studies (Cajal and Sánchez, 1915) see p. 40.

Evidence from locust

Work on locust compound eye includes fairly complete reports on receptor physiology. Angular sensitivity (Shaw, 1968 b), polarized light sensitivity (Shaw, 1968 b), spectral sensitivity (Bennett, Tunstall and Horridge, 1967), the relative absence of interreceptor coupling (Shaw, 1967) and the effects of light- and dark-adaptation on receptor properties (Tunstall and Horridge, 1967) are all known. Shaw also describes hyperpolarizations from the lamina region which are interpreted as originating from second-order units (Shaw, 1968 b). Higher order activity has been recorded

from a wide variety of units in the deep neuropiles of locust optic lobe (Horridge, Scholes, Shaw and Tunstall, 1965; Burtt and Catton, 1956, 1959, 1960) but the responses are not readily interpretable in terms of known receptor properties nor has their description clarified any mechanism of integration occurring within the optic lobes. Similarly units recorded from the ventral nerve cord in locust (Burtt and Catton, 1954) in response to light on or off and to optokinetic movements represent conveyance of visual information to particular sites in the motor system but have proved of little interpretative value in the analysis of movement perception. Ventral cord units are not synchronous with any recorded activity in optic lobe (Burtt and Catton, 1956). The properties of the ventral cord unit first described by Burtt and Catton were also analysed in the thoracic connective in response to rapid dimming of a light object in the visual field (Palka, 1967). The responses of the unit to rapid dimming of one object are blocked by a variety of procedures involving dimming and manipulation of two independant objects and the characteristics of the block are attributed to a single inhibitory process. Some interesting behavioural phenomena have been revealed by studies in which the performance of the visual system was tested using the optomotor responses. Thorson (1966 a) has shown that the eye can detect very small movements (20 seconds

of arc) over the whole visual field, implying extensive summation of many very small but temporally coincident signals. The acuity of the eye is limited however by the acuity of single facets, since responses are not elicited by stripe widths less than 3°. The eye responds to frequencies of stimulus oscillation from 0.0014 Hz to 6 Hz (Thorson, 1966 b) and reasons are suggested for the limitation of the frequency response at both high and low frequencies (see p.). One remaining phenomenon, that of an optokinetic memory in locust (Horridge, 1966 b) similar to that demonstrated in the crab Carcinus (Horridge, 1966 c) shows that the locust visual system is capable of retaining information concerning the visual field for many seconds in the dark.

Evidence from the skipper

Only one account of colour vision and physiology of the visual system of a skipper is available, Swihart (1969). From ERG responses it is claimed that receptors with two spectral sensitivities, one with a maximum at 440nm, the other at 540nm are present and simple behavioural evidence suggests that colour vision is present in these insects. A variety of optic lobe and protocerebral units were found but at present the significance of these is not known.

Evidence from *Pieris*

One piece of information relating to colour vision in *Pieris* is provided by behavioural evidence. *Pieris* apparently has colour vision, at least in the male, since during courtship recognition of the female of *P. rapae* has been shown to occur by the reflection spectra of the wings (Obara, 1970). Various parameters of the visual signal, such as pattern, size and markings, are dispensible for satisfactory sexual behaviour providing that near-ultraviolet wavelengths (380 - 400nm), those normally reflected from the females' wings, are present.

Evidence from water-bug and Notonecta

Some preliminary unpublished observations on the receptor physiology of water-bug are known (Ioannides, personal communication). The dark-adapted retinula cells have wide angles of acceptance (Walcott, 1971b) which narrow on light-adaptation. The directions of polarization sensitivity are in two orthogonal planes and the sensitivity ratio to light polarized in different planes is 3 : 1. The only spectral sensitivity data available are for cells with maxima in green and UV wavelengths (Ioannides, personal communication).

In the related genus *Notonecta* some differences from the retinal arrangement and sensitivity of *Lethocerus* are

known. The organization of the ommatidium is similar to that of <u>Lethocerus</u> but differs in having the microvilli of the two central retinula cell rhabdomeres arranged in parallel and not orthogonally; both are horizontal (Horridge, 1968 a). In <u>Lethocerus</u> polarized light and spectral sensitivities of the large retinula cells are similar to those of fly. In both, PL sensitivity ratio is approximately 3 : 1 (Scholes, 1969; Ioannides, personal communication), a similarity arising from the open arrangement of the rhabdomeres in these animals (Shaw, 1969 b). The only spectral sensitivity types so far encountered in water-bug retina (Ioannides, personal communication) are the same as those most frequently encountered in the fly (Burkhardt, 1962) which correspond with the absorption of rhabdomeres 1 - 6 (Langer and Thorell, 1967) and have peak sensitivities in green and UV wavelengths. In <u>Notonecta</u> on the other hand spectral sensitivity types are distributed in the dorsal half of the retina with very nearly the same frequencies as those of worker bee (Bruckmoser, 1968). <u>Notonecta</u> is curious, as the eye is known behaviourally to be colour responding in the dorsal half but colour blind in the ventral (Rokohl, 1942 in Burkhardt, 1964). Because of the backswimming locomotion in this genus this arrangement results in colour vision in the half of the visual field that is directed downwards, as in dragonflies and probably worker-bee.

MATERIALS AND METHODS

A. Animals

Eyes of the following animals have been used:-

Blowfly	Calliphora vomitoria	both fromm culture
	* Calliphora vicina (=erythrocephala)	
	Calliphora stygia, a local Australian species obtained wild.	
Locust	Locusta migratoria	both from culture
	* Schistocerca gregaria	
Honey-bee (drone)	Apis mellifera from culture	
Dragonfly (larvae and adults)	Aeschna cyanea from Haig's Aquatic Nursery, Surrey, and a small Australian libellulid collected locally.	
Water-bug	Lethocerus americanus	from Florida, U.S.A.
	* Benacus griseus	
	Lethocerus insulanus from Queensland and northern N.S.W.	
Backswimmer	Notonecta (glauca?) collected locally from St. Andrews, Scotland.	

* *Notonecta* spp. collected from
  Woods Hole, Mass., U.S.A.

Skipper * *Trapezites symmomus* collected from
South Coast, N.S.W.

Small white butterfly * *Pieris rapae* collected locally from Canberra.

Detailed results are presented for those species marked with an asterisk.

B. Fixation

A variety of fixatives have been employed, all buffered, at approximately pH 7.4. These included phosphate buffered osmium tetroxide without sucrose (Millonig, 1956), veronal acetate buffered osmium tetroxide (Palade, 1952), and veronal acetate buffered glutaraldehyde (Sabatini, Bensch and Barrnett, 1963) or cacodylate buffered paraformaldehyde and glutaraldehyde (the diluted form given by Karnovsky, 1965) followed by postosmication.

Generally material was fixed at 4°C and long fixation times have been employed (up to 12 hrs.) because they appeared to enhance the staining contrast of tissue. Occasionally material in fixative was placed under a slight vacuum to aid the

evacuation of the tracheal system and its subsequent filling with fixative.

No systematic study was undertaken on the effects of different fixatives but the material was selected for good visibility of axon profiles in sections for light microscopy and not necessarily for good ultrastructural preservation.

C. Dissection

The dissection of the various insect eyes has proved important in obtaining satisfactory fixation, and in particular the careful but thorough removal of tracheae and air sacs from around the optic lobes to facilitate penetration by the fixative and allow the tissue to submerge.

The fly

The cuticle of a severed head was cut around the eyes so that most of the head and mouth parts could be pulled away and detached from the eyes leaving intact the brain, paired optic lobes and retinae.

The locust

Each eye was sliced off by an oblique cut several millimetres under the peripheral rim of the cornea. The retina and adherent optic lobes were cleared of tracheae and placed flat on the cut surface and either horizontal or vertical longitudinal slices cut with a new razor blade through the retinal diameter. These slices contain ommatidia parallel

to the plane of section together with underlying optic lobe.

The bee and dragonfly

The eyes were dissected in a similar fashion to that of locust.

The water-bug and Notonecta

Halved heads were lightly prefixed in an aldehyde fixative and the retina and optic lobe dissected out before postfixation in osmium. The dissection required is more elaborate in the water-bug because the optic lobe, which is separate from the retina, is connected to it by fine strands of retinula axons.

Pieris

The eye is difficult to dissect and halved heads were aldehyde-fixed *in toto* after the hairy cuticle had first been wetted with a little fixative.

Fixed material was usually dehydrated in a graded acetone series at room temperature and embedded in Araldite (Glauert and Glauert, 1958). The staining of Araldite sections is more easily controlled than of either Epon or Vestopal which have also been used, and the absence of background staining made this the embedment of choice. In cases where the external axes of the eye needed to be known these were marked on the block which was then trimmed with a block face of unique shape. In other cases, the antero-posterior axis was inferred from the plane of the lamina-medulla chiasma.

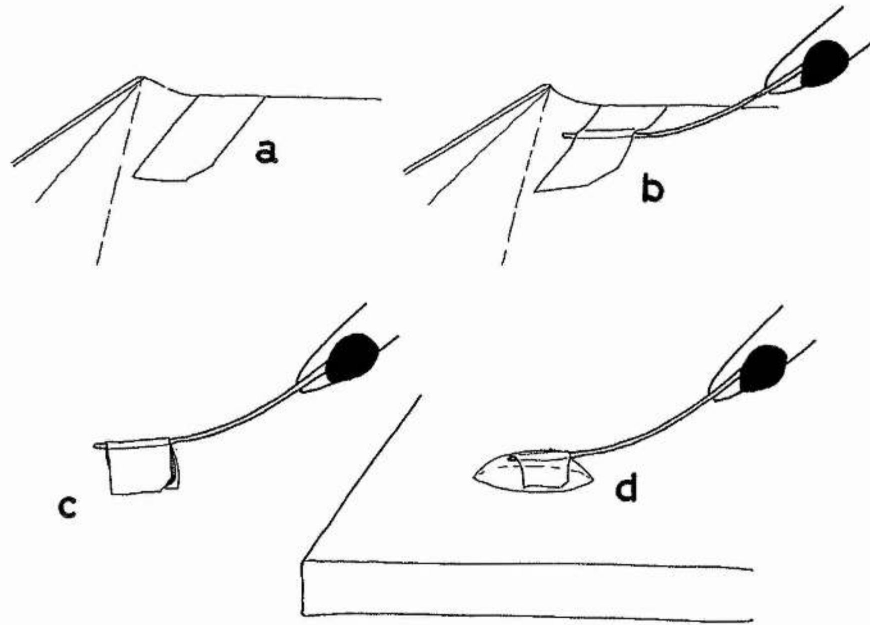


Figure 3

Serial sectioning method. (a) Sections are cut usually at 1 μm thickness on glass knives. The block is trimmed at one corner to identify the axes of orientation of the tissue. (b) An eyelash is placed under the middle of the section and lifted up to collect the section as in (c). A film of water (hatched) lies trapped between the halves of the folded section. (d) The section is placed on to a drop of water on a microscope slide and floats off.

D. Microtomy

Sections were cut on a Porter-Blum MT1 microtome usually at a thickness of 1 µm. At this thickness, near maximum resolution available by light microscopy is obtained with minimal confusion resulting from the superimposition of membranes and cytoplasmic organelles. The small gain in resolution with thinner sections is more than offset by the increase in numbers of sections and the difficulty in handling them.

Sections were picked up on an eyelash fixed to the tip of a mounted needle and each placed in sequence onto individual drops of water on a microscope slide. Once lifted from the water surface the section folds in two, apparently trapping a thin aqueous film between the two halves. When the section is set down on the water drop this film establishes fluid contact and the section instantly springs apart and floats (see fig. 3). This process occurs only if the water drop is deep enough. This quite critical point often required that slides be pretreated to produce the correct surface properties. Pretreatment in this case consisted of washing in a beaker of diluted toluidine blue (the washings of previous slides. The composition of the locust ommatidium was studied from one series of wax embedded Feulgen-stained sections prepared by Dr. M. Laing (Gatty Marine Laboratory). Some preliminary

observations on the optic lobe of the water-bug were made from wax embedded sections stained according to the method of Rowell (1963) by Mrs. E. S. Gunstone (Gatty Marine Laboratory).

E. Microscopy, photomicrography and tracing techniques

Completed slides were dried on a hotplate at 50°C for several minutes and stained with toluidine blue (1% aqueous solution in borax/boric acid buffer pH approximately 10; Trump, Smuckler and Benditt, 1961). Stained slides were mounted in Permount or more usually immersion oil. Sections of material in soft mixes of Araldite sometimes faded badly if mounted immediately after staining but this can be prevented by leaving on a hotplate for 24 hrs. to recure before mounting.

Series up to 1500 sections have been prepared in this way and approximately fifty series of 300-1500 sections have been used in this work. Detailed results are presented for only a few successful series. Successful series had the axon profiles of interest cut in near transverse sections for their entire length, with good staining contrast and less than 4% of the sections folded or lost.

Electron micrographs of optic lobe show a glial investment around the axons and it is this that presumably is stained by toluidine blue. Variations in the thickness and stain affinity of this layer could account for interspecific

variation in the appearance of axons.

Sometimes sections were gently etched in a solution of sodium hydroxide in ethanol (Berkowitz, Fiorello, Kruger and Maxwell, 1968) before staining, in order to improve their staining qualities.

Sections were photographed using a Zeiss Photomicroscope II equipped with oil immersion bright field and phase contrast 40/1.0 and 100/1.3 planapochromat objectives. Selecting corresponding areas from consecutive sections requires considerable experience and reference to series of low magnification survey micrographs of sections at 10 µm intervals or so. Additional aids to tracing are provided by subtle differences in the staining intensity of the axoplasm of particular cells within an ommatidium or cartridge, by differences in axon diameter and by constant spatial arrangements of axons. Latterly all micrographs for tracing axon pathways have been made with oil immersion 40 x objectives which provide near maximum resolution with reasonable field size. Only a portion of the greatly enlarged negative was printed. Approximately 15,000 micrographs have been used in this work, many of series that have proved unsuccessful.

An axon is followed through a projection by marking its profile at the first micrograph (of retina or lamina) and proceeding through the stack of micrographs marking the profile in transit. This repetitive method reinforces similarities

between axons but underemphasises differences. In regions in which many axons have nearly parallel pathways, transverse sections are required because it is impossible to follow axons from even approximately longitudinal sections in which any one axon may enter, leave and re-enter at different points in a single section.

F. Electron microscopy

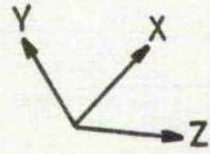
Thin sections of similar material were prepared by standard methods, using a Reichert UM2 ultramicrotome and were viewed with a Hitachi HU 11E electron microscope at 80kV. Sections were contrasted with lead citrate and 2% uranyl acetate.

G. Reconstruction of axon pathways

Reconstructions of the fly first projection were made by various methods. Reconstruction of all the nervous elements of this projection by conventional methods of transferring profiles to either sheets of cellulose acetate or glass piled in sequence is impossible because the whole volume of tissue is occupied by elements that were traced and no single element can be visualized in isolation. Models in which axons were represented as wires strung between sheets of Perspex proved useful in visualizing the spatial interrelationships of axons at different levels.

Figure 4

C. Stygia. Wholemount of cornea of left eye with the area equivalent to that shown in the retinal plan of fig. 5 outlined in the centre. The figure shows the small proportion of the first neural projection that was analysed.



DORSAL

POST.

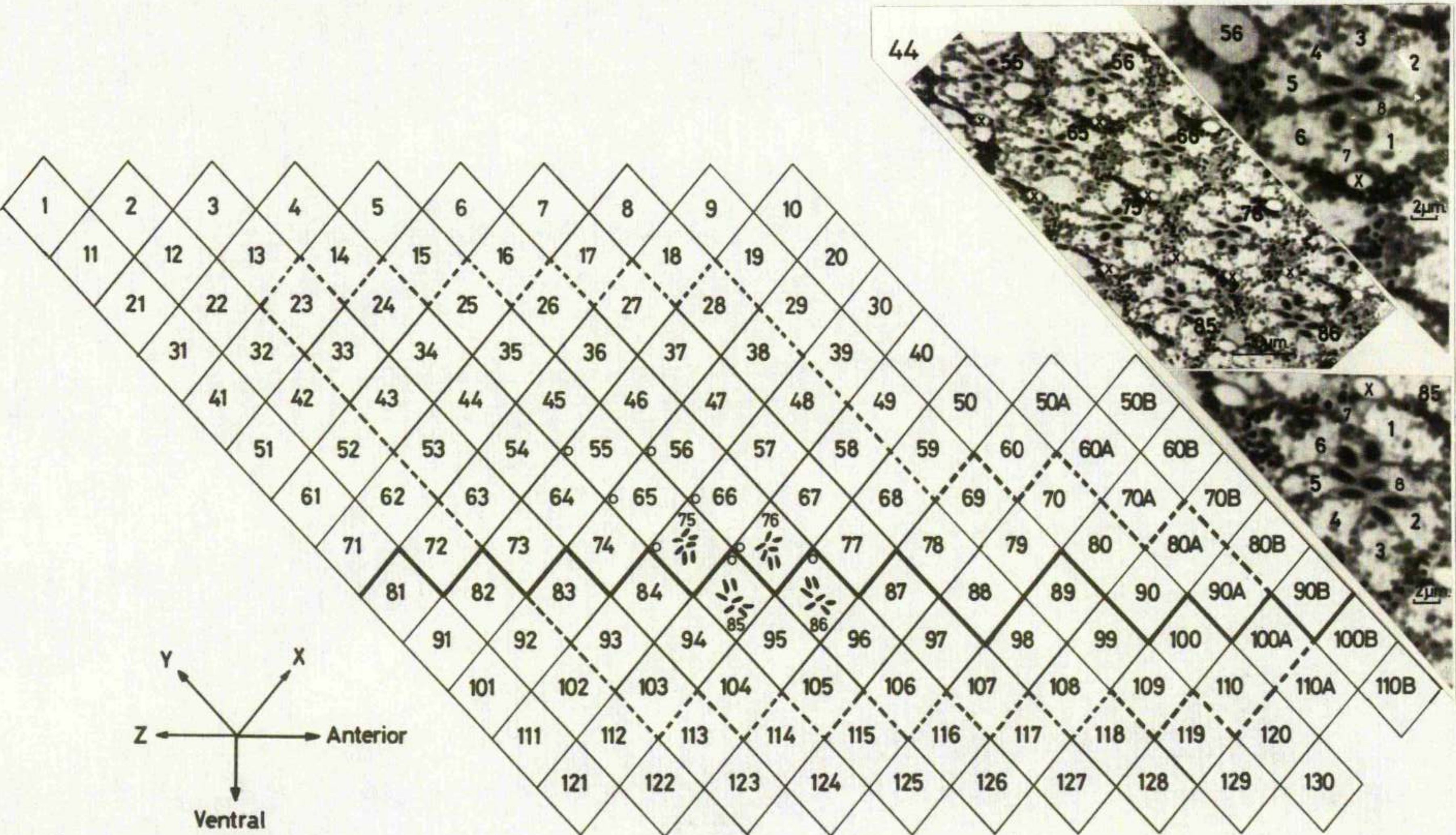
ANT.

VENTRAL

Figure 5

C. vomitoria Plan of the retina of a right eye from which the connections of the retinula axons have been followed. Each box represents an ommatidium and is given a number. Some or all of the axons from an area 10 x 13 ommatidia have been traced and it is this area that is marked in Fig. 4. The angles of the axes of the rhomboidal ommatidial rows are directly transposed from micrographs of the retina. The equator of the retina is marked with a heavy line between rows 71 - 90B and 81 - 100B. All axons from the central region of the field (between ommatidia 23, 28, 110 and 103) can be accounted for. The asymetrical rhabdomere pattern is shown on either side of the equator for ommatidia 75, 76, 85 and 86 and the position of the tracheole for each ommatidium is shown for ommatidia 55, 65, 75, 85 and 56, 66, 76, 86. At the equator a double row of tracheoles is formed.

Inset. Composite figure of the ommatidia numbered from section 44 of the series. Ommatidia 56 and 85 are shown enlarged with retinula cells 1 - 8 numbered in each. Comparison between these two shows that the rhabdomere patterns are mirror images with cell 7 in the equatorial quadrant and cell 8 in the anterior quadrant. Tracheoles are marked with a cross (x).


44
Y
X
Z
Anterior
Ventral
2μm

RESULTS

A. The fly Calliphora

A series of sections from a right eye of C. vomitoria was used to trace the connections of some of the first-order retinula axons (figs. 5 to 16). The results are similar to those of Horridge and Meinertzhagen (1970 a) and reconfirm the account of the generalized connectivity pattern of Braitenberg (1967). This specimen is of particular interest because it contains a number of connectivity errors associated with a dislocation of the equator. Errors of this type were not found in the tissue previously examined (Horridge and Meinertzhagen, 1970 a).

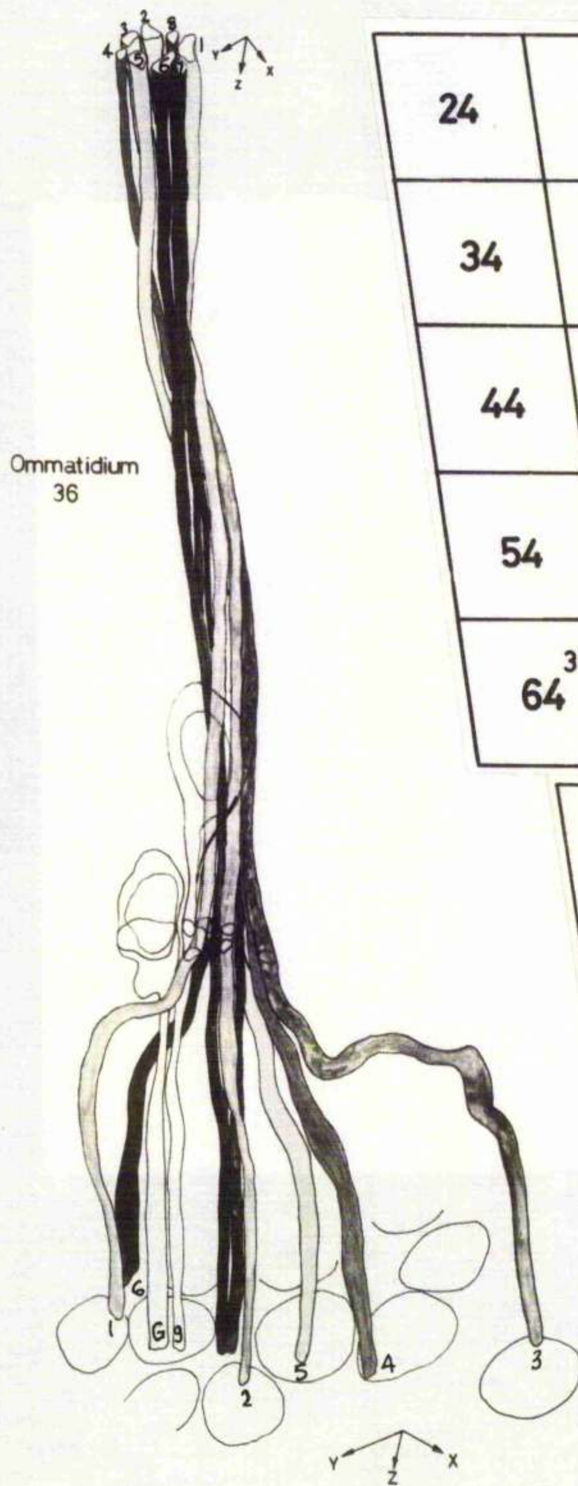
The area of the first projection that has been traced in this series is shown as a proportion of the corneal surface in fig. 4. It is positioned approximately in the centre of the retinal field at a place intermediate between the two hexagonal types of array of the anterior and lateral aspects, and consequently is derived from a rhomboidal ommatidial array (Braitenberg, 1967). Axons from between 1 - 2% of the ommatidia of the retina have been traced. These ommatidia correspond to the central portion of the retinal plan (fig. 5). The equator in the retina separates dorsal from ventral halves; the characteristic rhabdomere pattern of one half is the mirror image of that in the other

Figure 6

C. vomitoria. The projection of individual ommatidia.

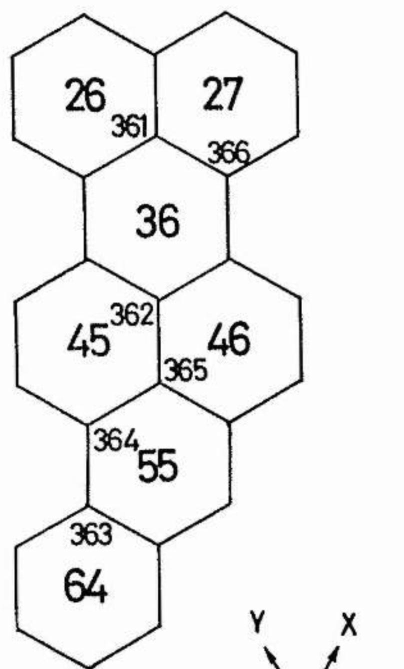
a. Left. Reconstruction of the eight axons of ommatidium 36; also shown are the two large lamina monopolar neurones.

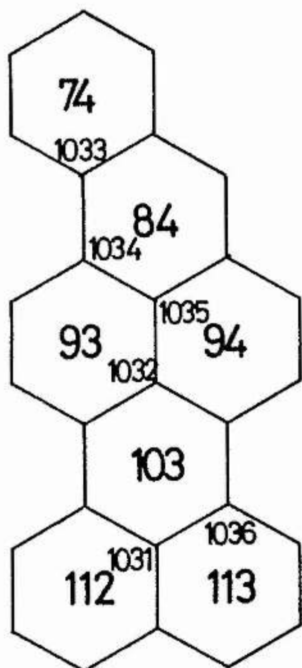
b. Right. Plans of the projection of the six short retinula axons on to six different lamina cartridges from two ommatidia 36 (above) and 103 (below), which lie on either side of the equator. These plans are taken from the lamina cartridge plan of fig. 8. The apparent lack of symmetry between the projections of these two ommatidia is a result of the distortion of the lamina cartridge array from a hexagonal to rhomboidal configuration (see fig. 7).



24	25	26 361	366 27
34	35	36	37
44	45 362	365 46	47
54	364 55	56	57
64 363	65	66	67

72	73	74 1033
82	83	84 1034
92	93 1032	1035 94
102	103	104
1031 112	1036 113	114





<u>Figure 7</u>

Plans of the projection of the six short retinula axons on to six different lamina cartridges from ommatidia 36 and 103 as shown in fig. 6 but converted to the hexagonal array characteristic of the lateral (posterior) region of the eye. The conversion makes apparent the complete symmetry about the equator of the projection patterns.

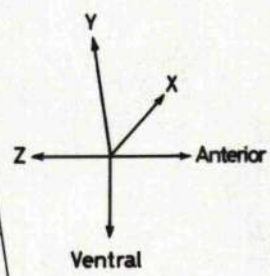
half (inset, fig. 5). Ommatidia 88 and 89 are interesting because the equator becomes dislocated at this point and in consequence the numbers of rhabdomeres with the same optical axis must vary from the generalized plans of Kirschfeld (1967) for the equator.

Each ommatidium gives rise to eight photoreceptor axons; cells 1 - 6 have short, and the central cells 7 and 8 have long, retinula axons. Each bundle of axons twists through 180° as it proceeds proximally (fig. 6a), in a clockwise direction in one half of the eye and anticlockwise in the other (Braitenberg, 1967). The consequence of this twisting is that the spatial arrangement of the visual axes of cells 1 - 6 is reestablished with respect to the animal after being rotated through 180° by the dioptric apparatus of the ommatidium (Kirschfeld, 1967). At the lamina ganglion cell layer each bundle is joined by the axons from the two large monopolar neurones L1 and L2 (g and G) (fig. 6a). At this level the axons in cross section have a chevron formation with axon 3 nearest the equator (see e.g. bundle 98 in fig. 11). The rotational sequence of axons is clockwise from 1 to 6 in one half of the eye and anticlockwise in the other. For the dorsal half of a right eye each bundle twists in a clockwise direction and its axons are arranged in an anticlockwise sequence. The ventral half of a left eye is identical.

The short retinula axons

At the top of the external plexiform layer the axons rapidly diverge approximately in a dorso-ventral direction (fig. 13). The pattern of projection of six short retinula axons on to six different lamina cartridges is radially asymmetrical (fig. 6b). The angular orientation of the axes of fig. 8 form a rhomboidal array with obvious rows along the y and z axes. The rhomboidal array arises by a horizontal stretching of the hexagonal arrays found in the anterior and lateral regions of the retina as a result of the transition between these two types of array (see Introduction, p.). As a consequence of this distortion, the projection patterns for ommatidia on either side of the equator are not mirror images (fig. 6b). In order to compare directly the patterns from the two halves the positions of the cartridges for one side must be computed with axes x and y transposed. Fig. 7 shows the two patterns plotted on a hexagonal array and demonstrates their mirror symmetry. The projection of short retinula axons is obviously comparable with that in the anterior region of the eye as described by Braitenberg (1967) when the distortion resulting from the rhomboidal array is corrected by this transposition.

One consequence of the projection pattern is that cartridges in most of the lamina contain six terminals (1 to 6) from six different ommatidia. These terminals surround the



	1	2	3	4	5	6	7	8	9	10		
	11	12	13	14	15	16	17	18	19	20		
6	21	22	23	24	25	26	27	28	29	30		
6	31	32	33	34	35	36	37	38	39	40		
6	41	42	43	44	45	46	47	48	49	50	50A	50B
7	51	52	53	54	55	56	57	58	59	60	60A	60B
8	61	62	63	64	65	66	67	68	69	70	70A	70B
8	71	72	73	74	75	76	77	78	79	80	80A	80B
8	81	82	83	84	85	86	87	88	89	90	90A	90B
8	91	92	93	94	95	96	97	98	99	100	100A	100B
7	101	102	103	104	105	106	107	108	109	110	110A	110B
	111	112	113	114	115	116	117	118	119	120		
	121	122	123	124	125	126	127	128	129	130		

Figure 8

C. vomitoria Plan of the projections of the short retinula axons of the ommatidia shown in fig. 5. Each box represents a lamina cartridge and so is numbered for the ommatidium to which it corresponds. The angles of the axes of the cartridge rows are transposed directly from the micrograph of section 177 shown in fig. 11. The equator of the lamina is marked with a heavy line between rows 71 - 90B and 81 - 100B. The large numbers 6, 7 and 8 at the left of each horizontal row indicate the predicted complement of photoreceptor terminals for cartridges in those rows up to the dislocation of the equator at ommatidium 87. Axons that terminate in an unexpected position are enclosed in a box.

cartridge in a particular rotational sequence of (in order) 1 to 6 (fig. 8) and the direction of this sequence is anti-clockwise for the dorsal half and clockwise for the ventral half of a right eye (Braitenberg, 1967). Another consequence of the projection pattern is seen in the cartridges bordering the equator. Because axons 2, 3, 4 and 5 spread towards the equator and only axons 1 and 6 spread away from it (figs. 8, 6b), three rows of cartridges on either side of the equator receive more than the regular complement of six terminals, an observation first reported by Cajal (1909) but not quoted thereafter. Thus on each side of the equator the first two rows contain eight-terminal cartridges and the third row seven-terminal cartridges. The number of terminals per cartridge is further complicated by the dislocation of the equator. The rotational sequence of terminals is preserved for those axons of the equatorial cartridges which start on the same side of the equator as they terminate. Axons added to these cartridges from across the equator occupy more variable positions e.g. cartridges 73 - 77 (fig. 8), see Discussion, p.215 .

The terminals of the short retinula axons are plotted on the plan of fig. 8 in a similar manner to that of Horridge and Meinertzhagen (1970 a). Each terminal is given a number, the last digit is the index number (1 - 6) of the receptor, the preceding digits indicate the ommatidia of

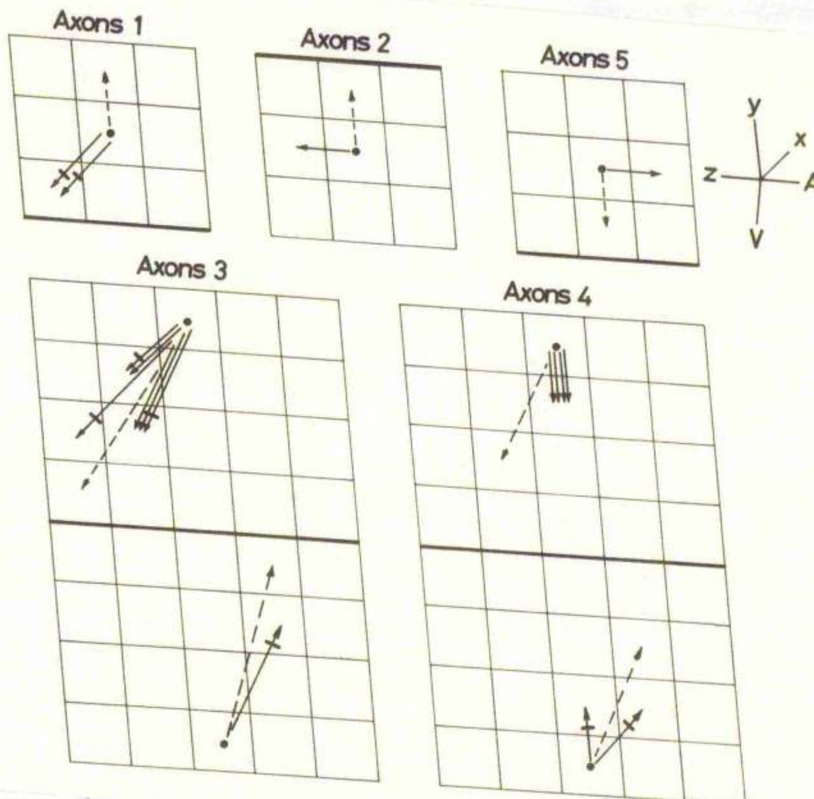
Table 2

The distribution of the seventeen short retinula axons that terminate in incorrect cartridges. The axons are listed on the left and for each axon the predicted and actual cartridge of termination are given. In addition the axons not accounted for in fig. 8 are listed. The predicted number of terminals for a cartridge containing errors may be calculated from the number of correct terminals (from fig. 8) and the number of mistaken terminals predicted in this table to go to that cartridge.

Figure 9

The projection patterns of all axons that terminate in an incorrect cartridge. Five patterns are shown for axons 1, 2, 3, 4 and 5 (no axon 6 terminated in the wrong cartridge). The point of origin for each axon is represented by a solid circle in each plan and the solid arrows connect the origin with the cartridges of termination. The interrupted arrows represent the correct projection pattern for each axon. Projection patterns above the heavy horizontal lines are from the dorsal half of the eye, and those below are from the ventral. Arrows crossed by short heavy lines represent the paths of axons that cross the equator to terminate.

	Axon No.	Predicted Cartridge No.	Actual Cartridge No.	Comment.
1	781	68	87	
2	894	70	79	
3	974	78	88	
4	1093	80	90	
5	901	80	99	
6	684	87	78	
7	775	87	78	
	785	88		missing.
8	60B3	90	80A	
9	70A4	90	80A	
10	100A2	90A	100	
	663	94		untraceable.
11	804	99	90	
12	70A3	99	80	
13	80A4	100	90A	
14	803	108	99	
15	80B3	110	100	
16	90B3	120	100A	
17	743	102	93	



origin. All the terminals of the central area of fig. 8 bordered by a broken heavy line have been plotted at their position of termination within their cartridges. Only two axons are not accounted for : 663 which was impossible to trace to its expected position in cartridge 94 (the unnumbered terminal between 851 and 754 in fig. 11) and 785 which is entirely missing from ommatidial bundle 78. (Ommatidium 78 and its rhabdomere pattern are otherwise normal). Axons that behave as number 3 which were not traced from their ommatidium are marked in cartridges 23 - 28; 103 - 110; 100A and 90A.

Seventeen axons that terminate in incorrect cartridges i.e. those departing from prediction, have been found. Their numbers are given in Table 2 together with both the cartridges at which they should have terminated and the cartridge to which they actually went. The distribution of errors in termination according to axon class is as follows:

Axon No.	1	2	3	4	5	6
No. of cases observed	2	1	7	6	1	0

Of the seventeen axons that terminate in wrong cartridges nine cross the equator to do so. The positions of the predicted cartridge of termination and the actual cartridge of termination for each class of axon (1 to 5) relative to the same ommatidium of origin are shown in fig. 9.

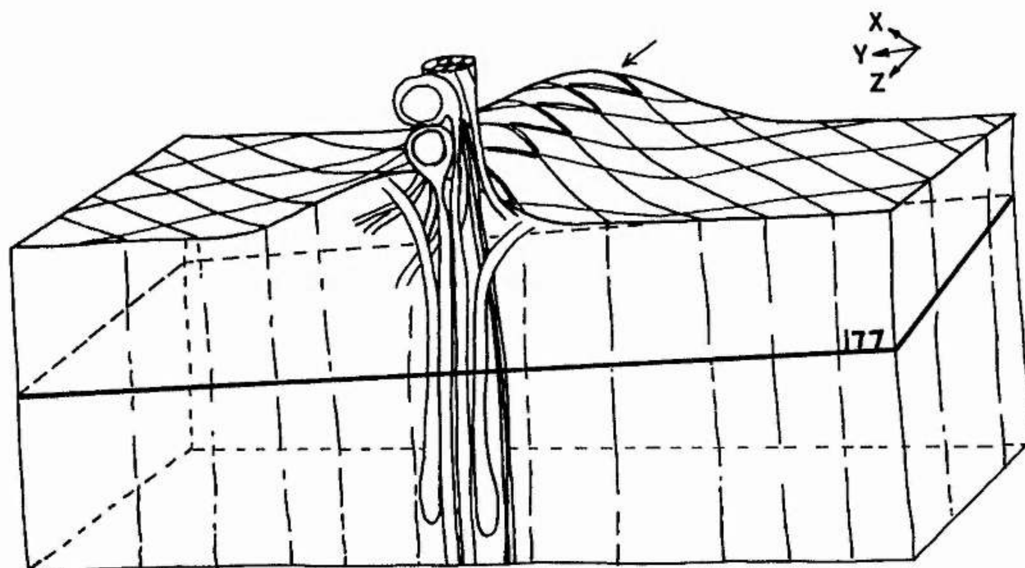
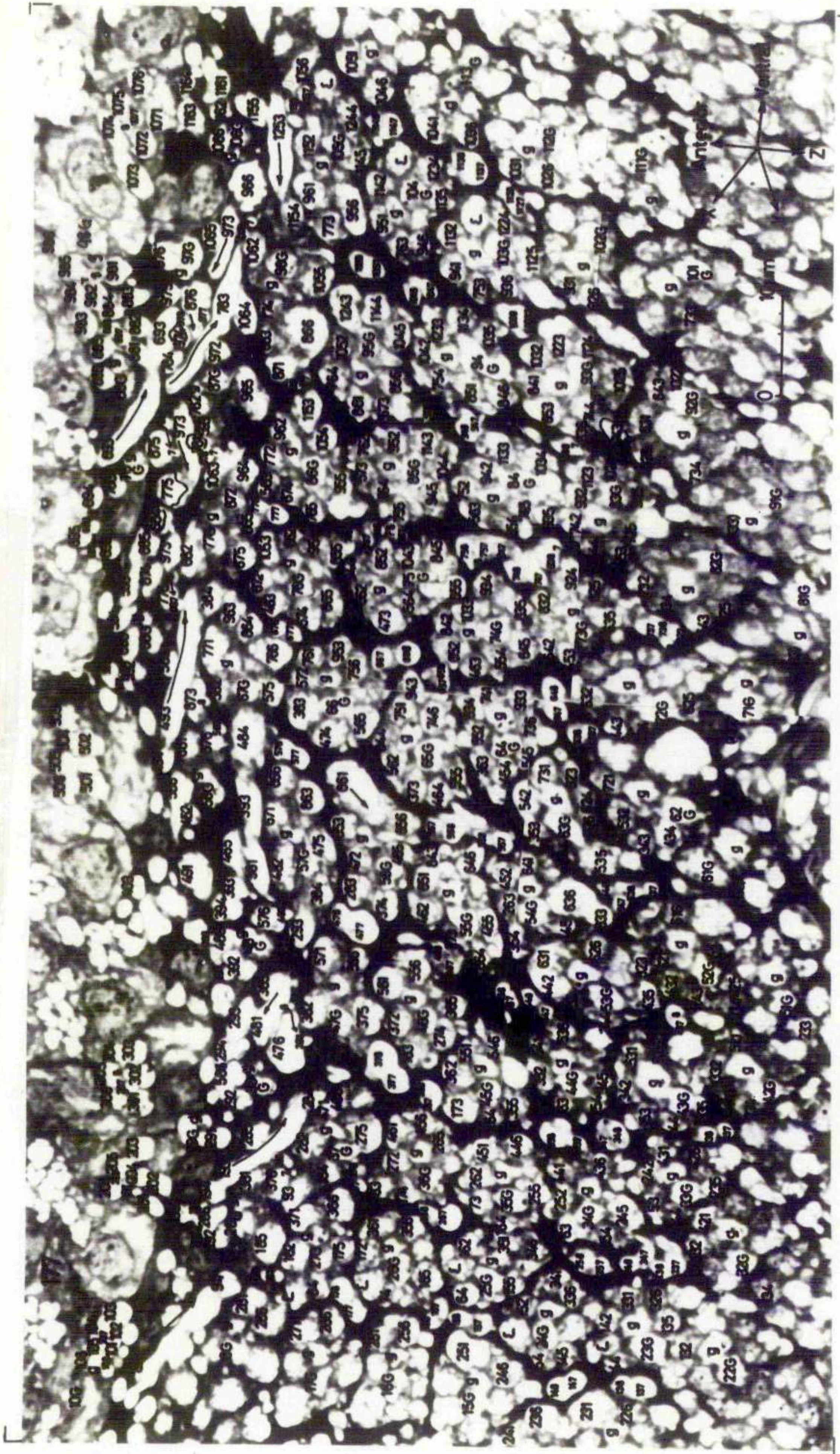


Figure 10

Block diagram of the lamina to illustrate the plane of section 177 (fig. 11). The equator of the lamina (arrowed) is shown as a double line, parallel to the z axis, running atop a ridge of tissue formed by the great concentration of axons in this region. The projection of one ommatidial bundle is shown on the front dorso-ventral face together with the two large monopolar cells. The plane of section of fig. 11 cuts the block in a tangential plane inclined to the right and to the rear.

Figure 11

C. vomitoria Micrograph of lamina from section 177, the plane of which is illustrated in fig. 10. The cartridges are visible as discrete aggregations of axons. At the top are the axons cut in longitudinal section as they spread over the top of the lamina. Above these are ommatidial bundles separated by monopolar cell bodies. On the overlay are marked the numbers of each axon profile. Every axon that is shown in fig. 8 is marked. Axons that terminate in error are outlined. The positions of the long retinula axons 7 and 8 between the cartridges are marked with small numbers. The position of the equator is obvious from the plan in fig. 8 and is marked by a double row of long retinula axon pairs. Axons that move into the field from outside the area traced and which are undoubtedly type 3, are marked ε. The two large monopolar axons are marked G and g and their number denotes the cartridge number.



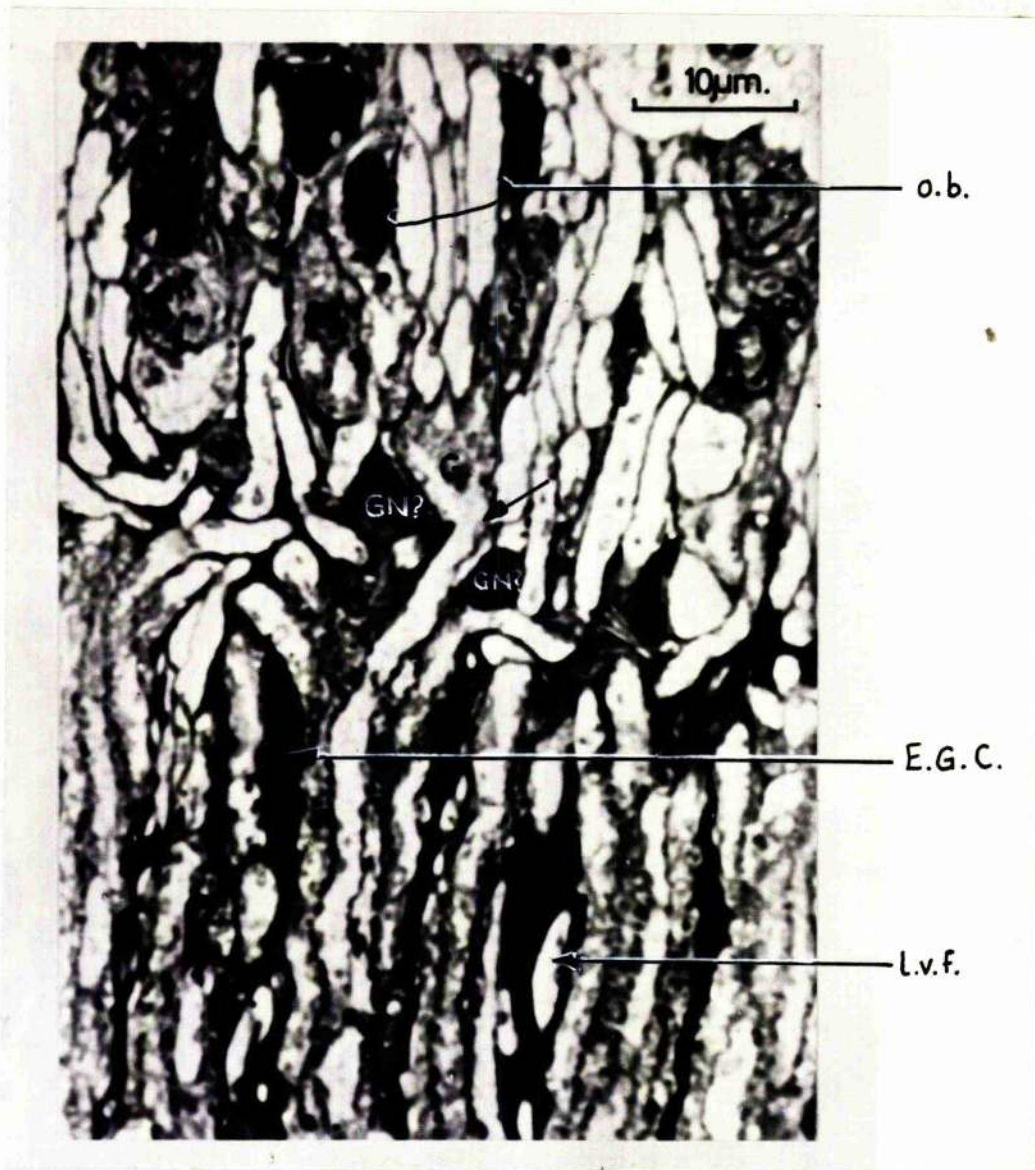


Figure 12

C. stygia HLS of first projection and lamina. The section shows ommatidial bundles (o.b.) of retinula axons alternating with columns of monopolar cell bodies (two large monopolar cell bodies are marked G and g, the former, arrowed, communicating with its axon). The smaller densely staining bodies (GN) are tentatively identified as the nuclei of glial cells, but might also belong to a type of monopolar neurone.
E.G.C. = epithelial glial cell situated in the longitudinal interstices between cartridges. l.v.f. = long visual fibres.

The long retinula axons

The preceding account refers to the projection of the six short retinula axons of each ommatidium. The pair of long retinula axons (7 and 8), after travelling to the top of the lamina at the centre of their ommatidial bundle, proceed vertically downward through the plexiform layer of the lamina where they proceed as a pair along the equatorial side of the cartridge underlying their ommatidium of origin. Axon 8 lies nearest the equator. After leaving the lamina they enter the chiasma and proceed to the medulla. Fig 11 is a micrograph of the portion of lamina represented in fig. 8 on which are marked the positions of the long retinula axons, and the terminals of the short retinula axons and the pairs of monopolar axons g and G. Characteristically the monopolar axons are orientated with their axes in an antero-posterior plane. The section is cut obliquely (fig. 10).

The spatial relationships of the axon lattice

Above the plexiform layer of the lamina a regular lattice is formed by the interweaving of short retinula axons as they travel to their appropriate cartridges after they diverge from their ommatidial bundles. The total depth of the lattice is approximately 7 μm (fig. 12). Although previously published pictures of tangential sections of reduced silver preparations (e.g. Pedler and Goodland, 1965 figs. 13, 14; Braitenberg, 1967 fig. 17) demonstrate well the rectilinear nature of

Figure 13

Fly. Drawing of a reconconstructed model of some of the pathways of the short retinula axons from the dorsal half of a right eye, to illustrate the spatial relationships of the axons as they spread out over the external surface of the lamina neuropile. Axons are shaded, according to their cell of origin, with a particular colour given in the colour code below. Ommatidial axon groups, arranged in triangular formations, diverge and generally pass on one side only of the axons that they cross, before reaching their cartridge (represented by a broken circular profile). In addition to the retinula axons the cell bodies and axons of g (= L1) and G (= L2) monopolar neurones are also shown together with another (unidentified) cell that occupies a position in the interstices of the diverging axon groups. The axons have well defined positions around this cell.

Colour code: short retinula axons 1, red; 2, yellow; 3, brown; 4, orange; 5, green; 6, blue. Long retinula axons 7 and 8, black.

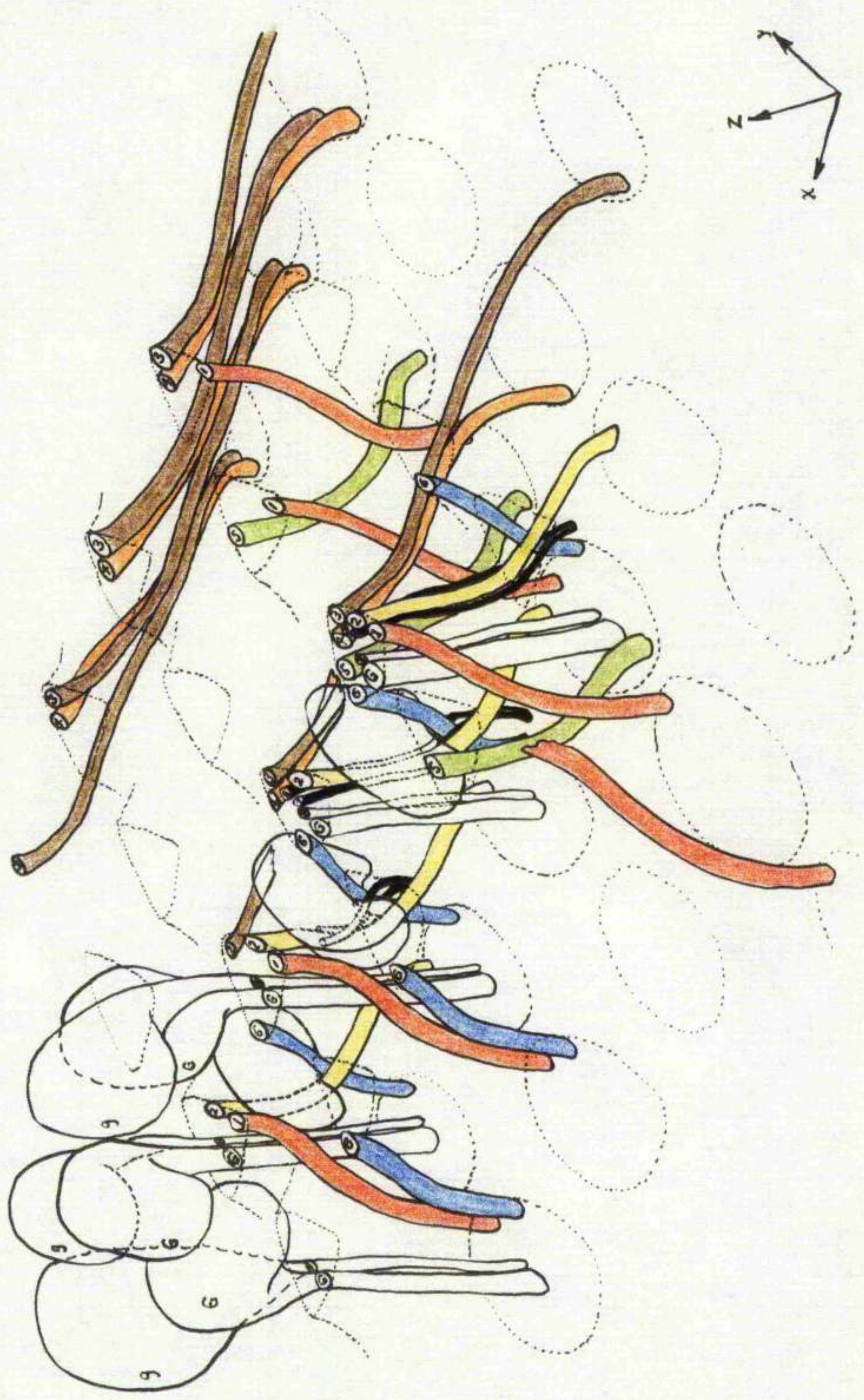
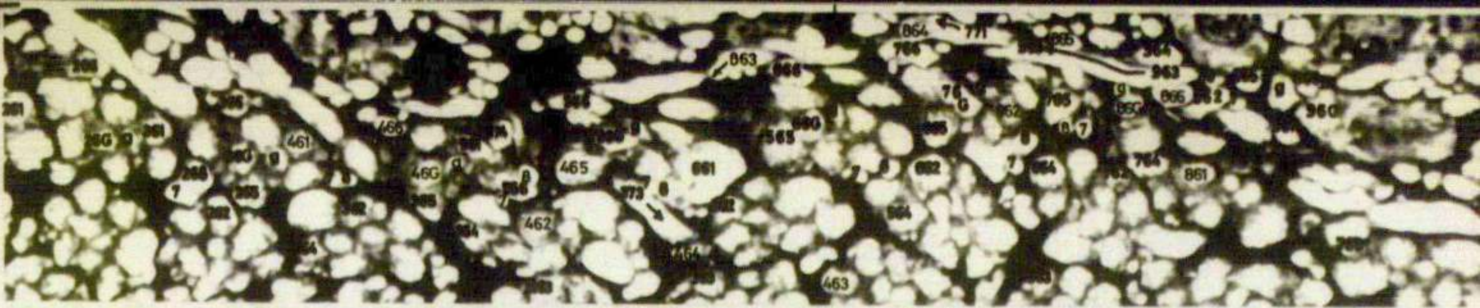


Figure 14

C. vomitoria. Pull-out series of micrographs from a selected region of the first projection to show the quality of micrographs from which axons have been traced and to show the repetition with which particular spatial configurations of axons recur for each ommatidial axon bundle. The micrographs cover a depth of 44 μm, starting from section 142, and describe the individual projections of ommatidia 26 - 106. To eliminate the confusion resulting from numbering all axons of each micrograph, each bundle is numbered on the micrographs only once every 4 μm. The numbering starts on those sections that cut the bundles just before their axons diverge. Micrographs on which bundles are first numbered are 26 (142); 36 (145); 46 (152); 56 (147); 66(146); 76 (145); 76 (145); 86 (148); 96 (159) and 106 (167). A measure of the predictability of the axon pathways is obtained by comparison of a bundle with another cut at the same level.



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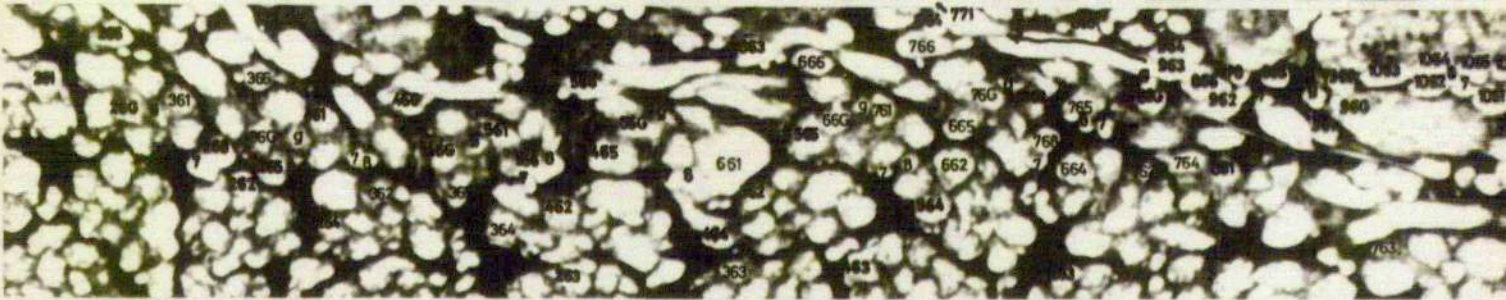

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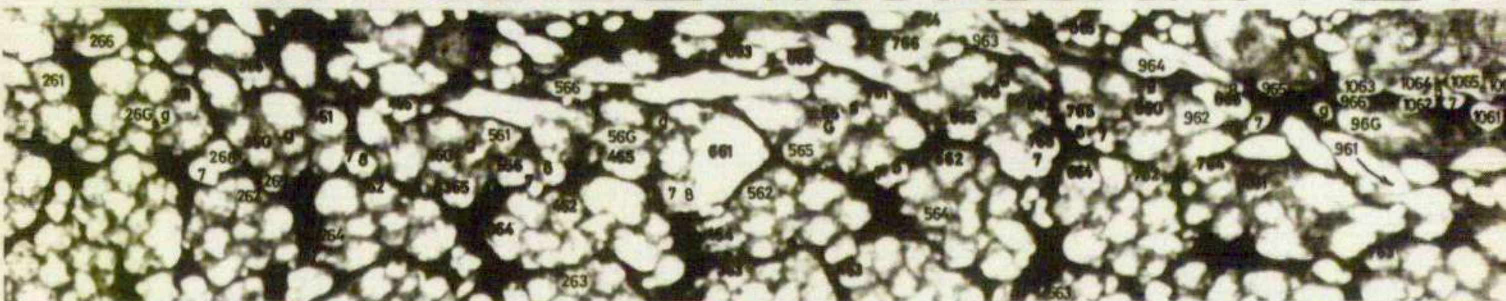

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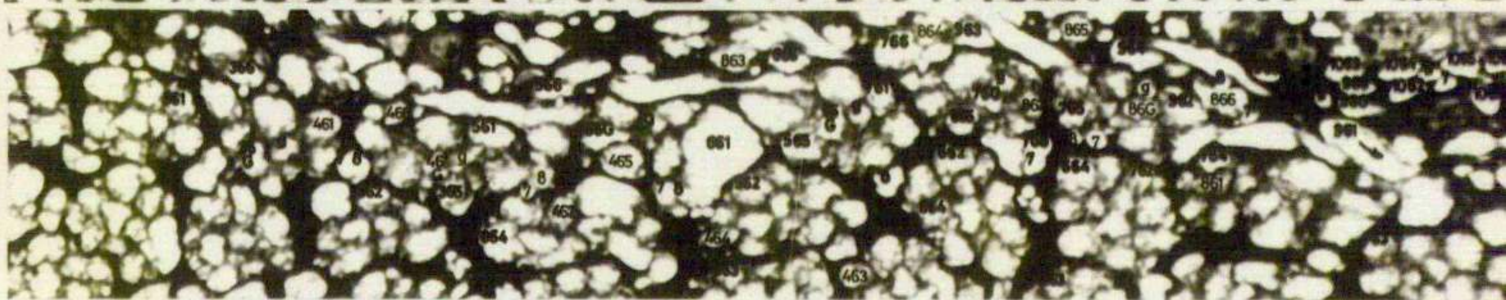

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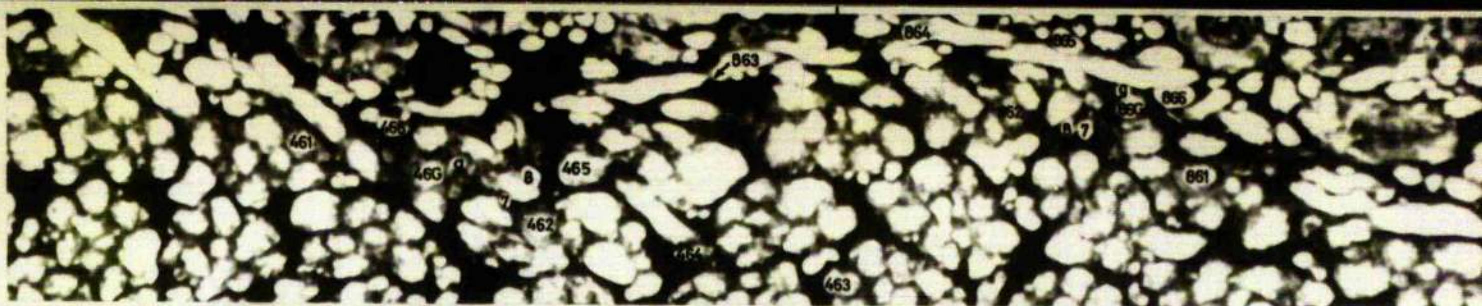

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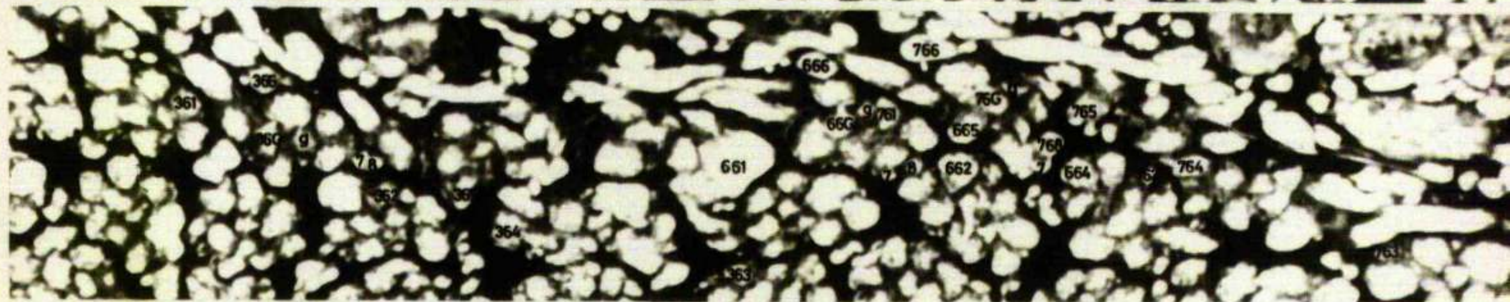

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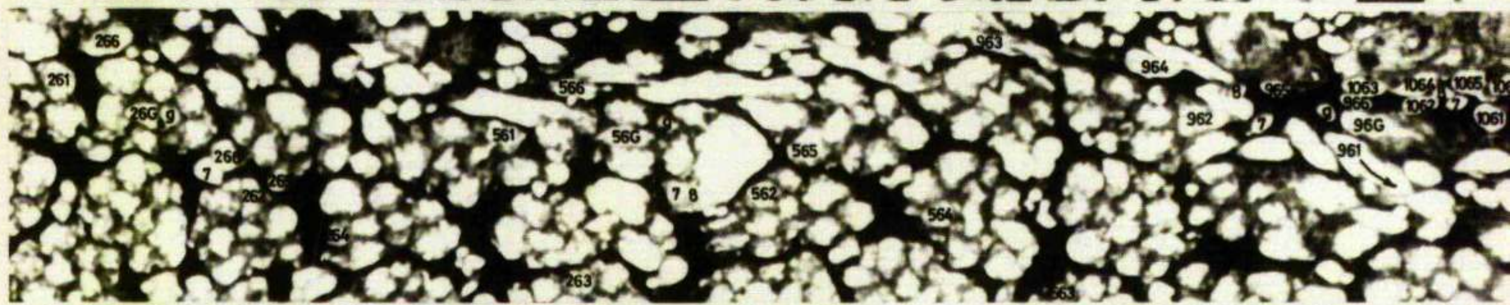

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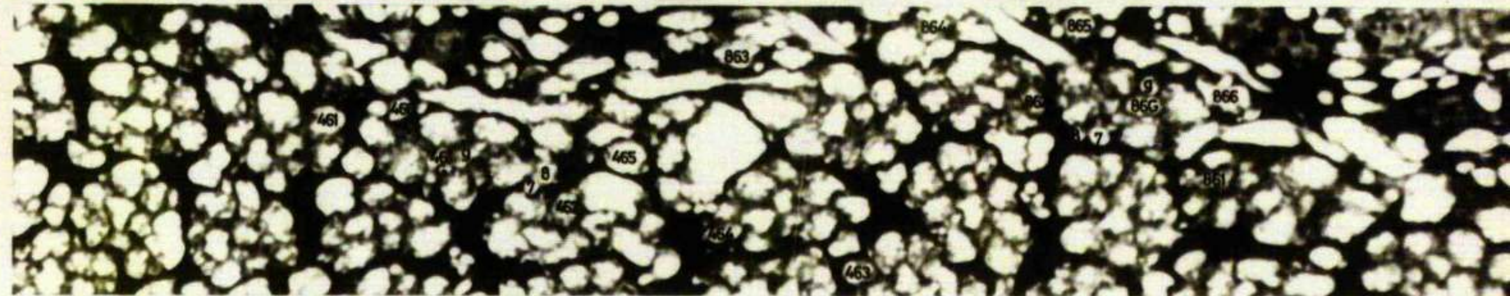

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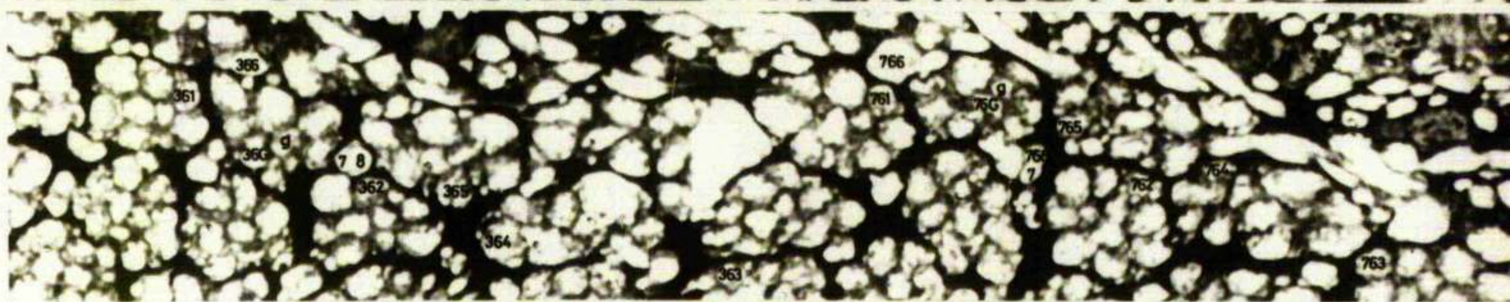

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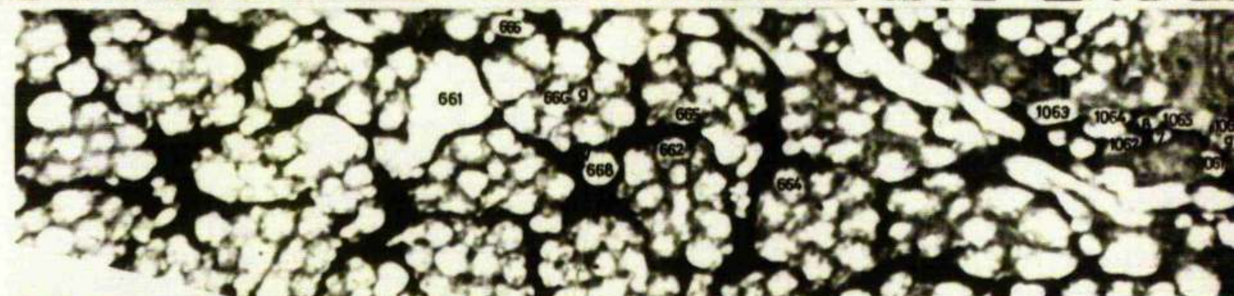

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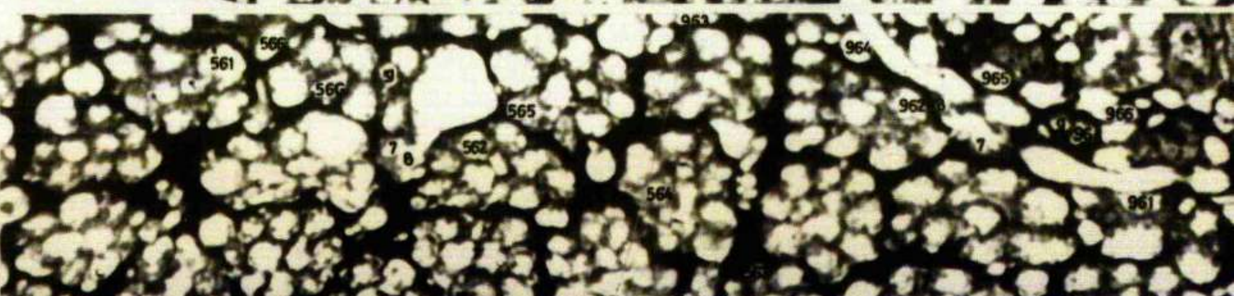

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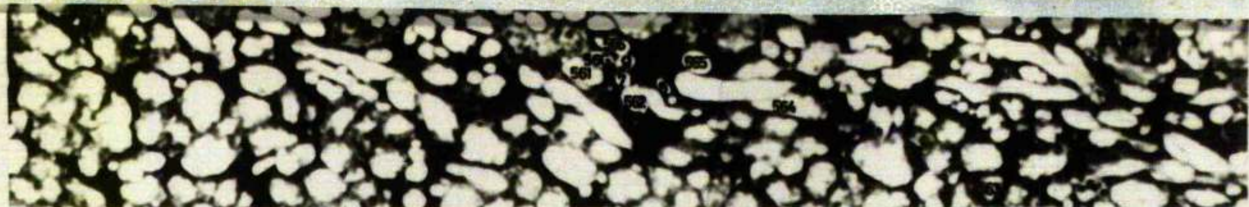

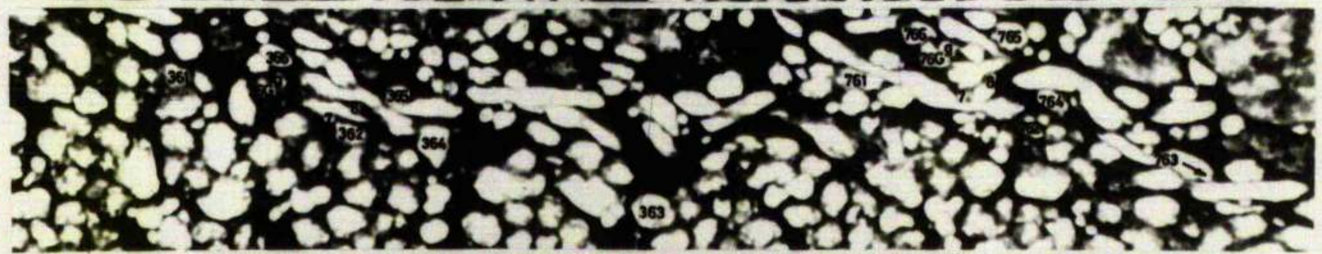
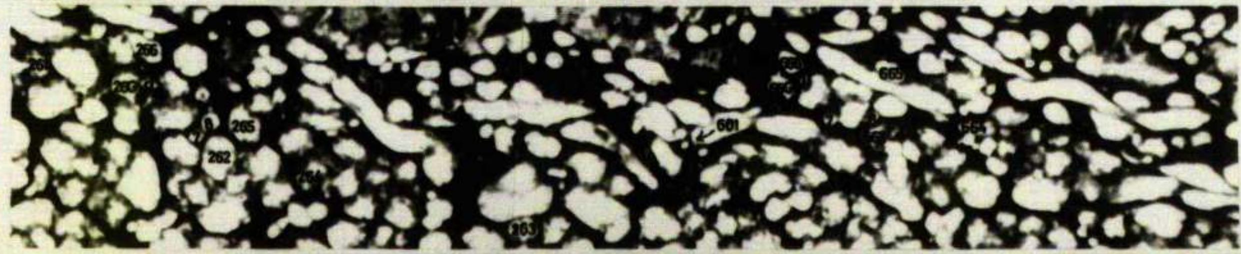
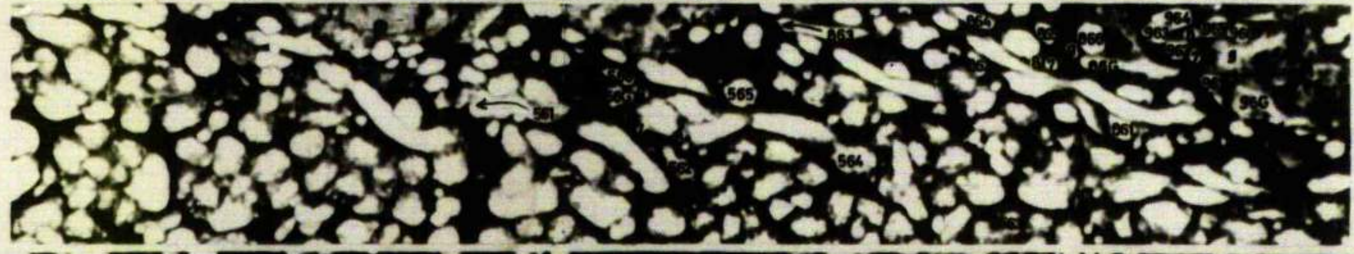
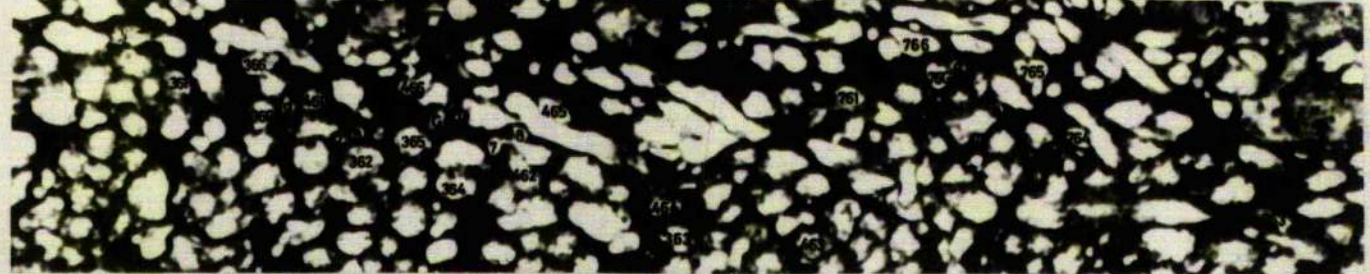
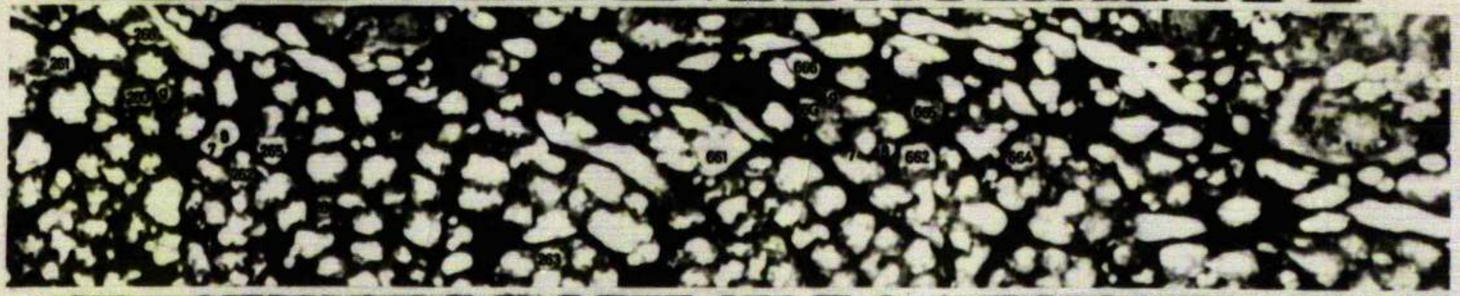
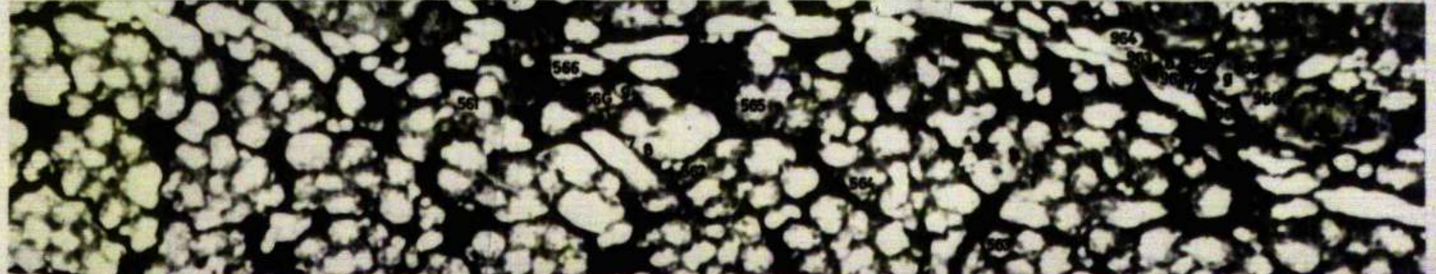

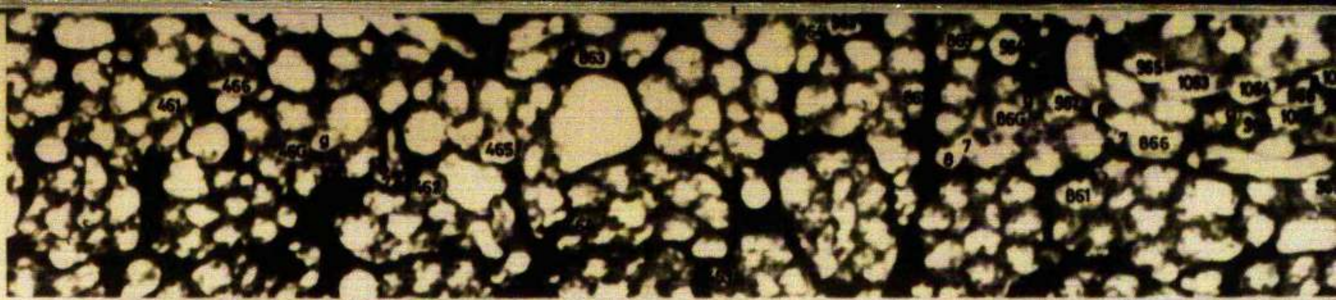
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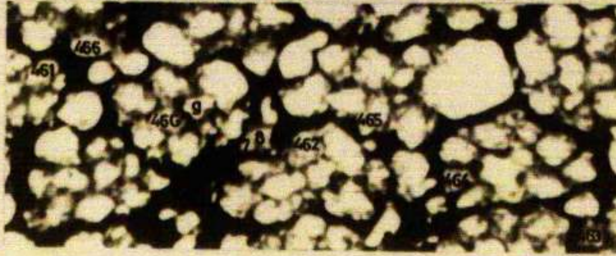
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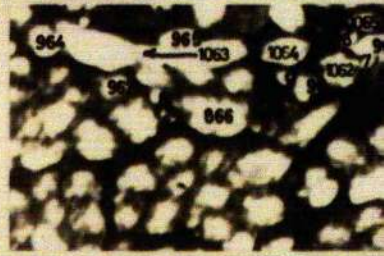

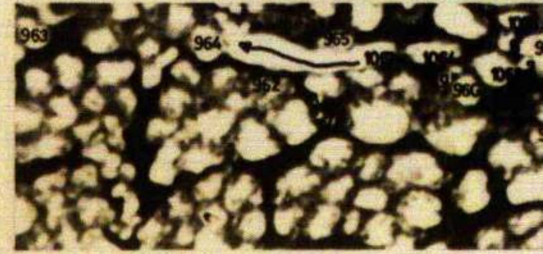
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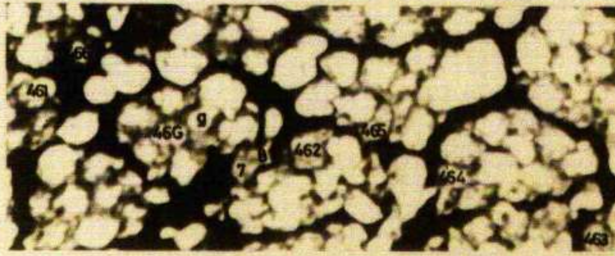
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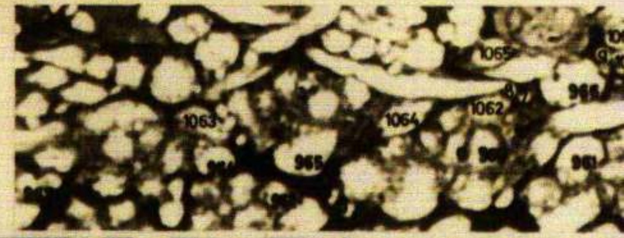
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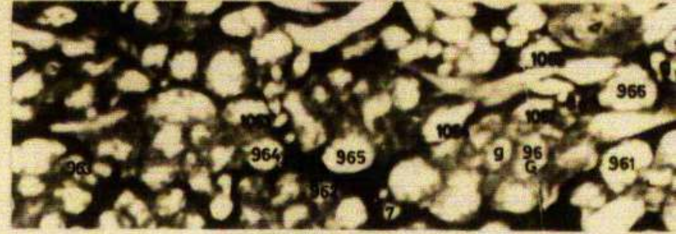
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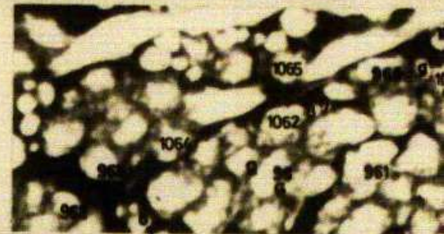
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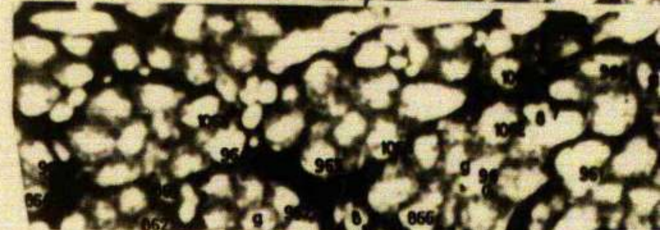
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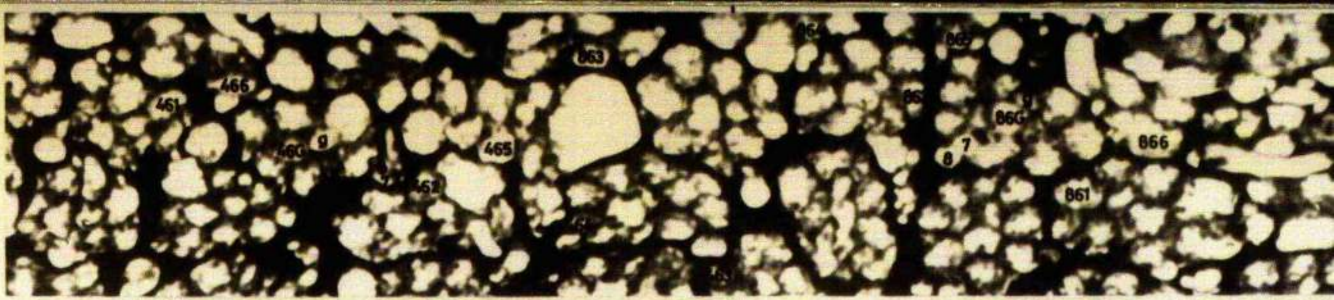

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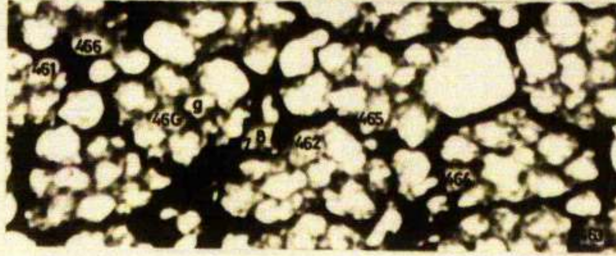

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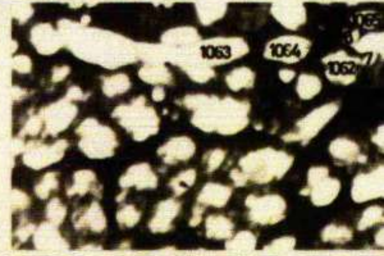

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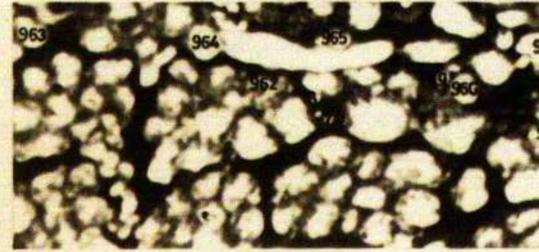

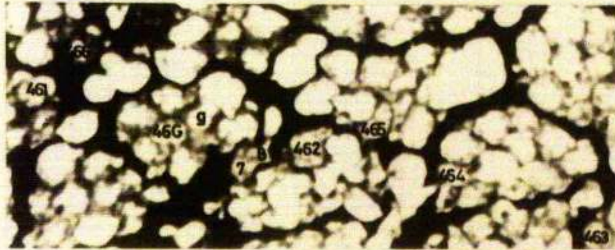

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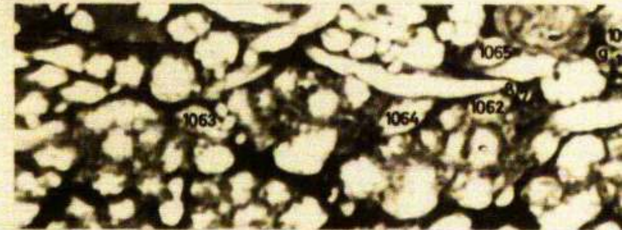
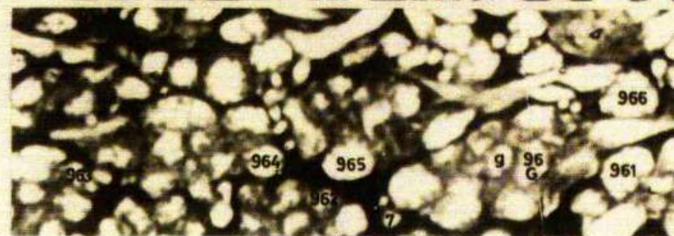


178

0 10µm.



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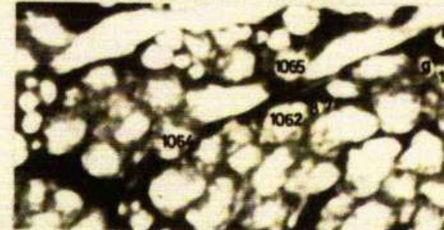

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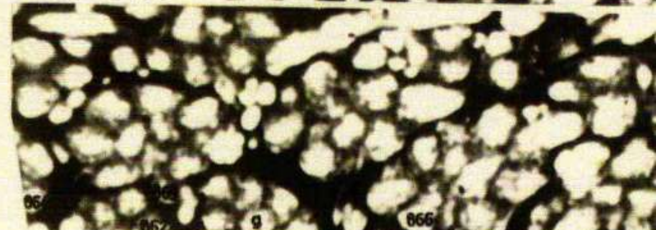

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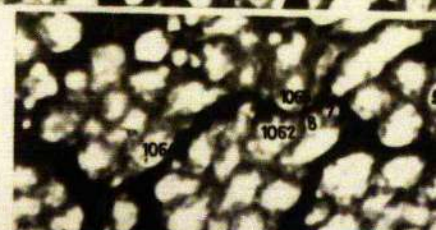

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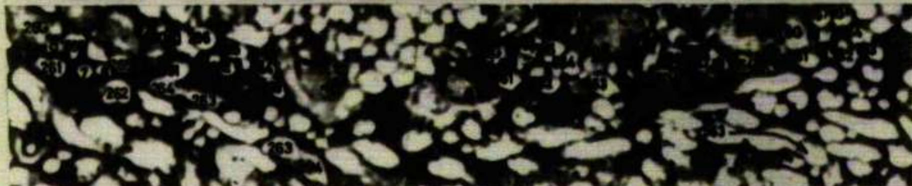

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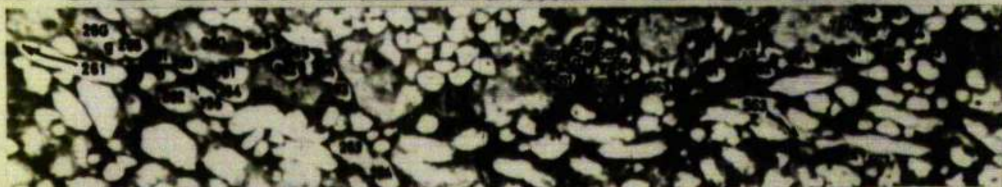
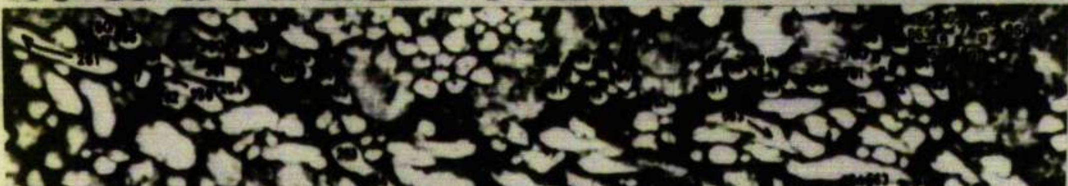


0 10µm

147



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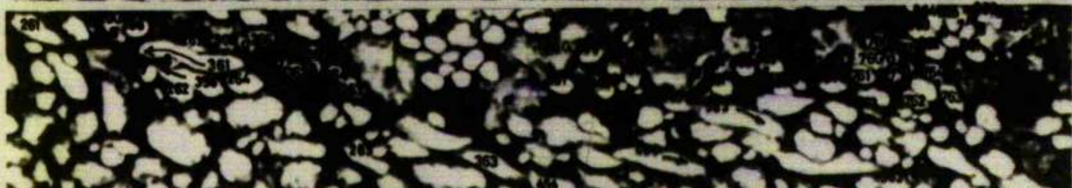

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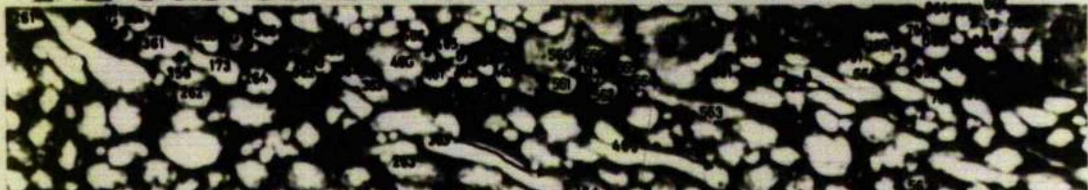

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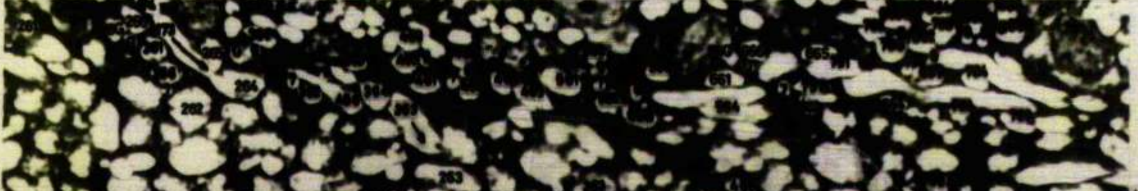

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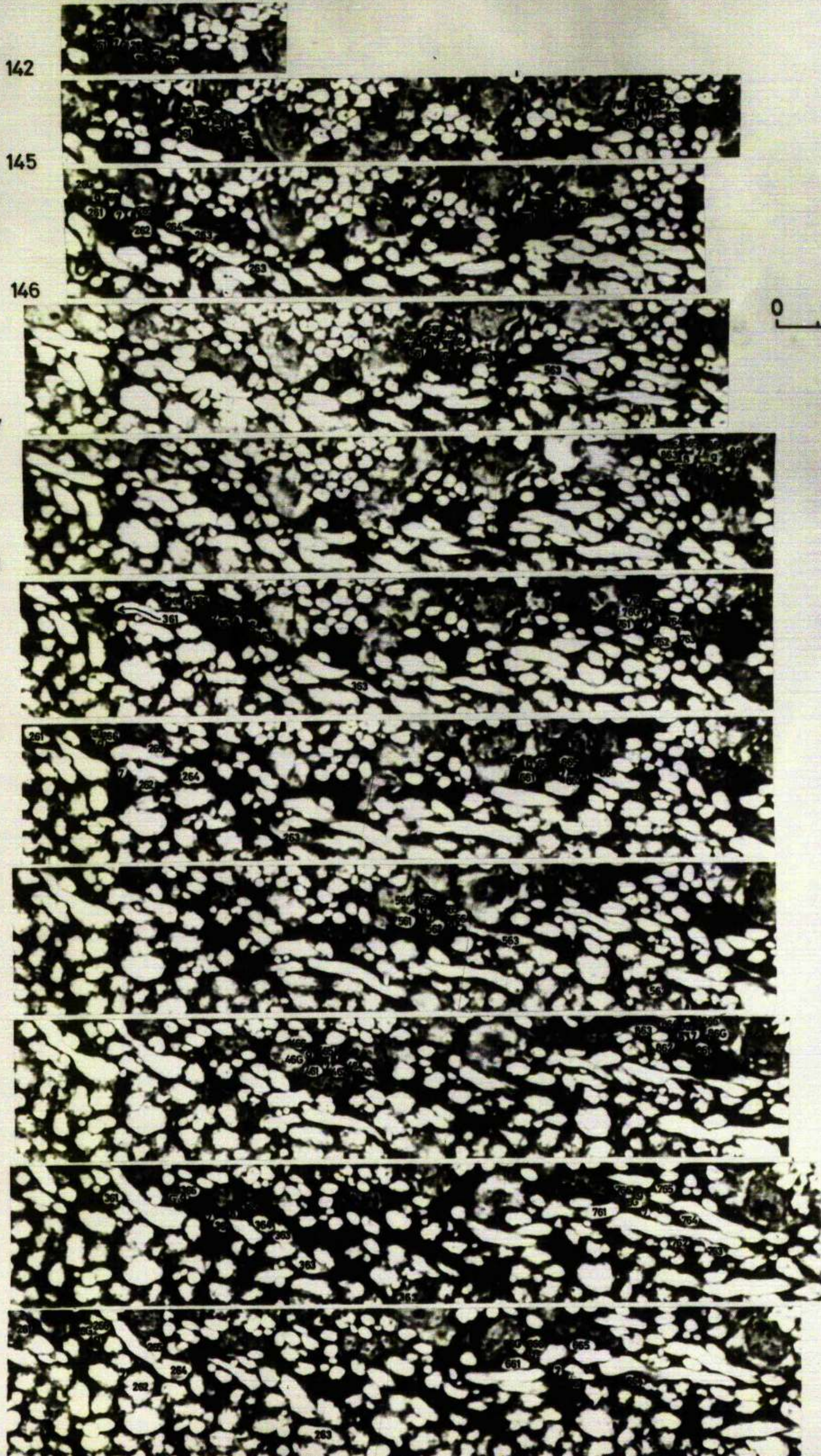
142
145
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152
153
154
0
10µm.

the axon pathways comprising the lattice, they do not reveal the spatial relationships of axons nor, due to shrinkage, the intimate relationship between the axons. These relationships are illustrated in figs. 13 and 14 and are predictable.

The four axons 1, 2, 5, and 6 from one ommatidium diverge to four immediately adjacent cartridges. They can be considered as two pairs, 1 with 5, 2 with 6. Axons 1 and 5 move in opposite directions along the y axis, 2 and 6 along the x axis. This results in a reciprocal relationship between one of the complementary axon pairs of neighbouring ommatidia, e.g. axons 1 and 5, from two adjacent ommatidia of the y axis (fig. 13).

The axons 3 and 4 both diverge more than 1 cartridge width and form parallel rows along the x axis, within which each axon (e.g. 3) is in close association with the other (i.e. 4) first from its own ommatidium and then from that of the next ommatidium along the x axis.

Axons 7 and 8 travel as a pair, first on either side of axon 2 from their own ommatidium and then on either side of axon 6 that crosses over to their cartridge. In crossing over, axon 6 always passes between axons 7 and 8.

At the equator the spatial relationships are modified as a result of the altered axon composition of this region. The third and second rows of equatorial cartridges are altered only by the addition of an extra axon 3 and

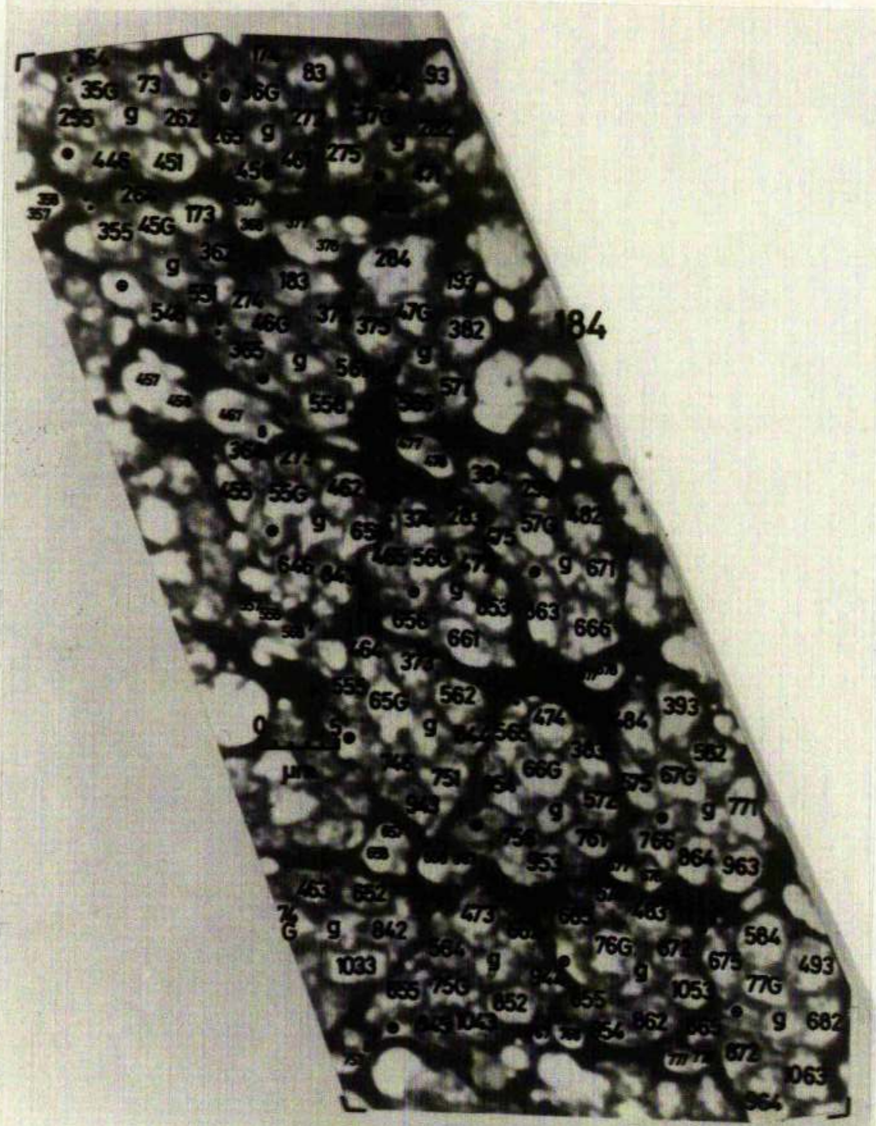
additional axons 3 and 4 respectively from the opposite half of the eye (fig. 8). After crossing the equator these axons travel between the retinula bundles of that side and have a superficial pathway overlying the other axons of the lattice until they reach their cartridge. In the equatorial row of cartridges however the addition of two more axons (2, 5) from across the equator (fig. 8) results in a more fundamental alteration of the lattice. One conspicuous feature is that axon 5 which crosses the equator passes between the long retinula axons 7 and 8 of the cartridge in which it will terminate in a manner identical to that of the axon 6 as it moves away from the equator in all other parts of the eye. In other words the path of axon 5 from one side of the equator exactly mimics that of axon 6 from the other side and vice versa. A similar equivalence exists for the axons 1 and 2 of both halves of the eye.

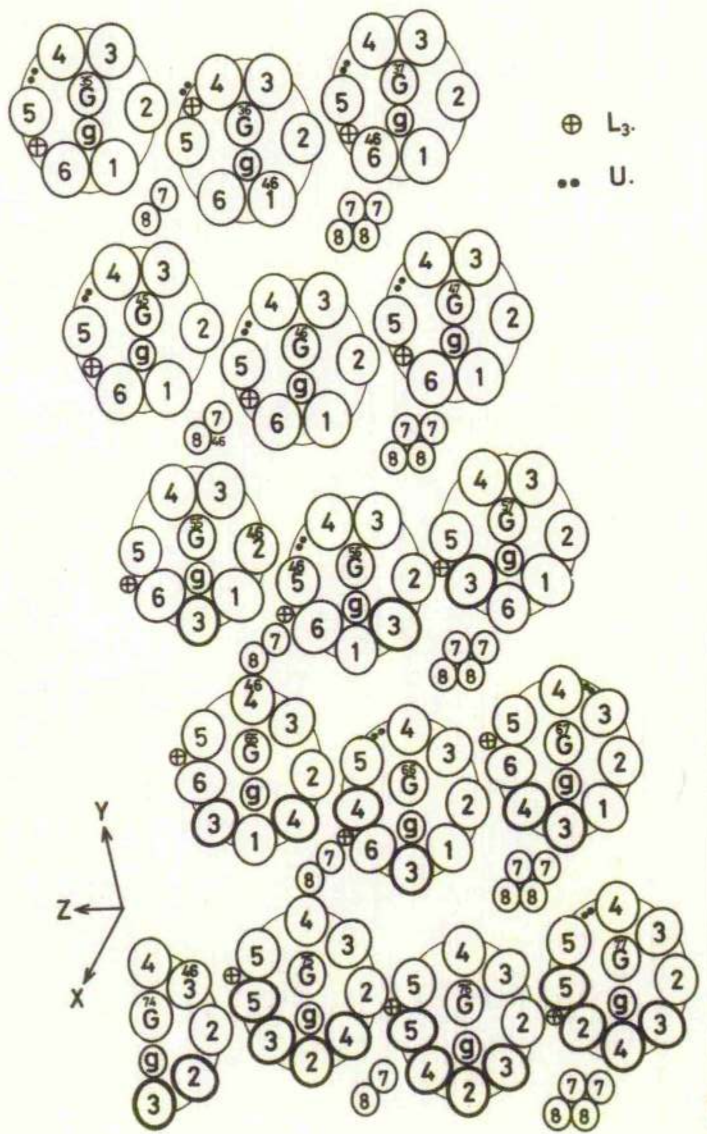
A conspicuous feature of the axon lattice is the occurrence of the nucleus of an unidentified cell body (figs. 12, 13). It has a periodic distribution and is situated in the interstices of the diverging axon groups. No axon can traced from it and its position does not seem to correspond to those of the four monopolar neurone somata described by Braitenberg or Strausfeld. In addition, it is darkly stained by toluidine blue and does not resemble the stained appearance of known monopolar cell bodies. It is tentatively identified

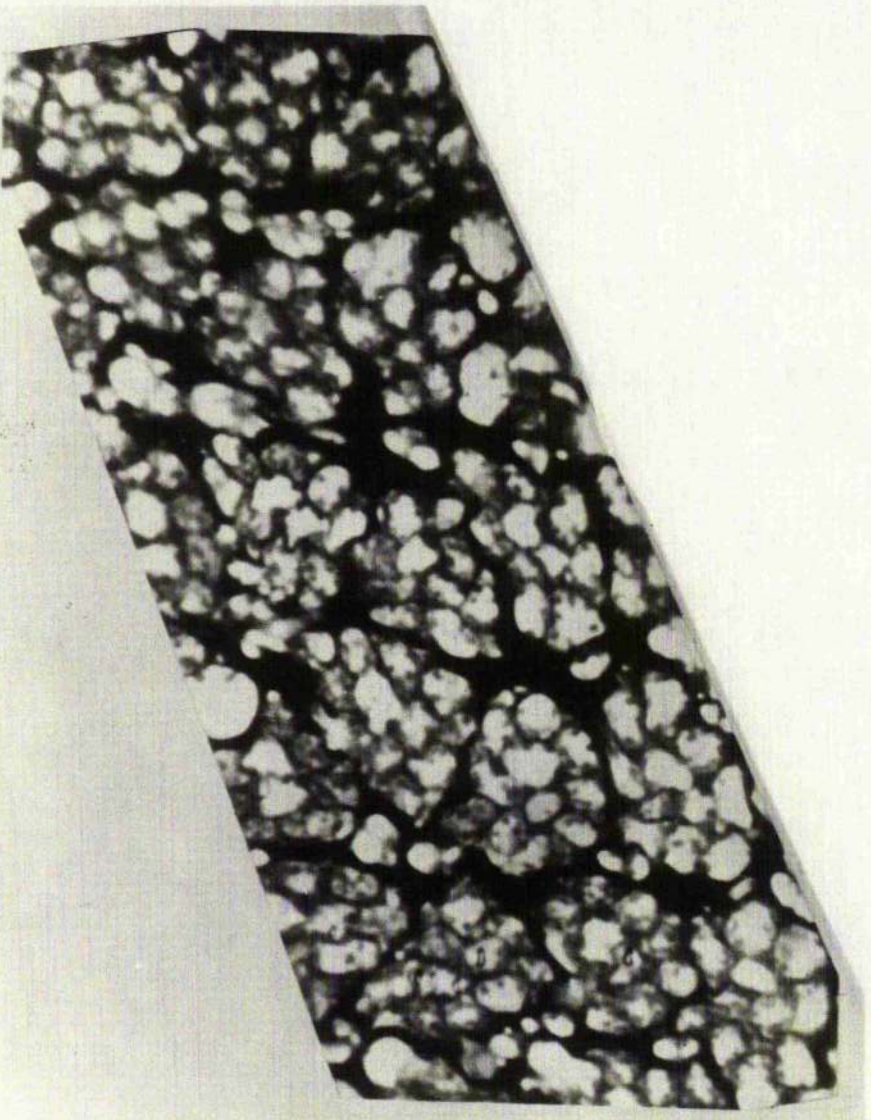
Figure 15

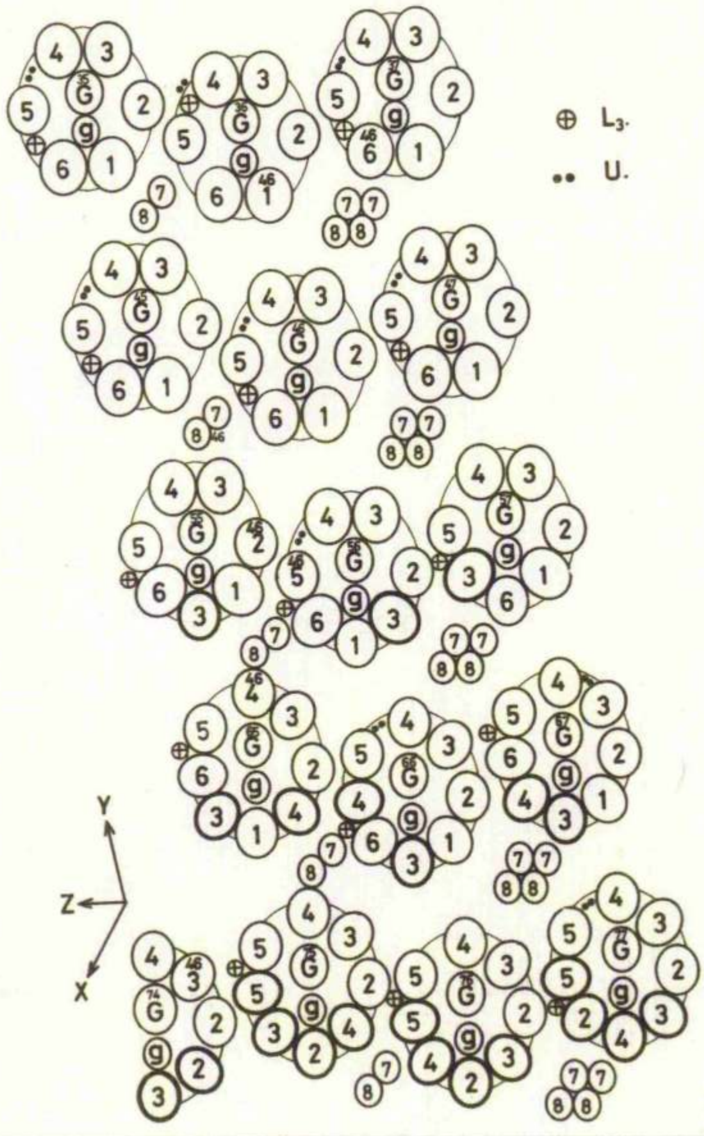
C. vomitoria a. Left. Micrograph from section 184 of five horizontal rows of lamina cartridges immediately dorsal to the equator. The retinula terminals of these cartridges are numbered in the overlay together with the long retinula axons and the monopolar axons g and G.

b. Right. Plan of the axon arrangement of these cartridges. There are eight short retinula cell endings in each cartridge of the first two equatorial rows (numbers 74 - 77, 65 - 67), seven in the next (55 - 57) and six in the last two (45 - 47, 35 - 37). Each terminal is marked with its class number (1 - 6) and those from ommatidium 46 are marked in full to aid comparison with the micrograph. The plan shows the rotational sequence of endings and those endings added to the sequence from ventral ommatidia are enclosed in a dark circle. On both the overlay to the micrograph and on the cartridge plan are also marked the positions of the profiles of the axon of L3 (crossed circles) and (dots) two unidentified fibre fragments (shown by Braitenberg, 1970).


184
0 5 µm


⊕ L3.
•• U.
Y
Z
X




⊕ L₃.
•• U.
Y
Z
X

as a glial nucleus in fig. 12, but may be the cell body of the fifth monopolar neurone described by Trujillo-Cenoz and Melamed (1970).

<u>The composition of the lamina cartridges</u>

The rotational sequence of short retinula terminals is predictable (Braitenberg, 1967) and the level of predictability is extremely high (Horridge and Meinertzhagen, 1970 a). Fig. 15 shows the terminal arrangement for a portion of the lamina shown in the plan of fig. 8 consisting of five rows immediately dorsal to the equator. Generally there is variation in the sequence of axons that cross the equator and they terminate anywhere in the ventral half of their cartridge sometimes interrupting the sequence of dorsal retinula axons which preserve their rotational sequence rigidly. In the dorsal retinula axons of fig. 15 the order is in fact perfect. In the third cartridge row from the equator one axon 3 is added (forming a seven-terminal cartridge), in the second from the equator axons 3 and 4 are added (forming an eight-terminal cartridge) while in the equatorial row axons 2 - 5 are added (also forming an eight-terminal cartridge). The position of the axon of the monopolar neurone L3 is also shown; in non-equatorial cartridges its position is between terminals 5 and 6 and with the exception of one cartridge (36) it rigidly adheres to its sequential position. In the equatorial cartridge rows more

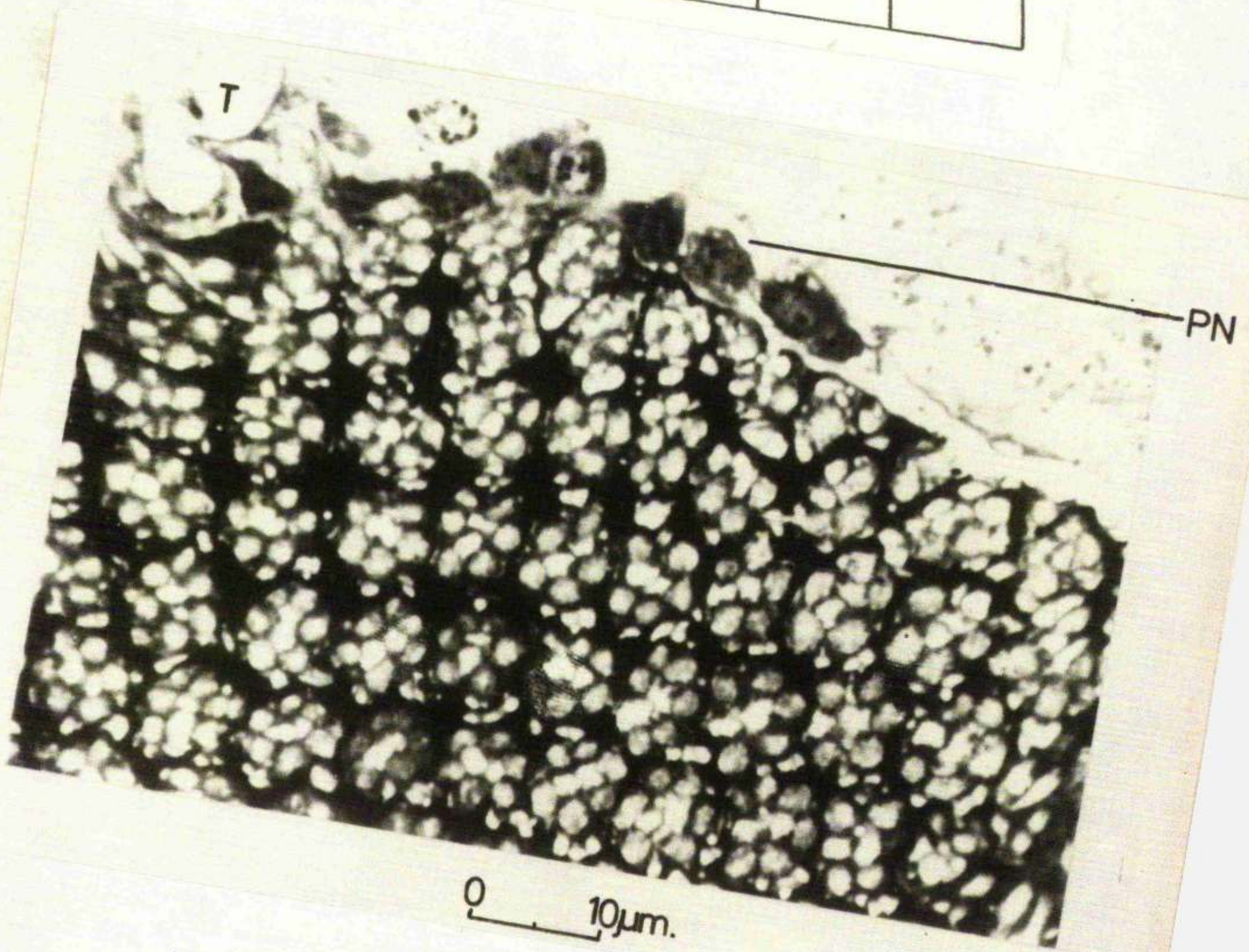
Figure 16

C. stygia. The cartridge array at the edge of the lamina.

a. Top. Plan of the predicted short retinula cell complement of cartridges B, F, J, N and R on the mid-dorsal or mid-ventral margin. The numbers at the left indicate the expected number of terminals.

b. Bottom. Micrograph of the edge of the lamina showing cartridges of reduced size. The cartridge array becomes disordered near the margin of the lamina and no axons have been traced in this region. PN = perineurium; T = tracheole.

6	A	1 6 E5 B F2 M3 I4	C	D
6	E	C1 B6 I5 F J2 Q3 M4	G	H
5	I	GI F6 M5 J N2 Q4	K	L
4	M	K1 J6 Q5 N R2	O	P
2	Q	N6 O1 R	S	T



terminals are added by axons crossing the equator so that, together with the remaining dorsal retinula, the axon of L3 comes to occupy a more dorsal position in the cartridge. Each cartridge row is staggered and interdigitates with neighbouring rows resulting in irregular interstices between them. Frequently long retinula axon pairs of any horizontal row aggregate into single and double pairs.

At the periphery of the lamina, cartridges of reduced size occur because they receive only the two axon classes (1 and 6) that travel away from the equator. Fig. 16b shows that cartridges of reduced size occur at the edge of the lamina, though their composition is unclear and their axons have not been traced. The predicted cartridge sizes from the known projection pattern are shown in fig. 16a.

The neurones of the lamina

Of the four lamina monopolar neurones L1 - L4 (Braitenberg, 1967; Strausfeld and Braitenberg, 1970; Trujillo-Cenoz, 1965) only the positions of the cell bodies L1 and L2 have been identified here. Pairs of cell bodies L1 and L2 (labelled g = L1 and G = L2) are situated in horizontal rows on the side furthest from the equator of the horizontal row of retinula bundles overlying them. The cell body g is situated above that of G and the two are further distinguishable because g has a finer calibre axon

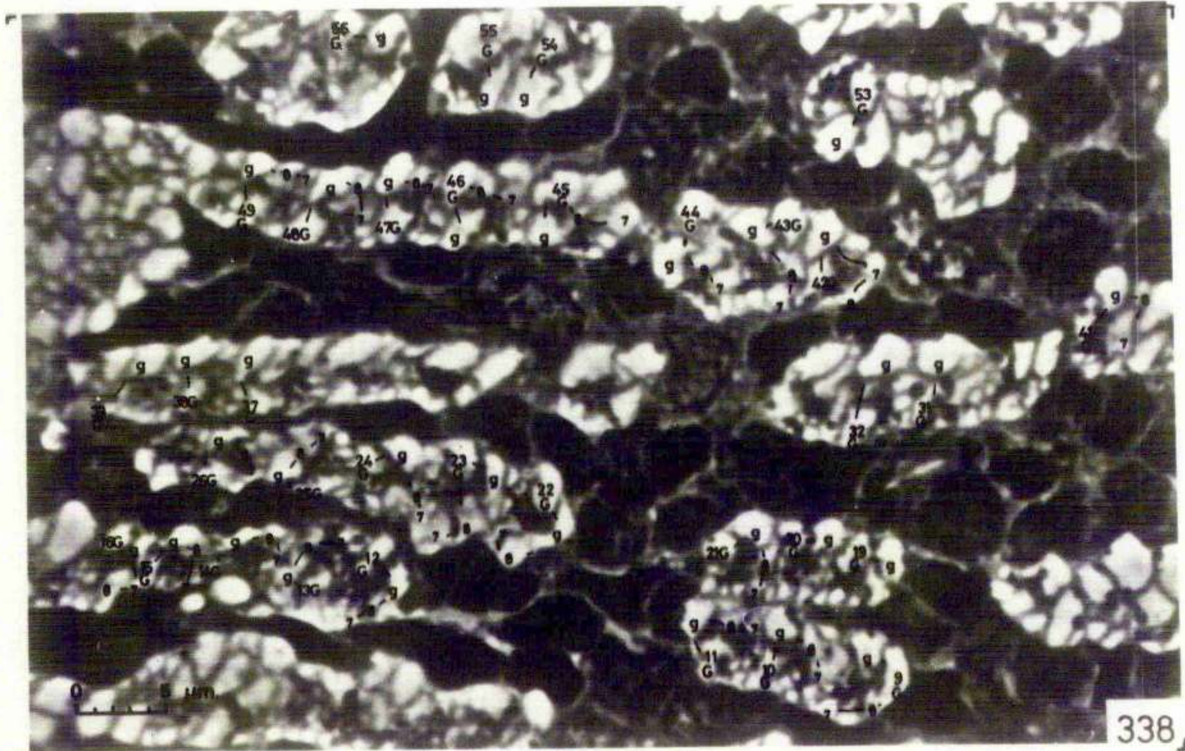
Figures 17 and 18

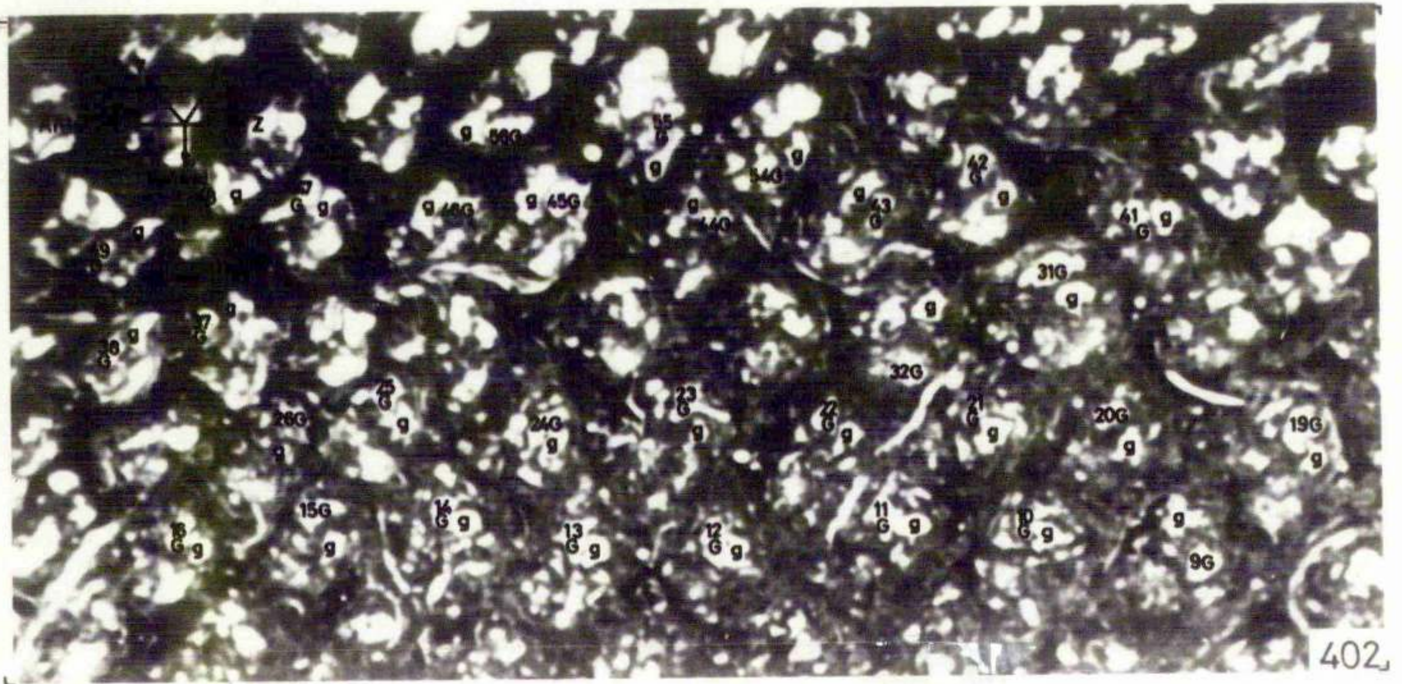
C. vicina Micrographs of four sections to show the area of the lamina-medulla projection traced for this series of a left eye. Only the two large monopolar axons (G and g) and the two long retinula axons that have unambiguously been traced through the chiasma are marked on the overlay and those originating from one cartridge are linked.

Figure 17

Top. Section 49. Mid-lamina region with array of cartridges and satellite long retinula axons, from the four rows immediately ventral to the equator. The number of retinula terminals in the cartridges of each row is shown on the left.

Bottom. Section 169. Top of the lamina-medulla chiasma to show the appearance of the horizontal stratifications of axon groups. Within the strata the axon groups invert by a clockwise twist. Scale and axes as fig. 18.


0 5 µm
338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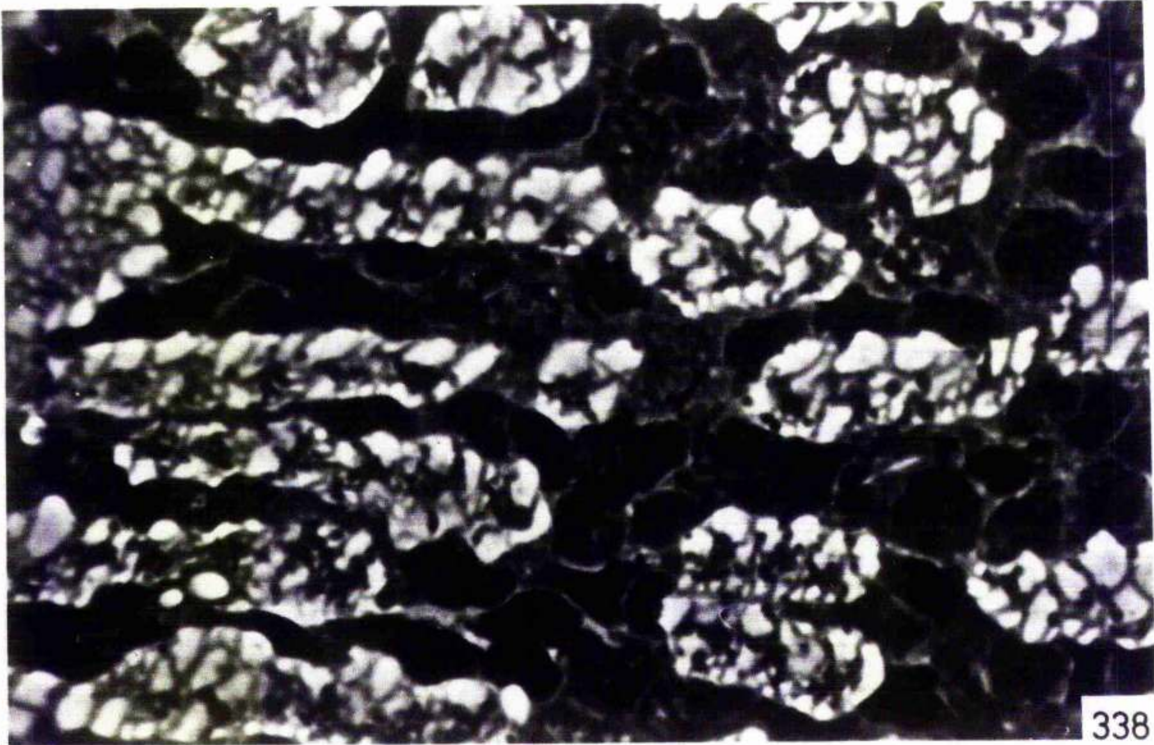
49

169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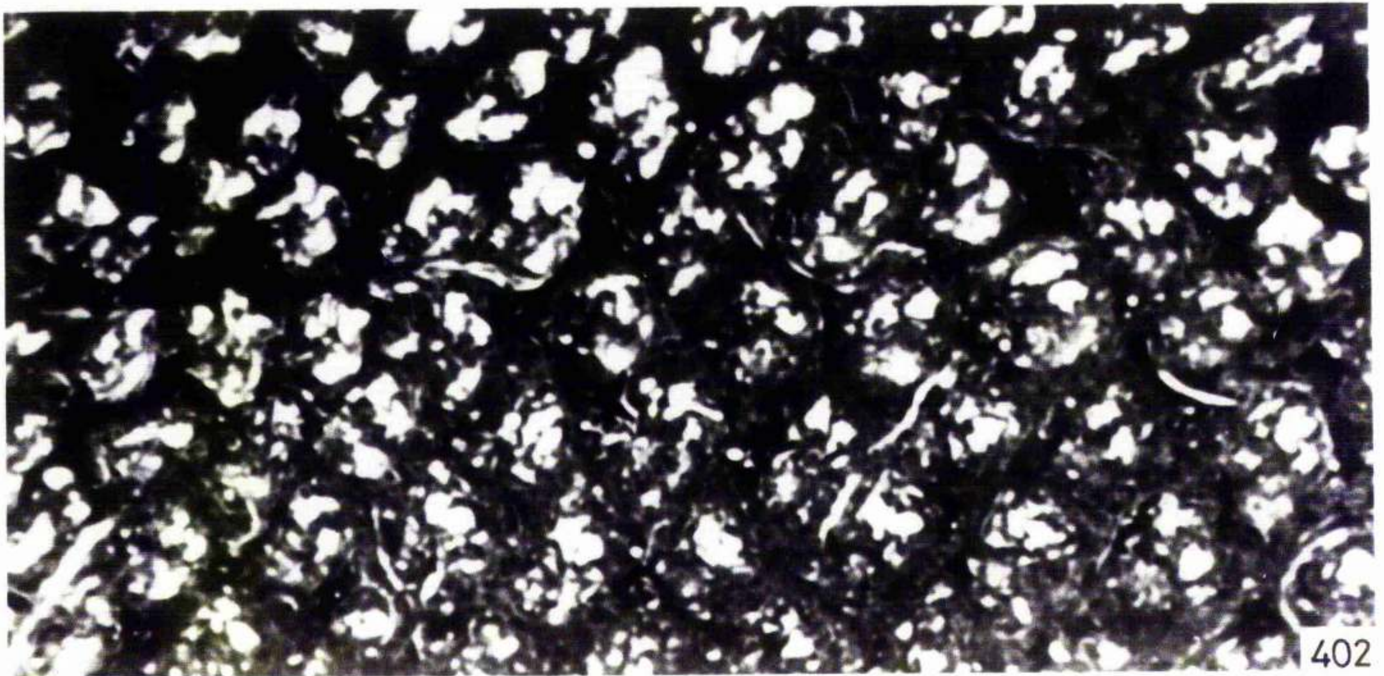

402

49

169

Figure 18

Top. Section 338. Top of the medulla to show bands of axons grouped into presumptive cartridges separated by columns of medulla monopolar cell bodies.

Bottom. Section 402. Neuropile of the medulla. The period of the medulla cartridge array equals that of the lamina. At the top of the micrograph the section cuts the medulla obliquely at its border which has numerous darkly staining nuclei surrounding the cartridges. The long retinula axon terminals are not marked on this section.

than that of G throughout the lamina (though this difference in calibre is somewhat reversed in the chiasma) and because the axon g is nearest the equator. The axons of these neurones have to move a little in an equatorial direction before becoming situated between the arms of the chevron of axons (e.g. bundle 98 in fig. 11). The position and path of the axonal isthmus of these cell bodies provides another example of mirror symmetry about the equator of the fly eye. At the equator above the lamina a double row of retinula bundles from each side interdigitates to form a conspicuous band of axons, flanked by horizontal rows of cell bodies. In the region with a dislocation in the equator this pattern is disturbed because the position of retinula bundle and cell body pair is reversed and the band of axons interrupted by the cell bodies of the dislocated cartridges.

The lamina-medulla projection

Axons from thirty lamina cartridges were traced from a series of sections of the left eye of *C. vicina* from a region near the equator and approximately halfway between anterior and lateral regions of the eye (figs. 17 to 20). The large monopolar axons can be traced over much larger areas and their projection is always homotopic (see below, fig. 21). The other four axons of each cartridge that have

been traced (two long retinula and two others, see figs. 19, 20) are much finer and more difficult to follow. For clarity only the two large monopolar axons and the two long retinula axons are marked on the overlays of figs. 17 and 18. All of these axons where they can be traced proceed to one medulla cartridge and none from one cartridge was observed to diverge to an adjacent cartridge. The medulla cartridge array exactly reproduces the lamina cartridge array but becomes reversed about a dorso-ventral plane by a clockwise twist in each horizontal stratum of the chiasma. None of the fine axons of the strata immediately dorsal to this area (i.e. the dorsal half of the eye) could be traced because they move laterally too quickly at the top of the chiasma to make tracing certain. The large monopolar axons which could be followed however invert by an anticlockwise twist to each stratum of the chiasma as reported by Braitenberg (1970). The projection pattern of these axons on to the medulla remains homotopic with respect to the ventral half of the eye in spite of the different lateral displacements that the axons of each stratum undergo in the chiasma. These displacements are a general problem in tracing through the chiasma of all insects both because the micrograph field size has to be large and because axons moving laterally are difficult to trace.

Cell bodies of other neurones classes are conspicuous

in the chiasma. Amacrine cells (Cajal and Sánchez, 1915; Strausfeld, 1970) have somata (diameter 5 μm) immediately under their cartridge, probably one per cartridge (arrowed in section 73, fig. 19). Other larger cell bodies at the top of the chiasma (diameter 10 μm) (section 169, fig. 17) are probably of lamina tangential neurones; their aperiodic arrangement has been discussed by Strausfeld (1970). No processes conspicuously emerge from any of these cell bodies so that their identification is inferred from Golgi preparations. Glial nuclei and tracheae (section 169, fig. 17) also permeate the chiasma.

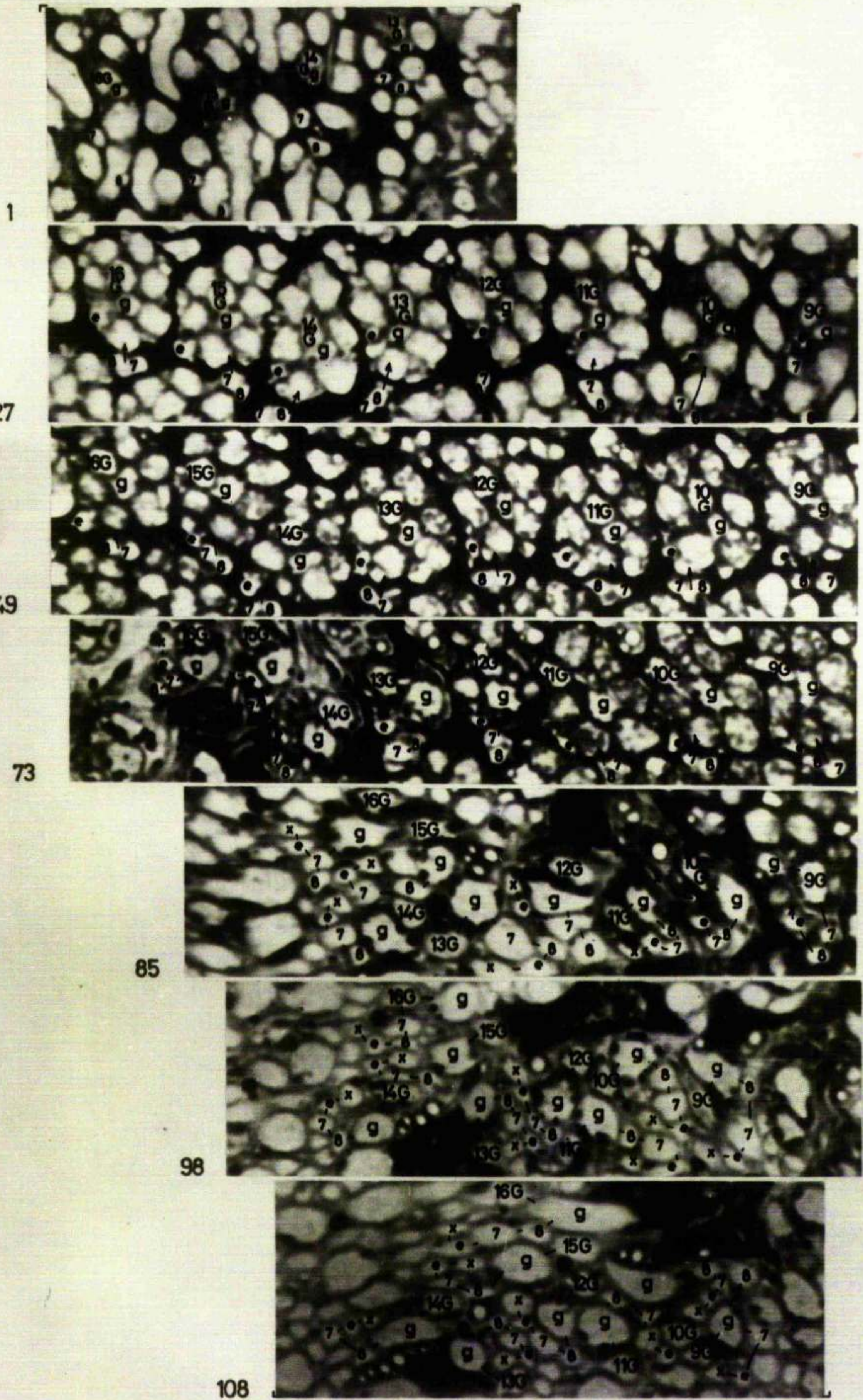
Having travelled the chiasma the field of axons is arranged in horizontal bands separated by columns of medulla monopolar cell bodies (section 338, fig. 18). Very often such a horizontal band consists of a highly ordered row of axon groups. Each group, which contains the axons of an individual lamina cartridge, is marked by the conspicuous axons g and G arranged in a dorso-ventral orientation and eventually forms a single medulla cartridge. At the level of the medulla neuropile in addition to the two large monopolar axons g and G the group contains at least eight fine axons some of which are not easily resolvable by light microscopy (see Trujillo-Cenóz, 1969, fig. 9). The axons of a number of medulla monopolar neurones are presumably also present in each bundle.

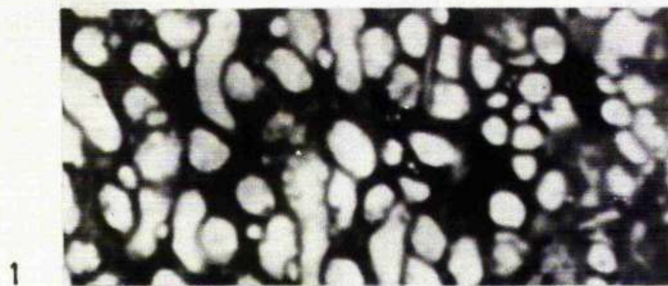
Figures 19 and 20

C. vicina Micrographs of one horizontal row of the lamina-medulla chiasma to show in detail the paths of six axons associated with each of eight lamina cartridges (9 - 16). The six axons are two large monopolar, two long retinula and two others marked e and x, and those of one cartridge are linked together.

Figure 19

Sections 1 to 108 are of the lamina and top of the chiasma. Bundles of axons (posterior portion of section 73) afferent or efferent to single cartridges emerge at the bottom of the lamina and enter the chiasma. Arrowed in section 73 is the nucleus of a presumed amacrine cell body. In the micrographs of lamina, more gradual progression of the axons through the lamina may be obtained by comparison of the cartridges within each section (the posterior cartridges are cut more proximally than the anterior ones).


1
27
49
73
85
98
108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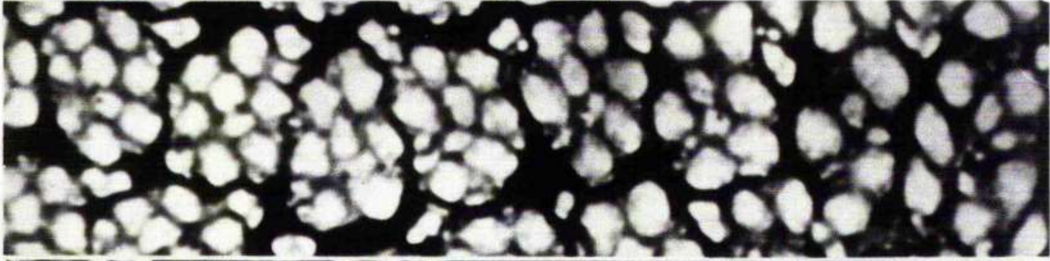
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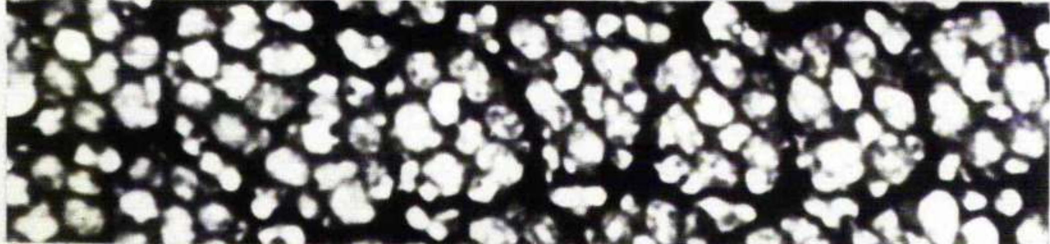
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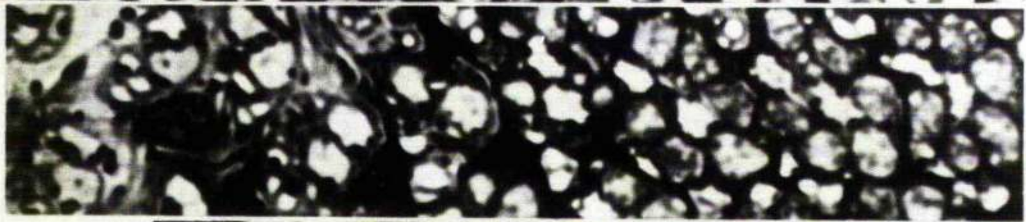
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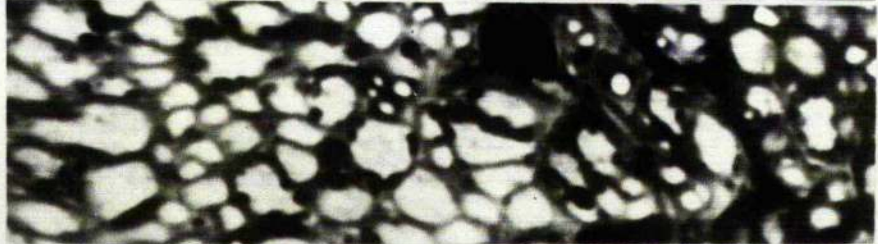
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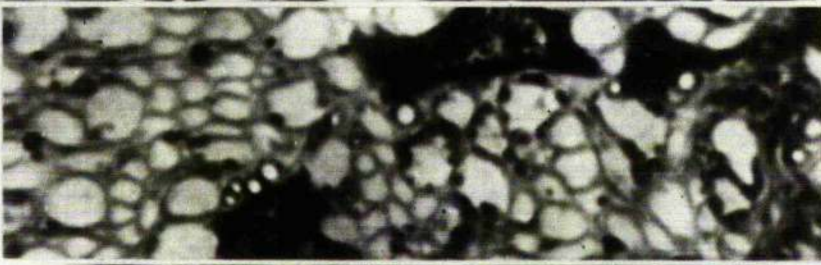
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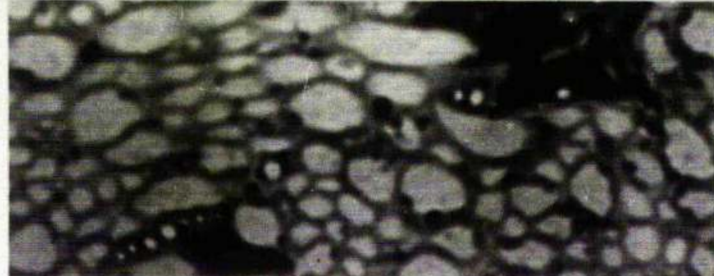
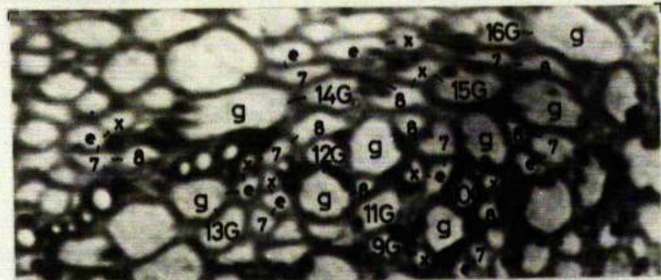

108

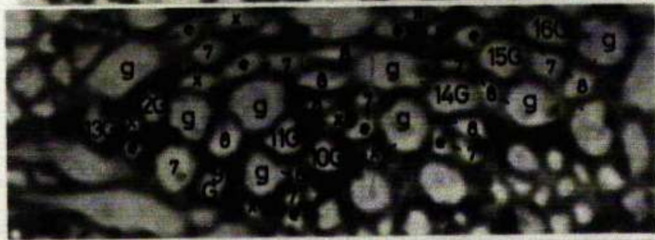
Figure 20

Sections 121 to 397 are of the chiasma and medulla. After inverting their horizontal sequence axons separate into their cartridge groups (Section 356).



121

0 10µm.



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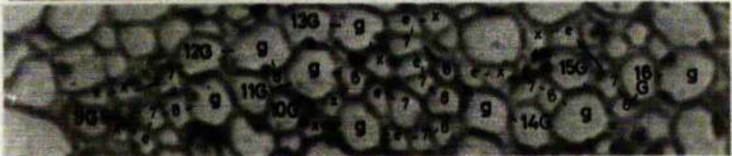

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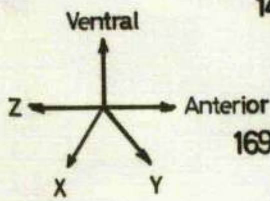

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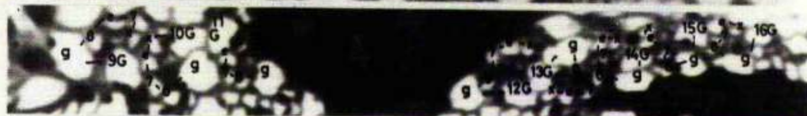

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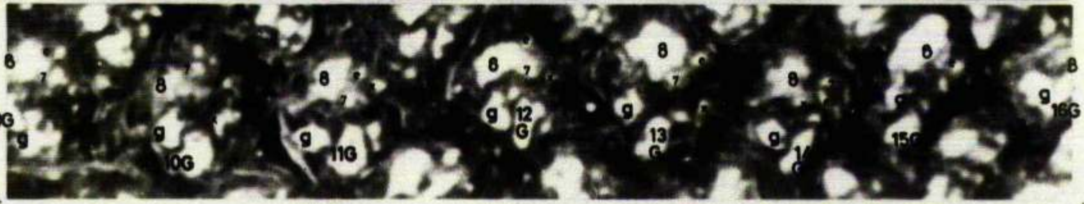

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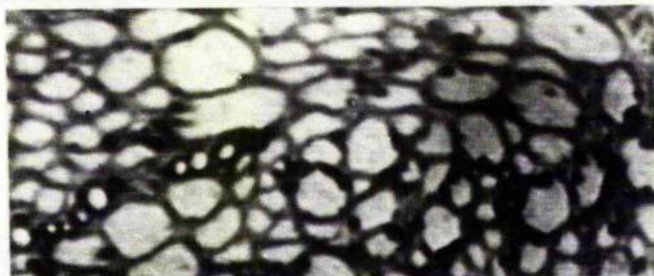

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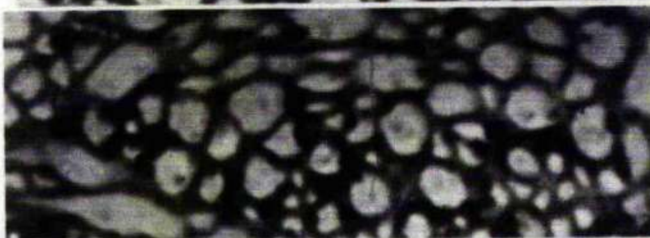
0 10μm.



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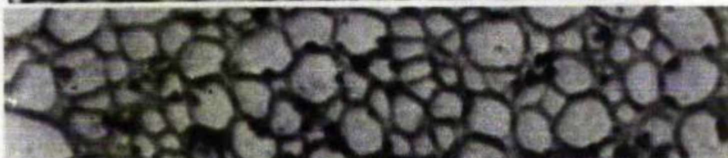

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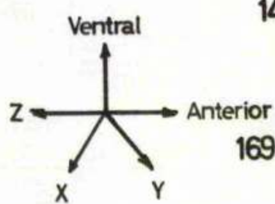

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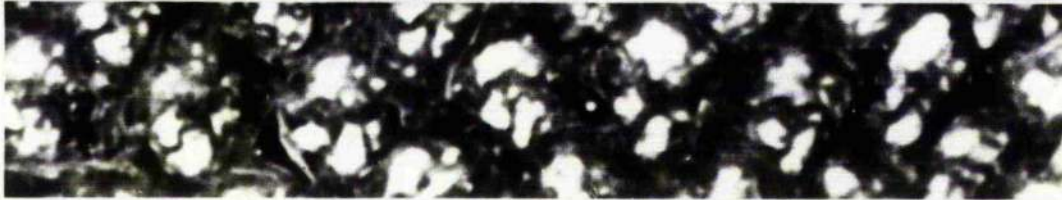


397

The paths through the chiasma of a group of six axons traced from each of eight cartridges within a single horizontal row of the lamina are shown in figs. 19 and 20; each axon is individually labelled as follows: two large axons G and g, two long retinula axons 7 and 8 and two other axons labelled e and x. The axon e (the fifth axon of the group mentioned by Horridge and Meinertzhagen (1970 a) can be identified as the third lamina monopolar axon L3 of Braitenberg (1967) by the position and size of its axon in the lamina cartridge. At the top of the lamina it becomes very slender and cannot be traced to its cell body (section 1, fig. 19), while at the bottom of the lamina it often has a close association with retinula axons 7 and 8. Another slender profile, that of the fourth monopolar axon L4 (Strausfeld and Braitenberg, 1970), can be seen alongside e in and about the plexiform layer of the lamina but is too fine and inconsistent to follow. At the top of the lamina the profiles of L3 and L4 are equally slender and that of L3 cannot always be unambiguously traced. Consequently both profiles are labelled e in some cartridges (e.g. cartridges 13, 14, section 1; cartridge 9, section 27; fig. 19). The axon x (not included in the account of Horridge and Meinertzhagen (1970b) is traced only from the bottom of the plexiform layer of the lamina where it apparently forms a network of very fine axons underneath the cartridge. It is

probably one of the centrifugal axons from the medulla, although no connecting fibres to a cell body at the side of the chiasma have been observed.

All six axons that have been traced from a single cartridge (fig. 19) can be followed into the medulla neuropile. They have an anticlockwise rotational sequence within their bundle of G, g, 8, 7, e and x. There is at least one other unidentified axon between G and x and other fine axons at the centre of each bundle.

At the level of the medulla ganglion cell layer the profiles of the small axons (0.5 - 1 µm diameter) become inconsistent and difficult to trace with absolute certainty in many cases, largely because the profiles of the medulla monopolar axons and of the other axons of the lamina cartridge which could not be followed through the chiasma become visible at this level. The horizontal rows of axons soon segregate into individual bundles and axons are not seen to pass between the bundles, so that all six axons contribute to one cartridge of the medulla (fig. 20).

Similar but less extensive results have been obtained with two other series both also for cartridges near the equator. This region is of particular interest because between two rows of lamina cartridges exists a double row of satellite long retinula axon pairs. In no case has such a pair diverged across the equator to a cartridge of the opposite side.

Figure 21

Reconstruction of the chiasmal paths of the axons G (L2) of seventeen cartridges of one horizontal row of the lamina. This row includes those x axons (41G - 49G) plotted in figs. 17 and 18, the remaining axons are numbered in sequence on either side. The group of seventeen cartridges represents just over one third of the width of that row, with a further seventeen cartridges anterior to it and twelve posterior to it. The positions of the axons between lamina (section 97) and medulla (section 313) are represented in five intermediate sections. In the top right hand corner the central border of the lamina is marked.

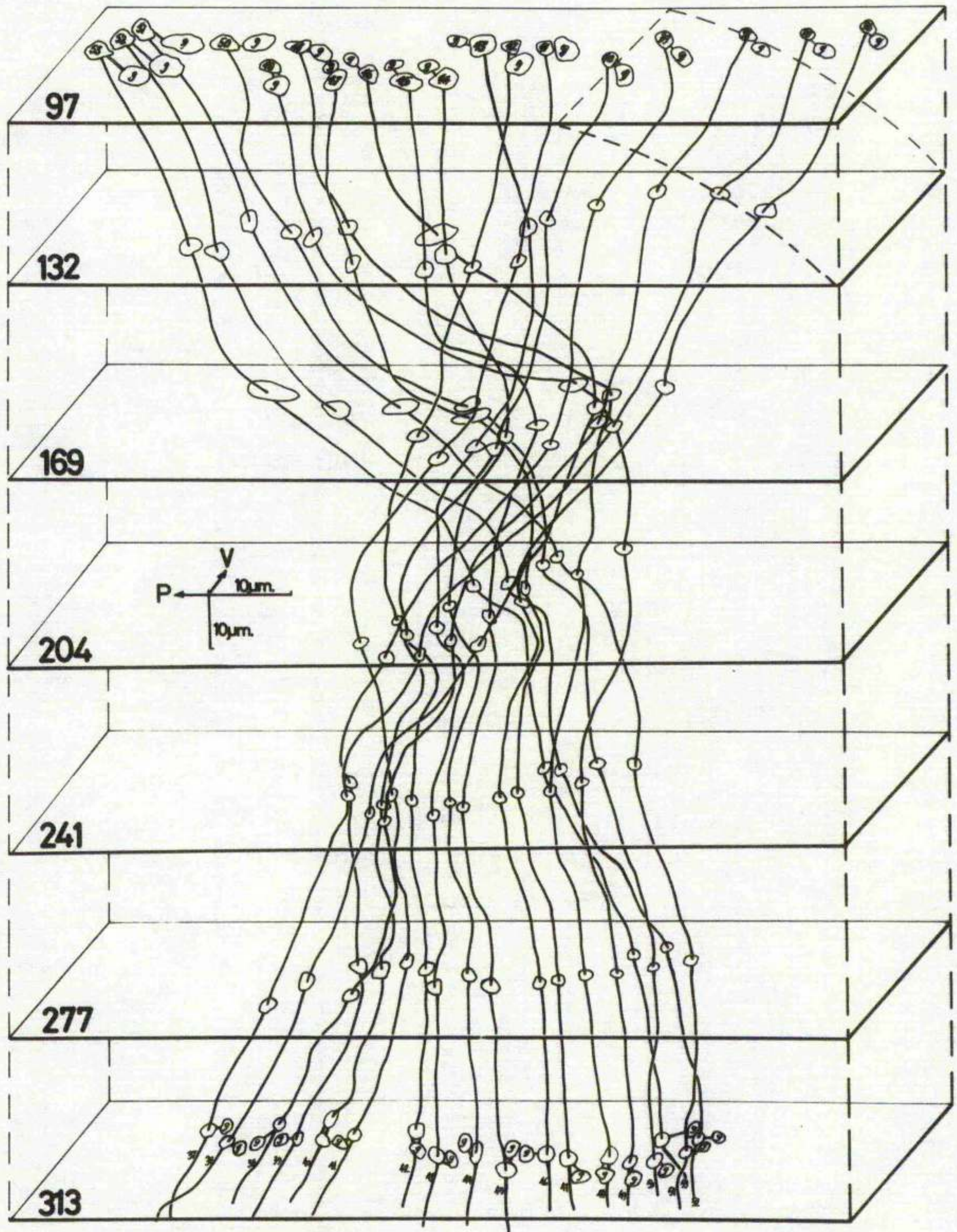
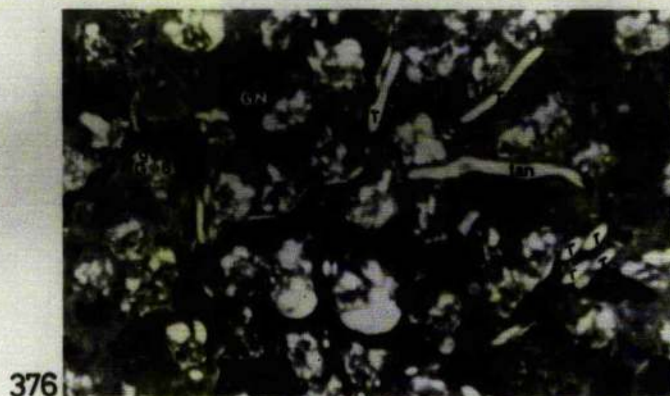

97
132
169
204
241
277
313
V
P
10µm.
10µm.

Figure 22

C. vicina Micrographs of the same area from a series of six consecutive sections nos. 376 - 381 (taken from a different region of the same series as that used for figs. 17 to 21) in the region of the superficial medulla neuropile. The periodic arrangement of medulla cartridges is obvious in addition to a single process (tan), the mother trunk of a medulla tangential neurone running amongst the cartridges along approximately the x axis. The process runs alongside three parallel rows of the x axis in the field of this micrograph and moves between them twice (arrowed in section 377). Three axon profiles within a single cartridge have been labelled G, g and 8 throughout the sections, but these identities are presumed from the appearance of the profiles and have not been traced from their peripheral origins. Similar profiles may be seen in many cartridges. Axon 8 which swells to a large diameter (4 µm) at one level in most cartridges is particularly obvious. GN = darkly staining nucleus of a presumed glial cell surrounding each cartridge at the edge of the medulla. T = trachea.



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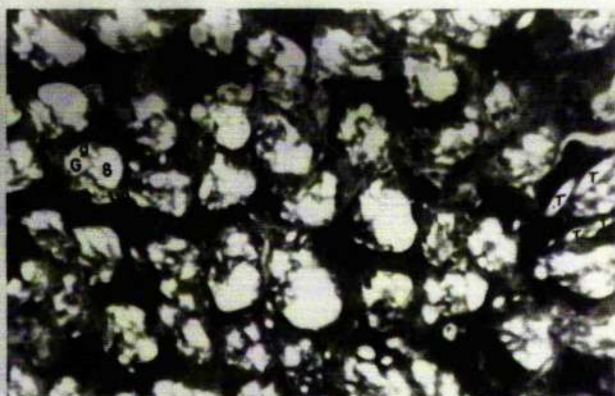

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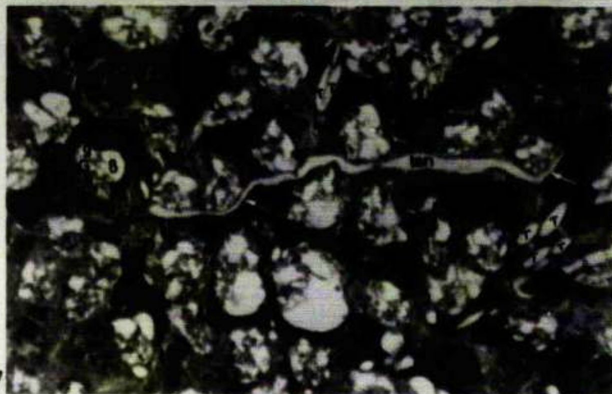

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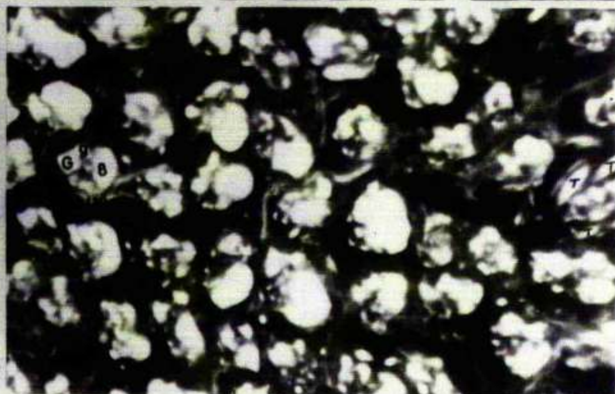

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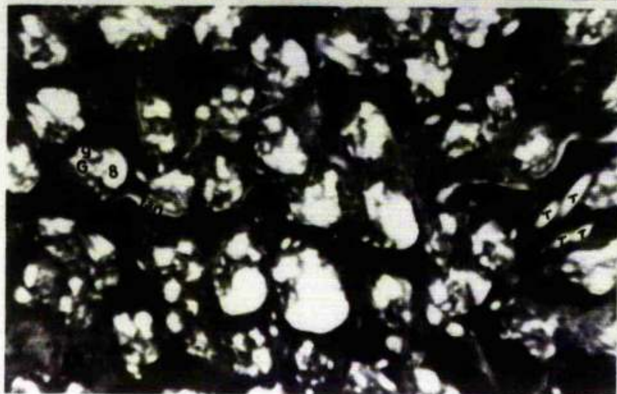

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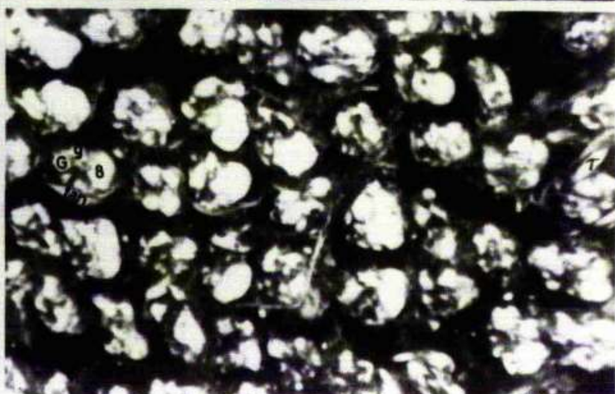
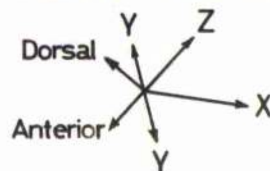


381

0 10µm.



Within the medulla neuropile axon 8 soon undergoes an expansion and partially surrounds the axon g within each cartridge, while at about this same level the axon G is also expanded. This arrangement of axon expansions is reminiscent of Strausfeld's reconstructions of quartets of medulla terminals found in Pieris. Section 397 (fig. 20) is at a level just a little central to the region at which all the axons 8 and G have their greatest diameter, and peripheral to the level at which one of the monopolar terminals divides into two branches as described by Trujillo-Cenóz (1969).

Also within the first synaptic stratum of the medulla neuropile are found processes of medulla tangential neurones which travel amongst the medulla cartridges with an orientation approximately along the x and y axes. One process is shown in fig. 22 which runs along cartridge rows of the x axis and abutts on to the axons of each cartridge. Where the process runs amongst cartridge rows however it establishes close contact only with the axons of one (the ventral) side of the cartridge. Some synaptic contacts between fine collaterals of a tangential process and one of the pair of monopolar terminals have recently been described by Trujillo-Cenóz (1969).

The existence of an uncrossed projection in parts of the chiasma has been claimed since the original account of

Cajal and Sánchez (1915). Figs. 17 and 18 indicate that this uncrossed projection does not occur at least for the six axons traced from each cartridge over a limited width of the chiasma. The possibility remains that a portion of the horizontal width of the chiasmal strata contains such uncrossed axons but has been missed. Trujillo-Cenóz (1969) proposes that the intermediate optic tract (the central region of the horizontal width of the chiasma) might be a region in which this type of projection occurs. The projection of the large monopolar axons of seventeen cartridges from the central region of one horizontal row failed to reveal an uncrossed projection (fig. 21) (53G in fig. 21 crosses 52G at a deeper level not shown in the reconstruction). It therefore seems unlikely that an uncrossed projection exists at all for the perpendicular neurones of the chiasma, and Strausfeld has also failed to observe them, although in Eristalis one of a pair of types of lamina tangential neurones apparently does traverse the chiasma against the grain of the normal decussation (Strausfeld, personal communication). The paths of axons through the chiasma (fig. 21) are not as rectilinear as depicted by Cajal and Sánchez (1915) and involve horizontal excursions through the whole width of the chiasma even though all these axons are from the central third of the row. Axons cross their neighbours at all levels between lamina and medulla and

there is little relationship between either height or horizontal position of cross-over and horizontal sequence in the lamina.

B. The water-bug *Lethocerus*

Some preliminary observations on the retina-lamina projection of the bugs *Lethocerus* and *Benacus* provide an interesting comparison with that of the fly, because the water-bug, at least in the dark-adapted state, apparently functions as an open-rhabdomere type of eye (Ioannides, personal communication).

Each ommatidium is composed of eight retinula cells with a 6 + 2 arrangement, fig. 23a and Walcott (1971 a). The two central cells 7 and 8 in the region beneath the rhabdome are different sizes, 8 small and 7 extremely large (fig. 23 a), and a large axon, probably from cell 7, is easily visible in the axon groups of the first projection (fig. 23 b). The eight axons of each ommatidium penetrate the basement membrane as bundles (fig. 23 c), coalesce into prominent bundles and enter an extremely long (approximately 2mm) first projection (Ioannides and Walcott, 1971) before penetrating the lamina which overlies the separate optic lobe. At the top of the lamina, bundles form consisting of eight axons; centrally a large axon (number 7 in fig. 22f) is closely apposed to a smaller one, with two sets each of

Figure 23

Benacus Micrographs of sections at different levels in the retina and optic lobe.

a. Retina. Cells 1 - 6 with peripheral rhabdomeres are situated in an arc, around the edge of the ommatidium. Two cells 7 (large) and 8 (small) have separate rhabdomeres, with orthogonal microvillar orientations, at the centre of the ommatidium. Cell 7 has a nucleus at this level.

b. Groups of bundles of retinula axons between retina and optic lobe. Each bundle has one large axon which remains conspicuous till the optic lobe.

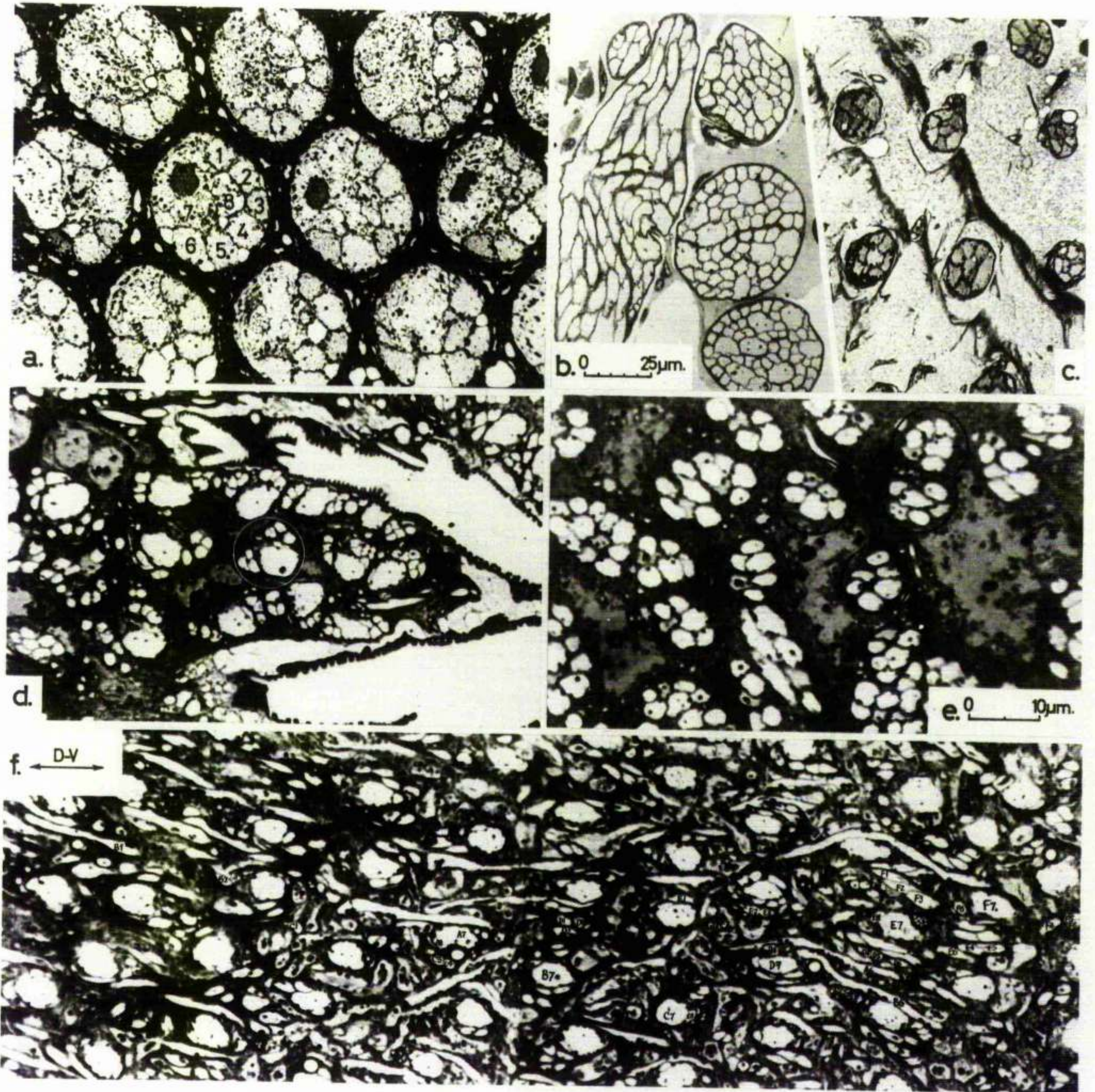
c. Bundles of eight retinula axons penetrate the basement membrane.

d. At the top of the lamina the retinula axons re-form into groups (centre circle) containing one large and seven small axons.

e. At the bottom of the lamina, bundles containing six prominent axons (circled) enter the chiasma.

f. In the superficial lamina neuropile there is an array of retinula axon pairs (7 and 8) which proceed vertically through the lamina and retinula axons (1 - 6) which travel horizontally around about them. The eight axons of six groups (A - F) are marked on the micrograph. The retinula axons are numbered according to the retinula cell to which it is anticipated to belong. The relationship between axon and cell of origin is not yet proven.

Scales a - d, as in b; e, f, as in e.

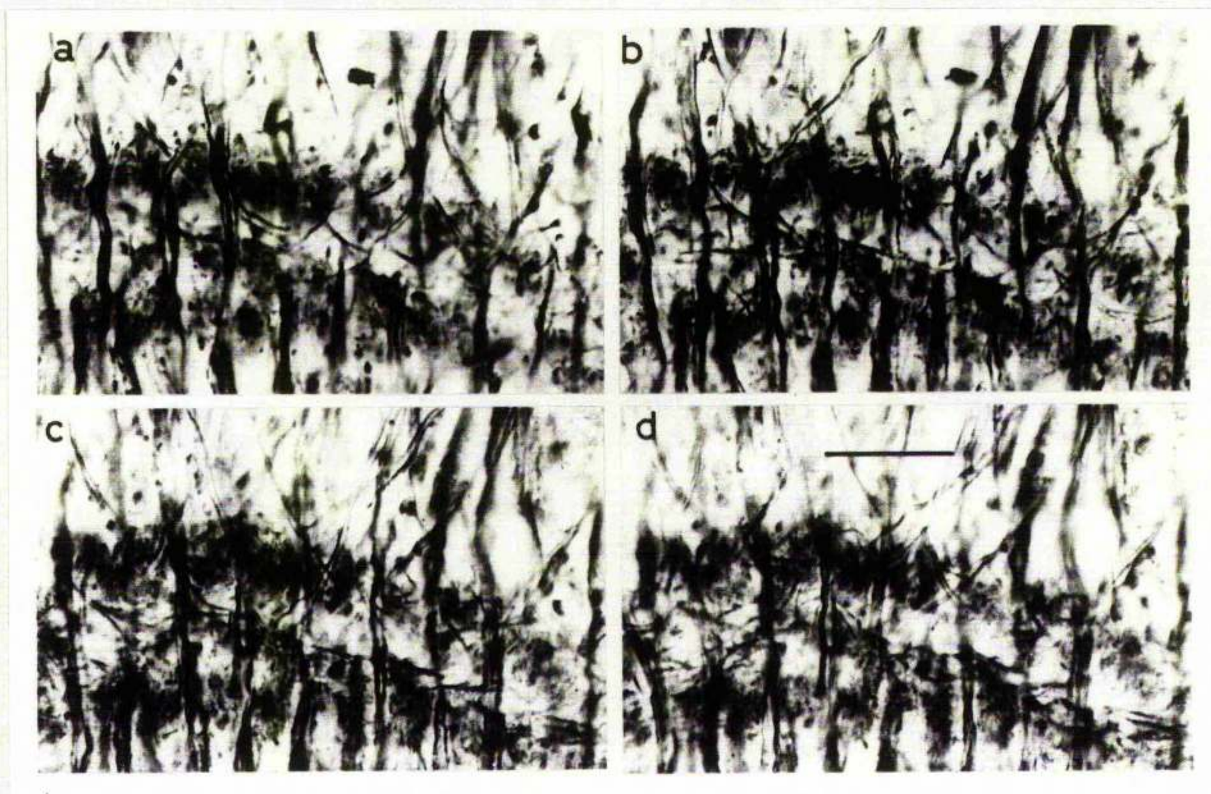

a.
1
2
3
4
5
6
7
8
b. 0 25µm.
c.
d.
e. 0 10µm.
f. D-V

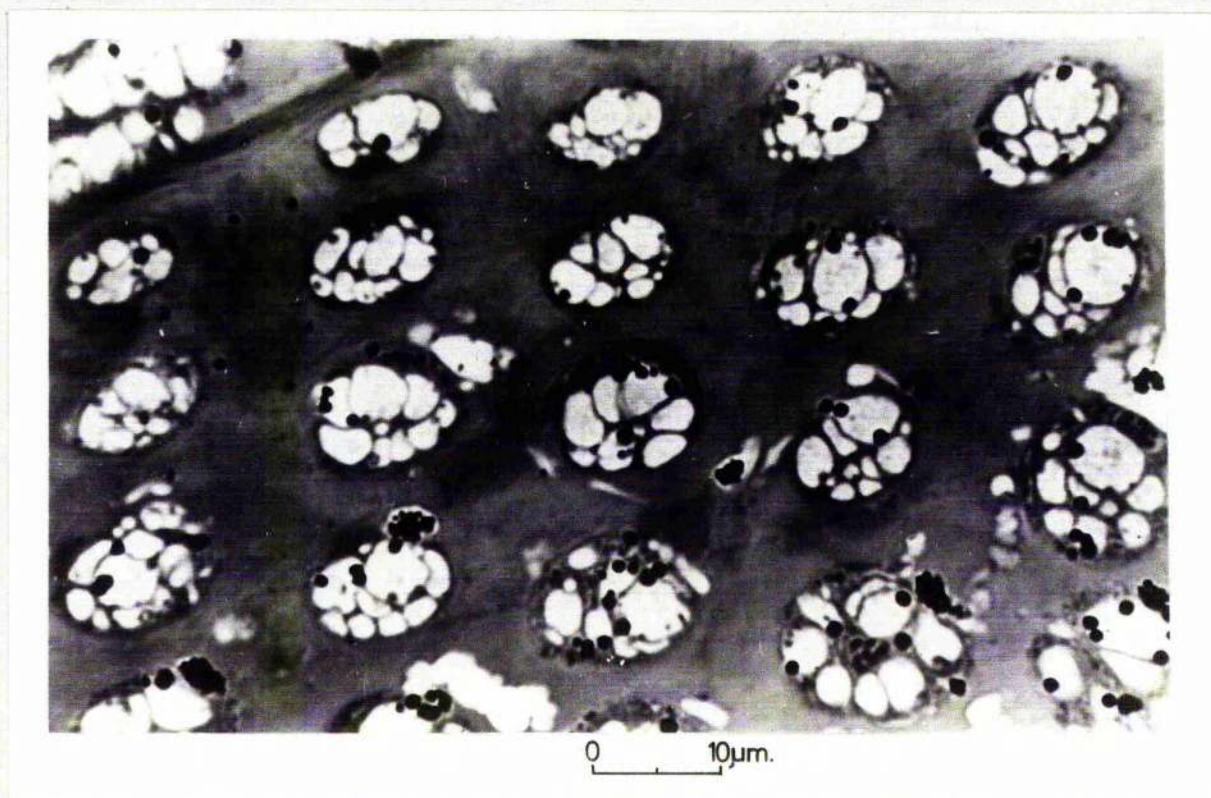
three small axons arranged along a dorso-ventral plane on either side. The two axons at the centre of each group form a prominent feature of the lamina as they proceed vertically downwards, arranged in regular rows. The two sets of three axons flanking the central pair diverge considerable distances in opposite (dorso-ventral) directions to redistribute among the central axon pairs. The divergence pattern is shown in relation to a prominent horizontal band of neuropile in the lamina in fig. 24 a (from a reduced silver preparation). Each triplet of diverging axons travels through the superficial neuropile as a single group until it reaches the second horizontal row of cartridges either dorsal or ventral from its origin. The triplet then splits, one axon (numbered either 1 or 6 in fig. 23 f) travelling tangentially until it reaches at least the fifth horizontal row of cartridges, while the remaining pair (numbered 2, 3 and 4, 5 in fig. 23 f) stay together for a short vertical distance (10 µm or so) in close association with the central axon pair of the cartridge of the second horizontal row. They then move outward at least a further cartridge row. They do not appear to separate. The spatial relationships of the diverging axons are seen in fig. 23 f. Groups of axons moving ventrally necessarily pass close to other groups moving dorsally as they interweave amongst the cartridges. Unlike the fly, the lamina organization is open

Figure 24

a. Top. Lethocerus 14 μm paraffin embedded section stained by Fraser-Rowell's (1963) reduced silver method, focused at different levels (a - d) in the section. The section is slightly oblique so that the perpendicular axons of two rows of cartridges are included together with two bands of superficial lamina neuropile (one from each row). Portions of the diverging retinula axons are obvious in the section, but no single axon is present from origin to termination. Scale in d, 25 μm.

b. Bottom. Notonecta Tangential section which grazes the basement membrane, showing the arrangement of ommatidial bundles of retinula axons. Each bundle consists of eight axons though the number of these is not consistently obvious in each (other small profiles of the bundle are probably the spaces left by lost pigment granules).


a
b
c
d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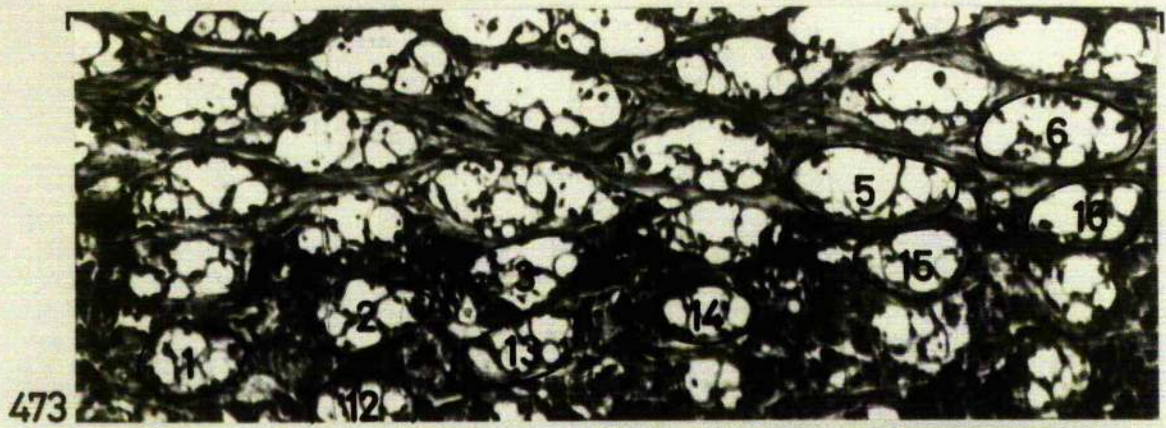
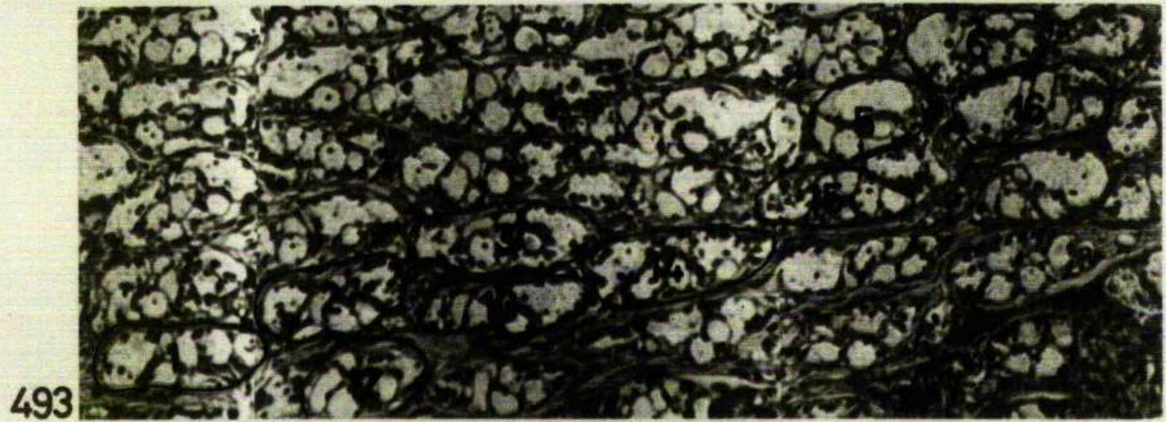
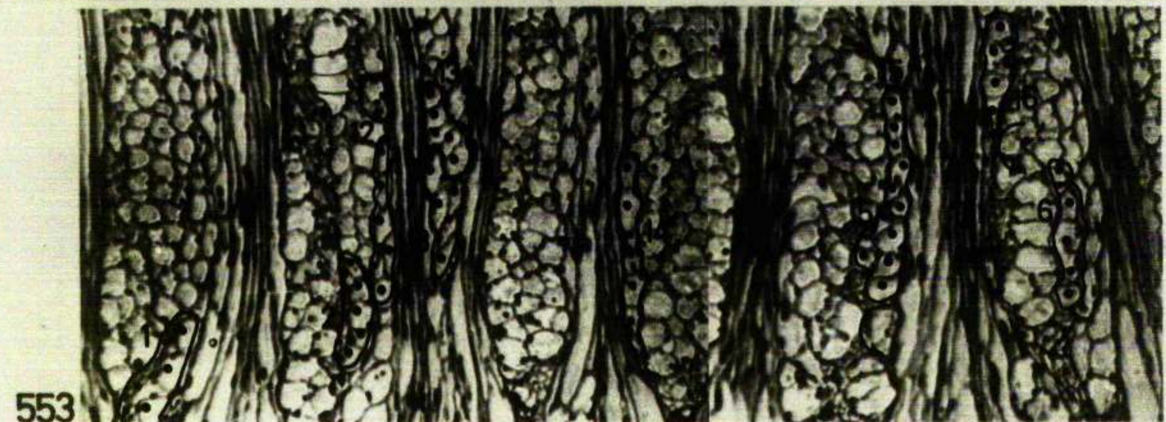
10μm.

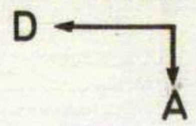
Figure 25

Notonecta The lamina-medulla projection of ten lamina cartridges. Section 473 shows a portion of lamina and section 633 the corresponding area of medulla. The array of lamina cartridges is reproduced in the medulla in inverted sequence. The direction of twist in each stratum of the chiasma is indicated by arrows in section 553. The six axons traced for each cartridge through the chiasma are marked in sections 553 and 633.









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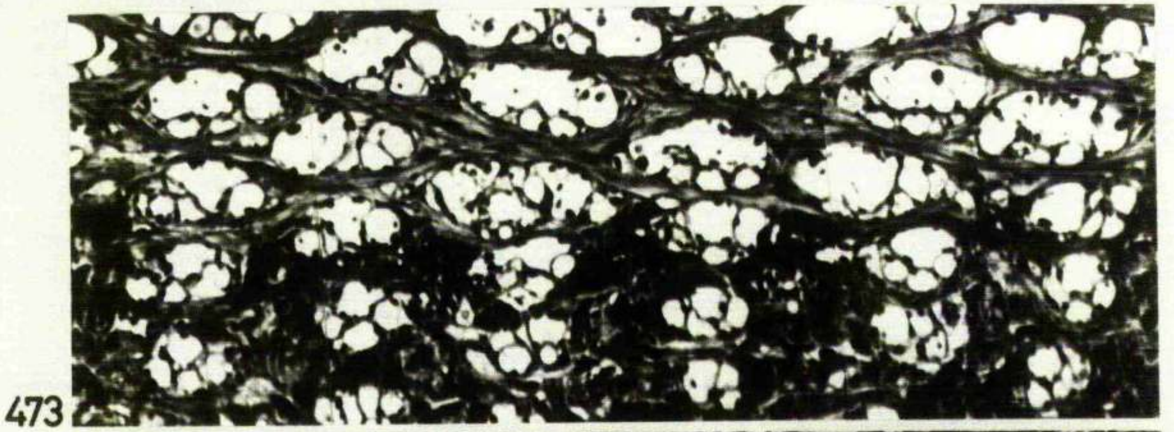
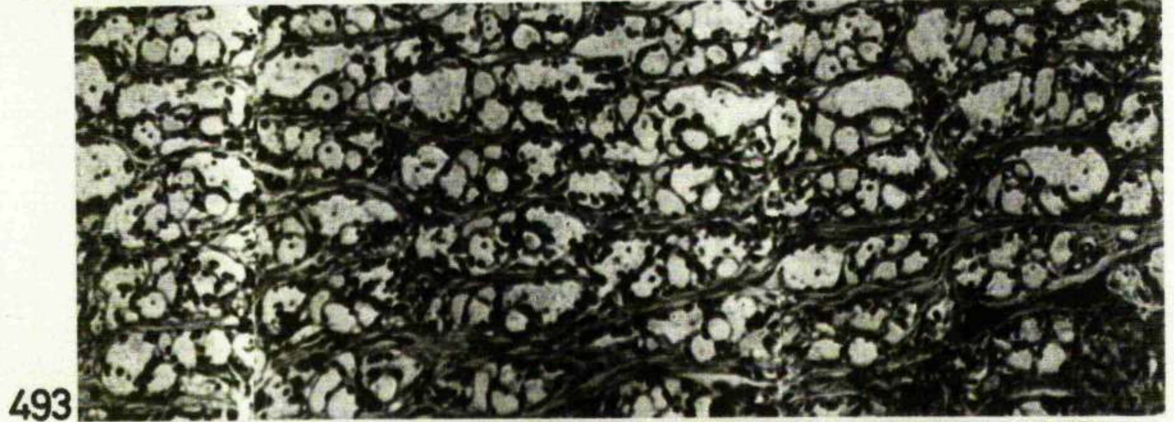
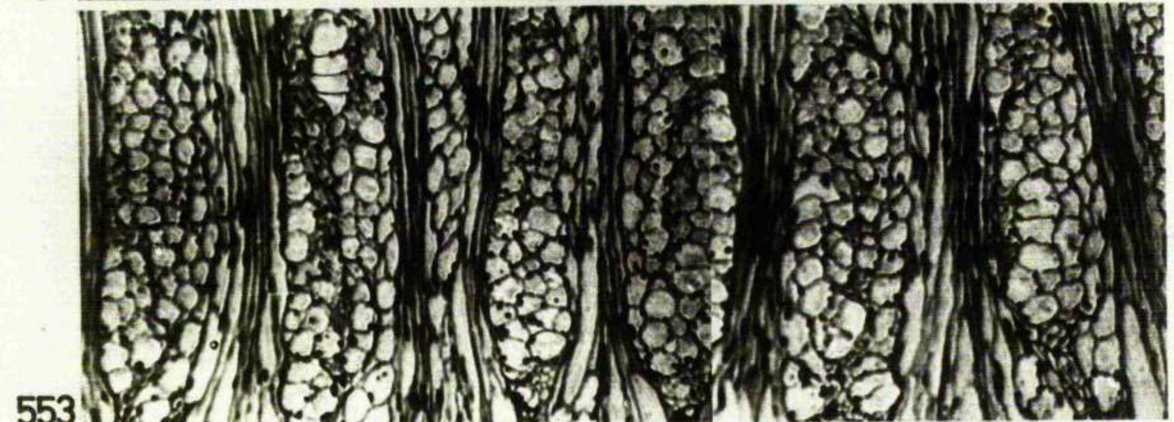
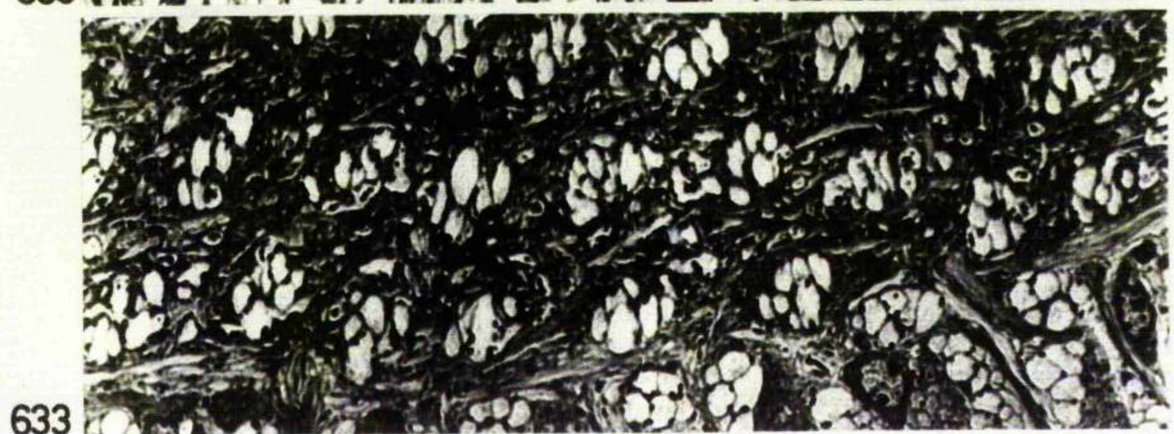
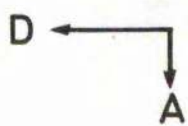










0 10µm.

and the neuropile appears to be arranged in a continuous sheet around a central core of axons, rather than subdivided into cartridges. Consequently it is possible that there are extensive lateral connections between retinula terminals of different origins and the end position of the retinula terminal may not be the sole feature in the flow of activity.

Lamina monopolar neurones are not obvious. At the bottom of the lamina, bundles containing six conspicuous axons leave each cartridge; possibly other axons exist in each bundle.

C. Notonecta: the backswimmer

The ommatidium of Notonecta (Horridge, 1968 a) is similar to that of the related water-bug. Ommatidial bundles of eight retinula axons (fig. 24 b) project on to the lamina but the axons cannot be followed because of their inconsistent size and appearance.

The organization of the lamina of Notonecta is not known and the profiles of axons in this region (Section 493, fig. 25) are inconsistent. None of these profiles can be followed through the lamina to either cell bodies or retinula axons.

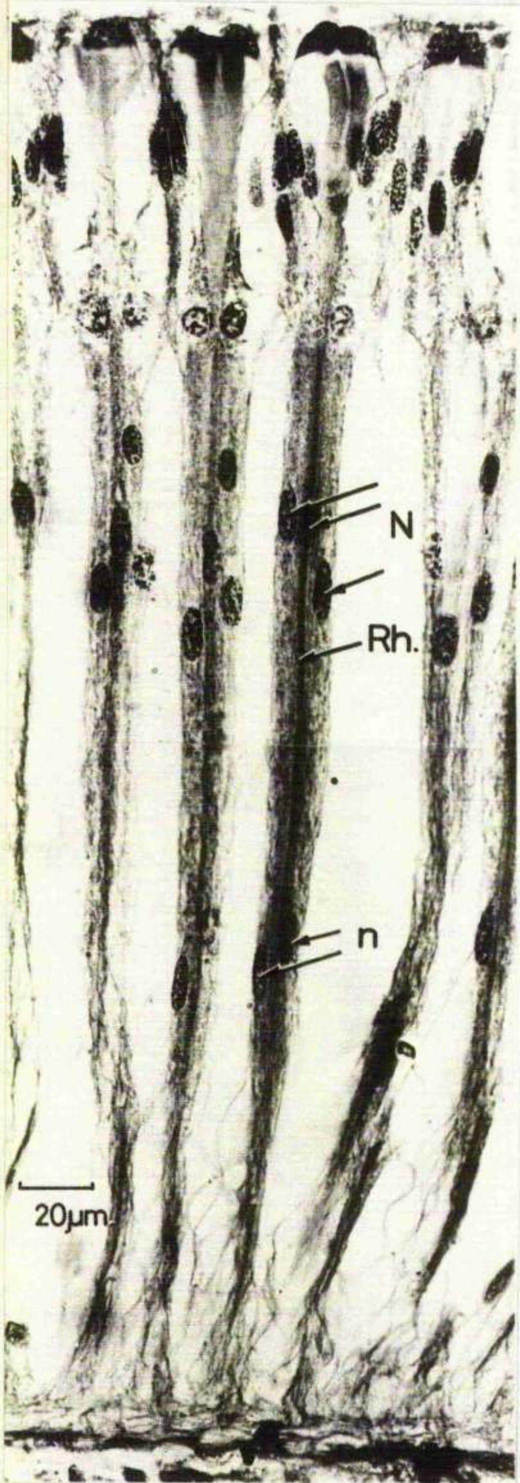
There are six chiasma axons associated with each lamina cartridge that can be followed to the medulla (fig. 25). These project homotopically on the medulla in an inverted

Figure 26

Locusta retina. 10 µm paraffin sections stained by Feulgen reaction.

a. Left: L.S. Retinula nuclei occur at two levels, 80 µm below the cones (N) and 75 µm above the basement membrane (n).

b. Right: T.S. At the top, the section cuts the six long retinula cell nuclei (N), and at the bottom the two basal retinula cell nuclei (n). Rh = rhabdome.


N
Rh.
n
20µm

10µm.
N
Rh.
n

horizontal array, and each group projects to a single cartridge of the medulla. Chiasma strata with both clockwise and anticlockwise twists have been observed.

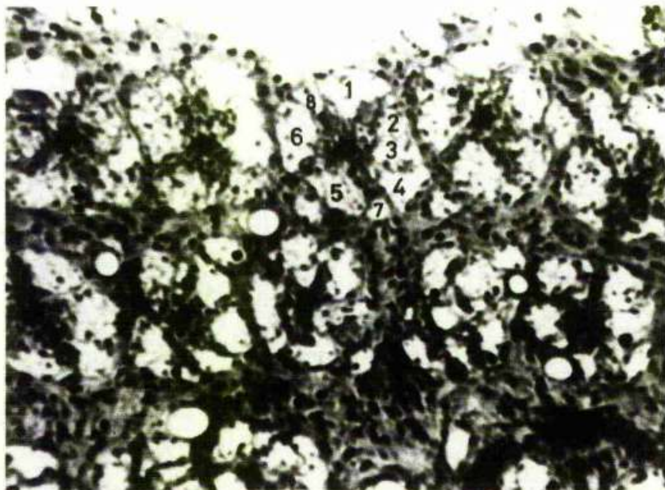
D. The locust *Schistocerca*

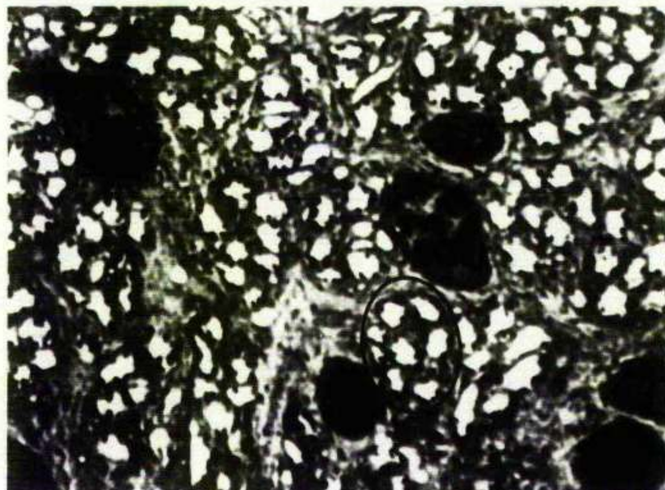
The ommatidium of the locust has a fused rhabdome and a 6 + 2 composition of retinula cells (Horridge and Barnard, 1965). There are six long retinula cells with nuclei in the top half of the ommatidium, and two smaller basal retinula cells (called eccentric cells by Horridge, 1966 a) in the proximal part (fig. 26 a). The basal cells are situated on opposite sides of the ommatidium approximately along the dorso-ventral axis (fig. 26 b). Their position is not absolutely constant, but there are always two in approximately opposite quadrants. In addition one long retinula cell is smaller in diameter than the other five and is numbered cell 3 in this work.

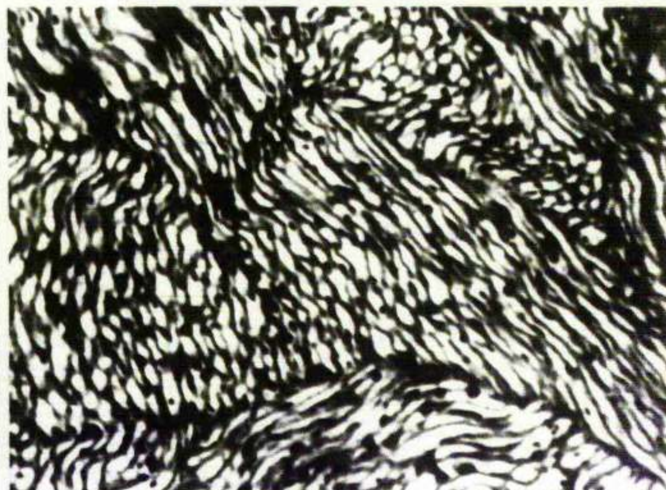
The numbering convention adopted is illustrated for the eight cells of one ommatidium in the micrograph of the retina (fig. 27). The cells 1, 2, 4, 5 and 6 are numbered in a clockwise sequence from the most ventral (number 1). Cells 3, 7 and 8 which give rise to recognizably smaller axons are numbered independently of the other five, numbers 7 and 8 (the basal cells) in the ventral and dorsal quadrants respectively and number 3 in the posterior quadrant. The

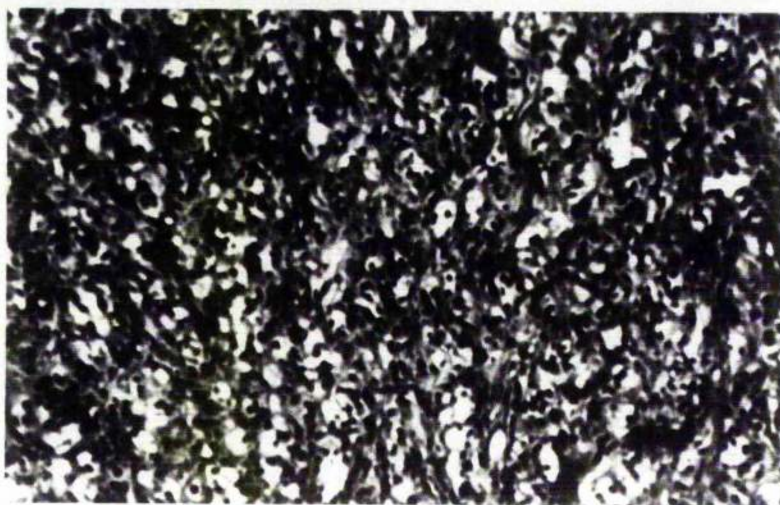
Figure 27

Schistocerca Micrographs of randomly selected areas at different depths in the eye. Top left: retina. Middle left: top of the lamina with one cartridge circled. Bottom centre: the deep lamina neuropile. Top right: the transition between lamina and chiasma. Circled are two groups of axons, the presumed derivatives of two cartridges. Middle right: lamina-medulla chiasma.









0 10μm.

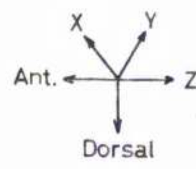
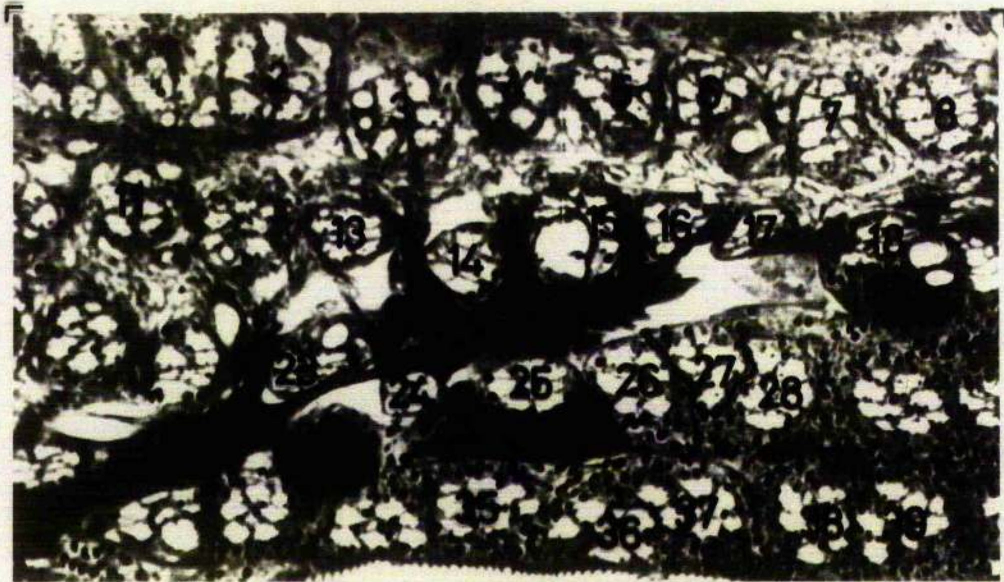


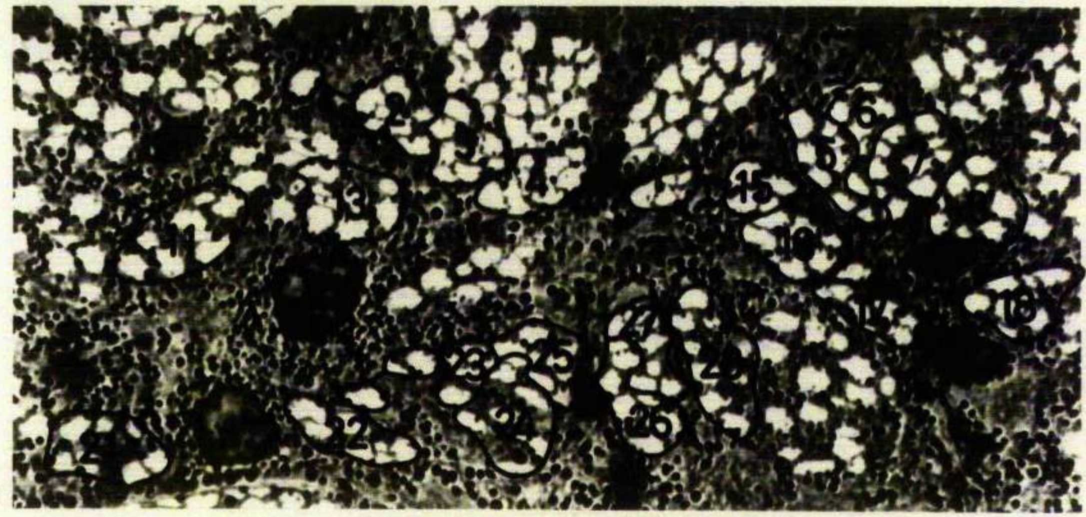
Figure 28

Schistocerca Micrographs of the whole area of twenty ommatidia traced from a series of sections from the retina of a right eye. (a) Section 341 at the level of the basement membrane which separates the retina in the antero-ventral corner from retinula axon bundles in the postero-dorsal corner. (b) Section 424 shows the partial coalescence of ommatidial bundles separated by glial cells. (c) Section 576 shows the formation of a wide tract of axons with interspersed glial cells. Each tract is separated from its neighbours by a structureless stroma which contains a trachea at a level between sections (b) and (c). (d) Section 676 shows the top of the lamina containing loosely separated cartridges.

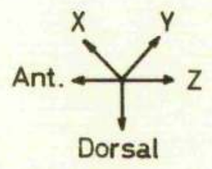


0 10μm.

a

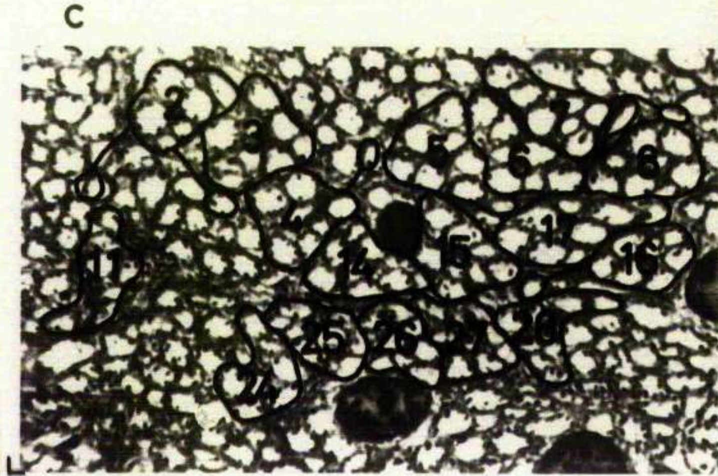


b



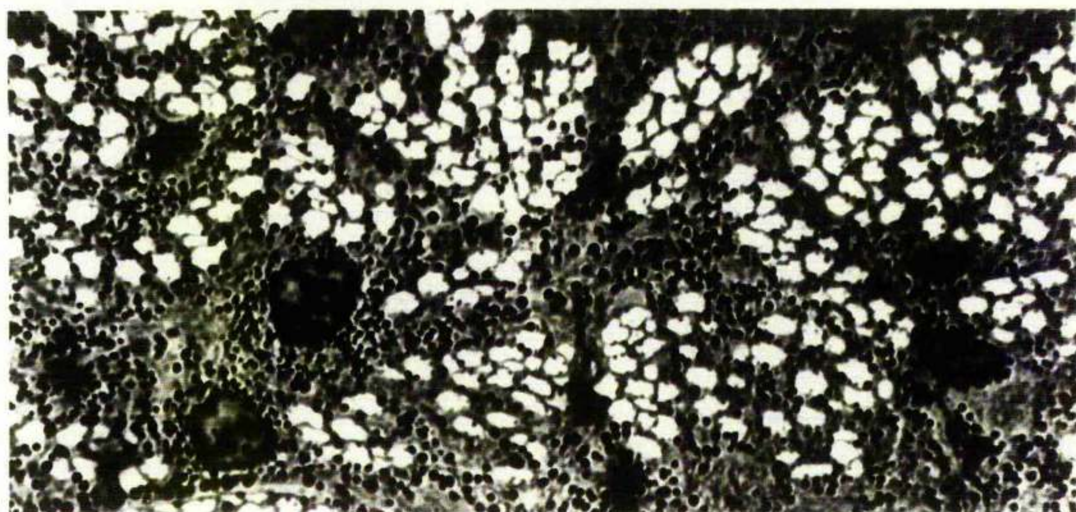
d

c

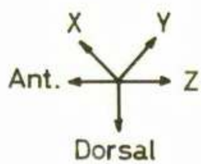


0 10μm.

a



b



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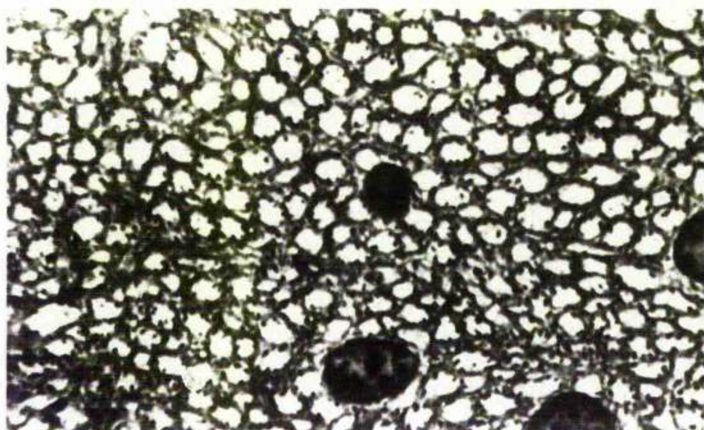
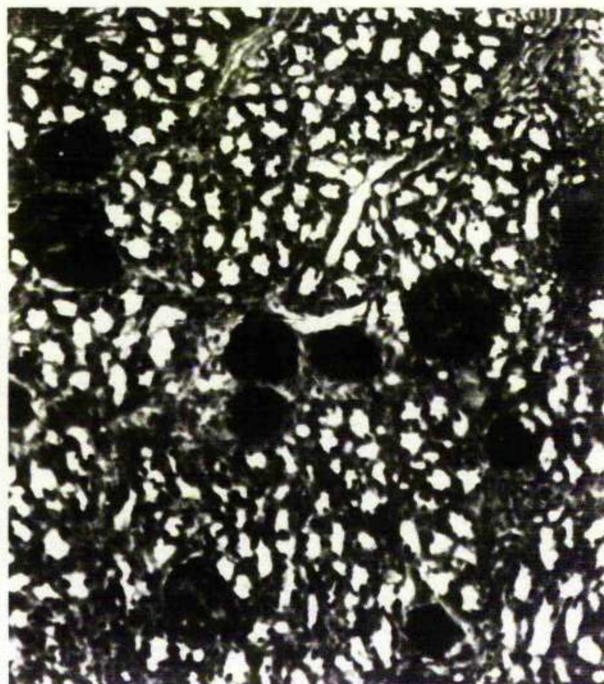




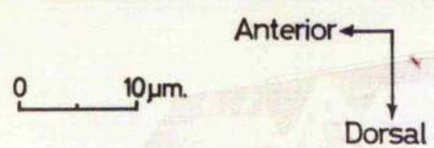
sequence of retinula cells around the periphery of the ommatidium is 1, 2, 3, 4, 8, 5, 6, and 7 and this sequence is reflected approximately in the arrangement of axons beneath the basement membrane. The positions of axons 3, 7 and 8 may vary somewhat relative to the other five axons but maintain their position relative to each other.

The appearance of the cartridge array at the top of the lamina is seen in fig. 27. The ommatidial projection has been traced to this level of the lamina (figs. 28 and 29). The cartridges are less well separated than in other insect optic lobes and contain eight axons derived from a single ommatidium. These axons are the only profiles conspicuous at this level. Deeper in the lamina neuropile the tissue is closely packed and homogenous in appearance and it has not been possible to trace any axons through the lamina. At the bottom of the lamina (fig. 27) groups of axons again become obvious, each group, presumed to be derived from a single cartridge, containing at least six profiles. The chiasma contains fine axons that have proved impossible to follow to the medulla.

The retina-lamina projection of twenty ommatidia from a series of sections of a right eye of Schistocerca has been studied and is shown in micrographs of four sections in fig. 28. Ommatidia give rise to retinula axon bundles which pierce the basement membrane and travel centrally through

Figure 29

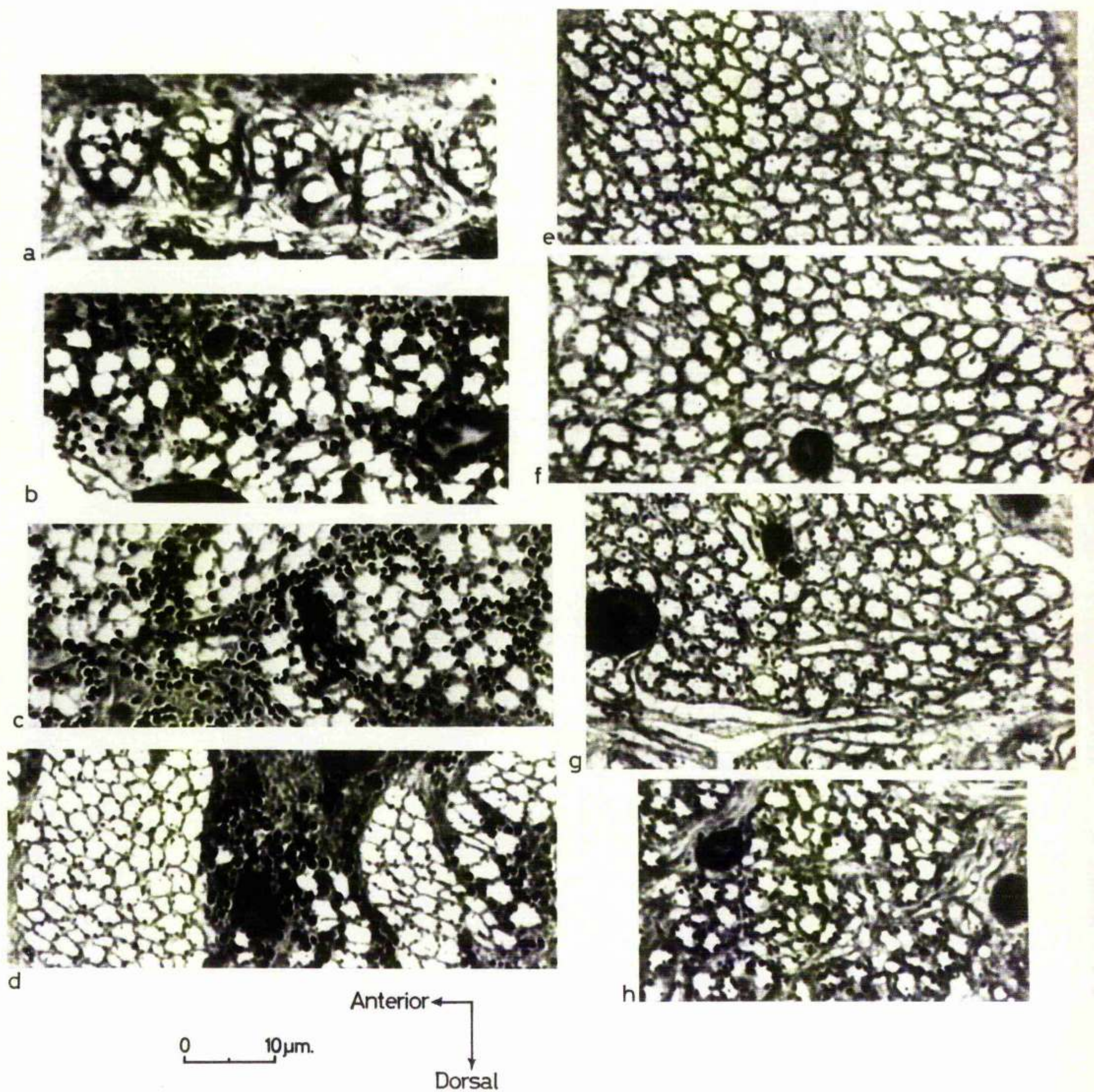
Schistocerca Micrographs of part of the area of fig. 28 containing (from left to right) ommatidia 4 - 7 with each axon individually numbered. Section numbers are a. (341), b. (381), c. (421), d. (461), e. (540), f. (576), g. (600) and h. (673).



Anterior

0 10μm.

Dorsal

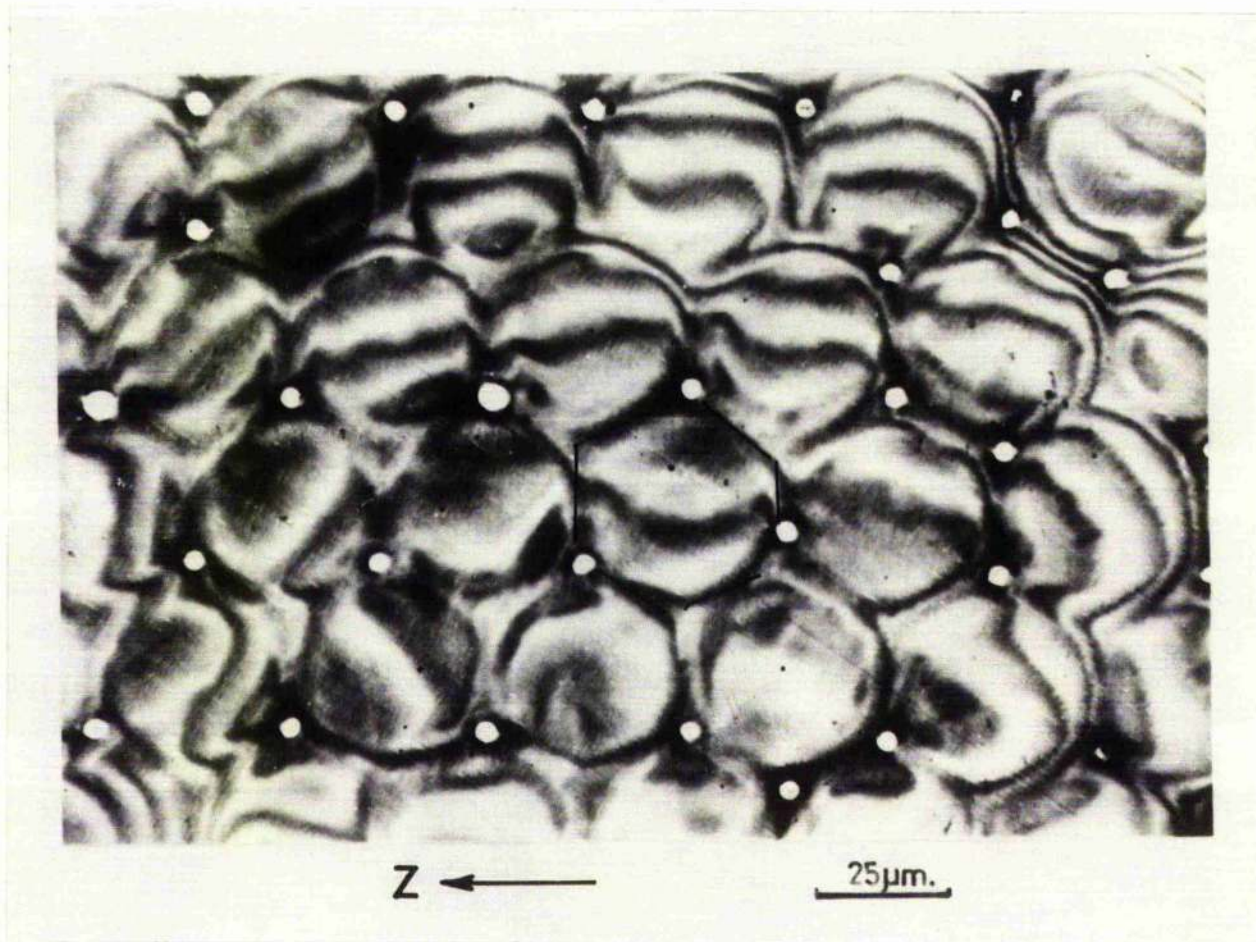

a
b
c
d
e
f
g
h
Anterior
Dorsal
0
10µm.

a stroma containing pigment granules and glial cells. Sometimes an ommatidial bundle may become divided between different areas of axons but the group always reforms within the large axon tract deeper down (e.g. bundles 2 and 15). Gradually the axon group becomes separated by lamina neuronal somata (presumed to be the monopolar neurones depicted by Cajal and Sanchez 1915). Beneath the ganglion cell layer the cartridges are separated in parts by glial cells and although in any one section not all the cartridges are discretely separated most are separate for one part of their length.

Each ommatidial bundle has axons of two diameters (fig. 29). Axons 1, 2, 4, 5 and 6 are 2 - 3 µm and axons 3, 7 and 8 only 1 µm. As the axons proceed centrally, the axon tracts in which they travel often undergo relative displacement which results in the loss of their rotational sequence. At the top of the lamina the axons sort out and the rotational axon sequence becomes reestablished as the axons move laterally back into their own cartridges (e.g. axon arrowed in section g, fig. 29); in particular, axon 3 often meanders from its neighbours before reaching the lamina. No recognizable axons of monopolar neurones can be seen in the lamina, although that of the giant monopolar type of Cajal and Sánchez (1915) ought easily to be resolved. In section h (fig. 29) the fine axons running horizontally

Figure 30

Apis Micrograph of a section through the central portion of the cornea to show the distribution of corneal hairs represented by spaces between the facets. The outline of a single facet is marked in the centre of the micrograph.



amongst the cartridges (arrowed) may be the narrow isthmuses of monopolar neurones.

The results of tracing of the axons from this and two other less extensive series may be summarized as follows: in some cases all eight axons from one ommatidium can be followed unambiguously to one cartridge in the lamina, in many cases six or seven axons may be followed and only in a few cases have less than six been successfully traced to one cartridge. Axons 1, 2, 4, 5 and 6 can always be followed. In no case has an axon been observed to diverge to a neighbouring lamina cartridge. Thus it may be taken as a rule that eight axons from one ommatidium go to one cartridge in the lamina. Because of the indistinct nature of the cartridges in locust it is perhaps only justifiable to say that axons of one ommatidium occupy neighbouring positions in the lamina in a predictable rotational sequence.

E. The drone bee *Apis*

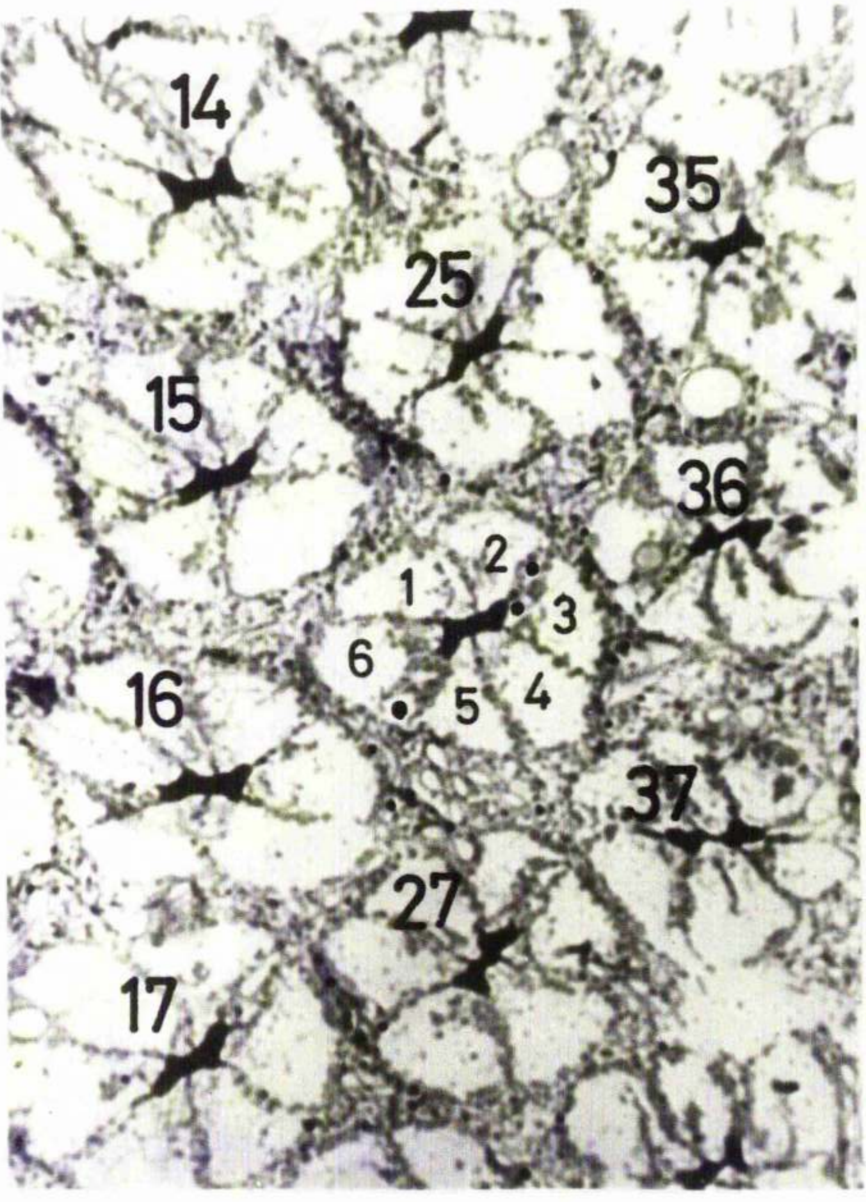
The cornea of drone bee is covered with sensory hairs the positions of which are seen in fig. 30 by the spaces left between the facets. There is approximately one hair per facet, but the distribution is not regular, although more frequent than shown by Perrelet (1970).

Underneath each facet are a total of nine retinula cells, six long and three small. The numbering convention adopted

Figure 31

Apis Micrographs to show retinula cell numbering. a. Left. Micrograph of a portion of section 109 through the retina, plotted in fig. 32, showing ommatidia from three rows. Ommatidium 26 has large retinula cells numbered 1 - 6 and three small retinula cells marked with a spot.

b. Right. Micrograph of bundles from a different area illustrating a further size difference which is seen in some areas between axons 1 and 4 (marked with a spot) and the other four short retinula axons.



109

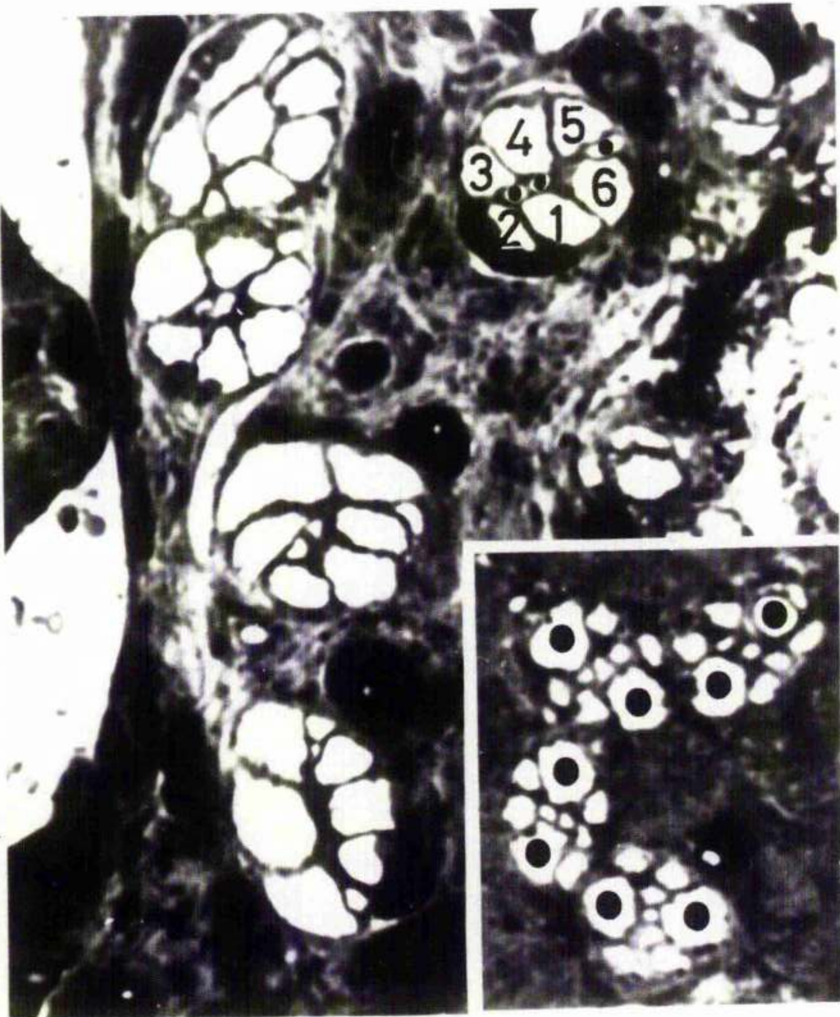


Figure 32

Apis Plan of the area of retina from which the short retinula axons from twenty-six ommatidia have been followed to their termination in the lamina. The angular orientation of the longest axis of the rhabdome cross section is shown as a diametric line.
Inset top left: an idealised ommatidium, cut near its base, with the rhabdomere microvilli orientation drawn for the six cells with short axons.

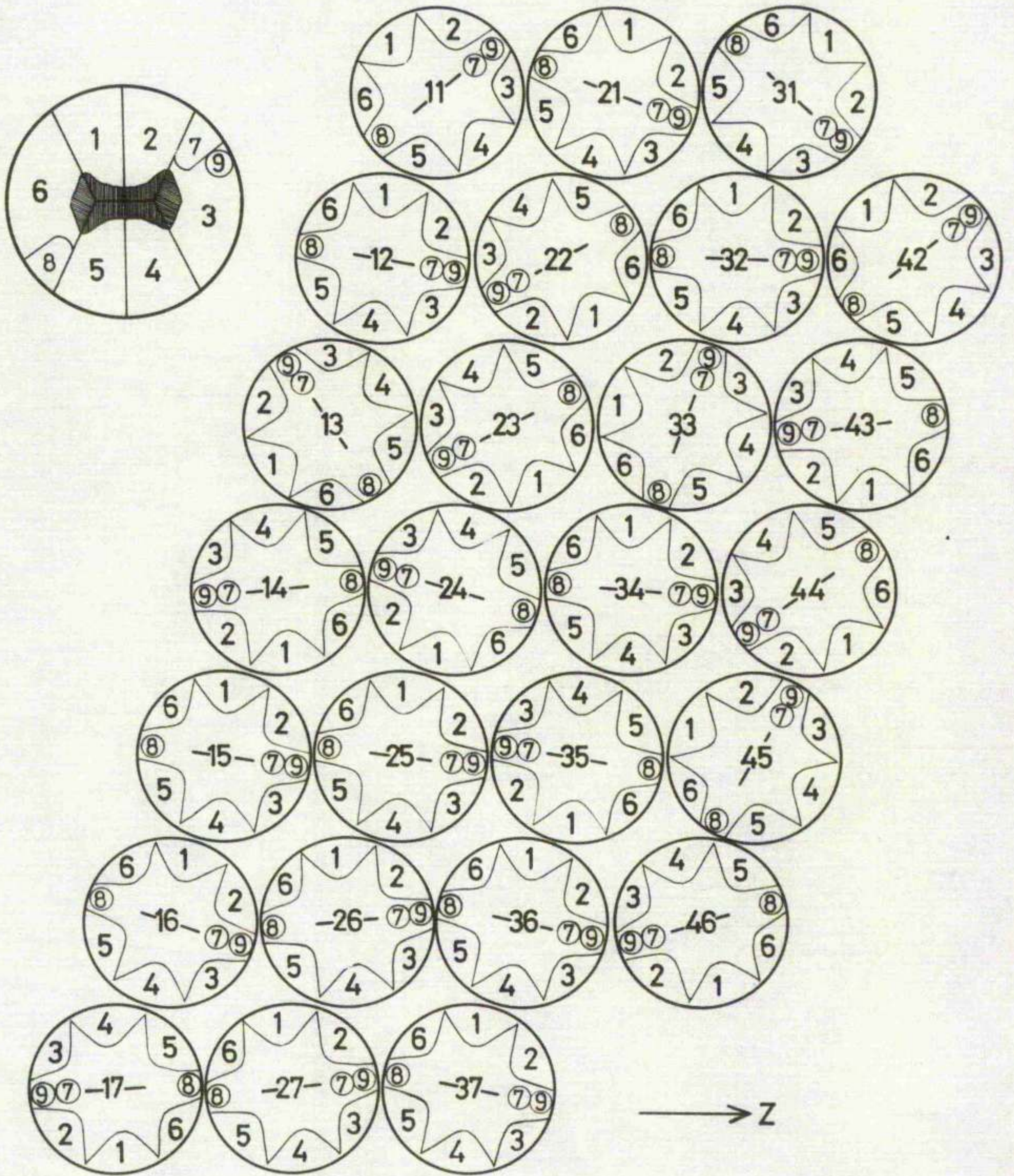

11
21
31
12
22
32
42
13
23
33
43
14
24
34
44
15
25
35
45
16
26
36
46
17
27
37
Z

Figure 33

Apis Plan of the area of lamina corresponding to the retina plan of fig. 32. The positions of the terminals of the six short retinula axons are shown for each ommatidium together with the positions of three long retinula axons in the centre of each cartridge. The cartridges are given the same number as the ommatidia that they underlie. The arrow encircling each cartridge indicates the angular rotation undergone by the ommatidial bundle.

11 21 31 12 22 32 42 13 23 33 43 14 24 34 44 15 25 35 45 16 26 36 17 27 37

→ Z

is shown in fig. 31. The positions of the long retinula cells 1 - 6 are defined in relation to the positions of the two small retinula cells 7 and 8, and the basal cell 9. There is a size difference between the two types of axons of these cells and the symmetry of the bundle is obvious from the position of the pair of small diameter axons on one side.

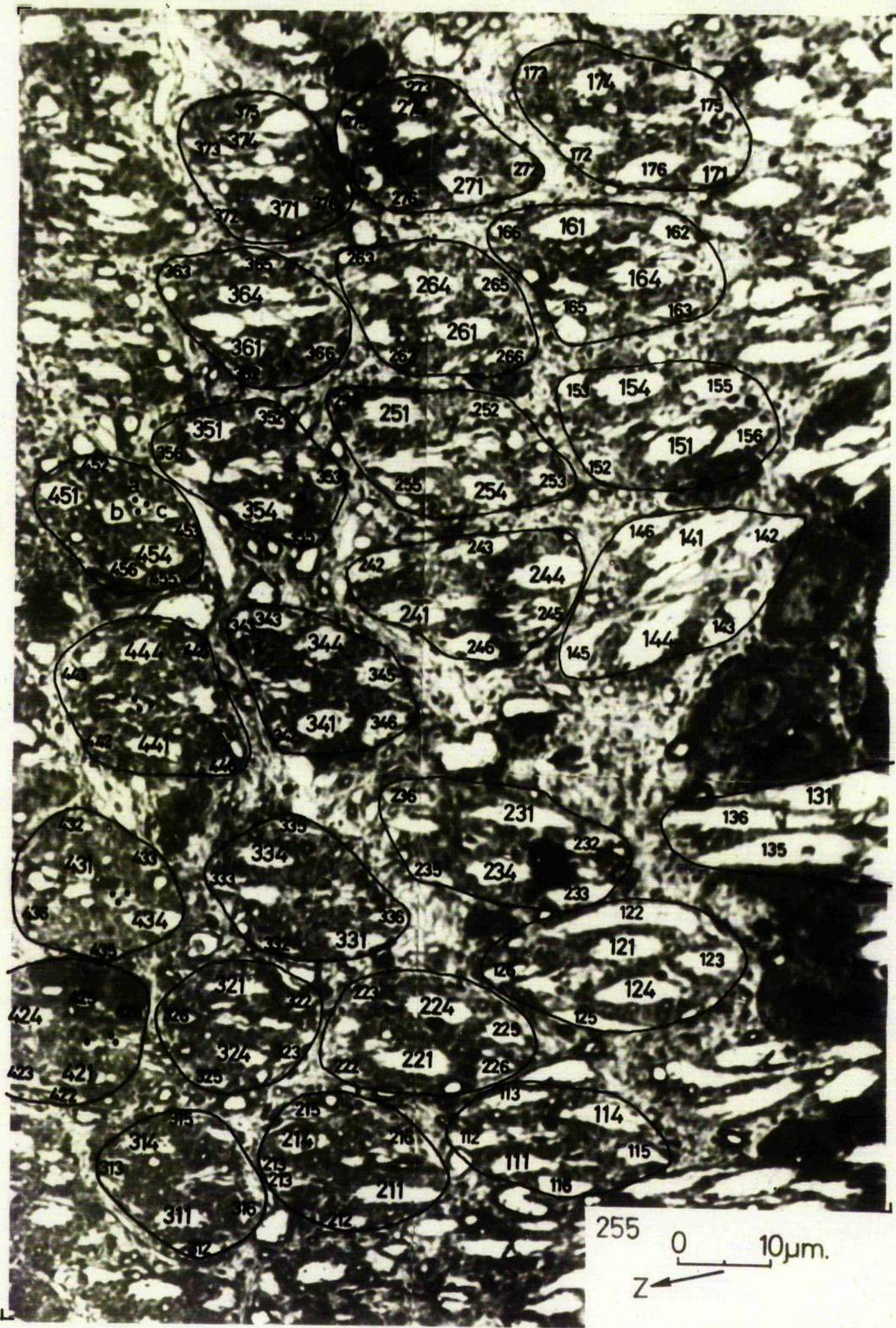
In some areas a further size difference exists between the short retinula axons 1 and 4 which are larger than axons 2, 3, 5 and 6 (inset, fig. 31). The angular orientation of the ommatidia varies and is represented as a diametric line (the long axis of the rhabdome cross section) for each ommatidium in fig. 32. The variation is apparently random but may consist of a number of preferred classes. Within each ommatidium, rhabdomeric microvillar orientation is predictable from the published electron micrographs of Perrelet, (Perrelet and Baumann, 1969 b; Perrelet, 1970).

The retina-lamina projection

The axons of twenty-six ommatidia have been followed to their positions in the lamina. The six long retinula cells have short retinula axons that terminate in the lamina. Two of three axons of the small retinula cells from each ommatidium pass through the lamina at the centre of their own cartridge, whilst the third presumably ends in the lamina (see Discussion, p. 173).

Figure 34

Apis Micrograph of the area of lamina from section 255 shown in the plan of fig. 33. Axons are numbered with the first digit(s) from the ommatidium of origin and the last digit from the retinula cell of origin. For the sake of clarity, only short retinula axons are numbered, except in cartridges 42 - 45 in which the long retinula axons are marked with a dot and in cartridge 45 in which three presumed monopolar axons are marked a, b and c. The section is slightly oblique and where axons are cut longitudinally in the section faint transverse striations are visible. These may be the agranular tubules described by Perrelet (1970) in drone bee retinula axons or alternatively parallel septa such as are described by Perrachia (1970) for some axons of crayfish abdominal root and nerve cord.



174
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361
366
154
155
153
151
156
152
251
252
254
253
351
354
451
b
c
454
146
141
142
244
243
242
241
245
246
144
143
145
444
441
344
345
341
346
131
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231
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233
431
434
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333
331
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122
121
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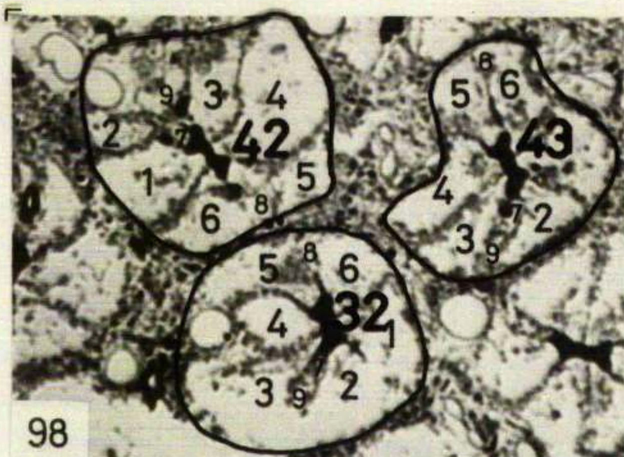
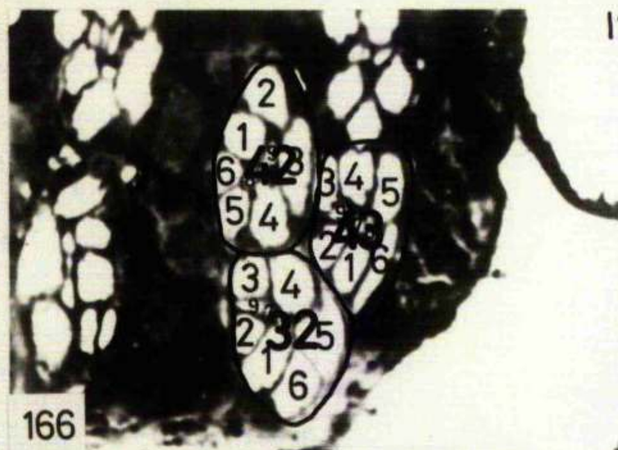
124
125
321
324
224
223
225
221
226
222
114
113
115
112
111
116
314
313
311
211
216
215
212
421
424
423
255
0
10μm.
Z

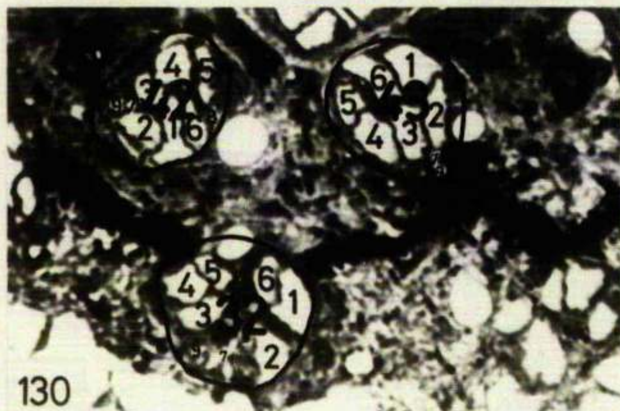
255
0
10μm.
Z

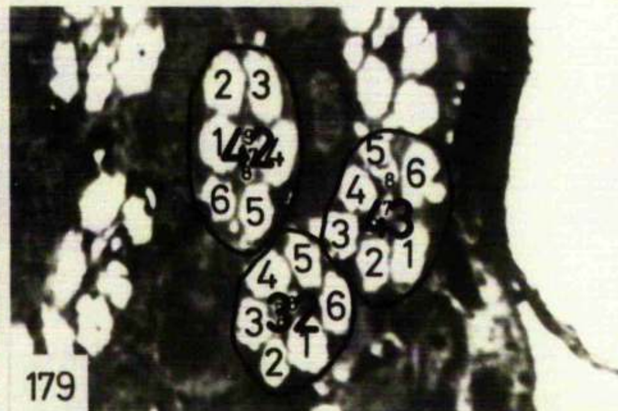
Figure 35

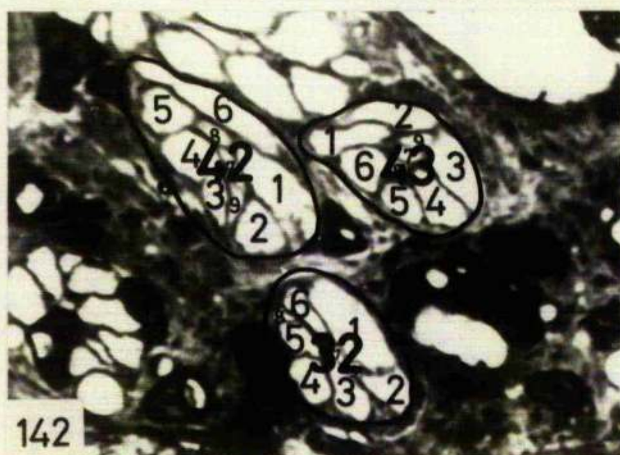
Apis Micrographs of the retina-lamina projection of three ommatidia 42, 32 and 43 of fig. 33. The series shows both the distribution of all nine axons from each ommatidium in section 98 on to the cartridges of the lamina in section 220, and the rotation of the bundles as they proceed centrally. The ganglion cell layer occurs from sections 1o6 to 190.

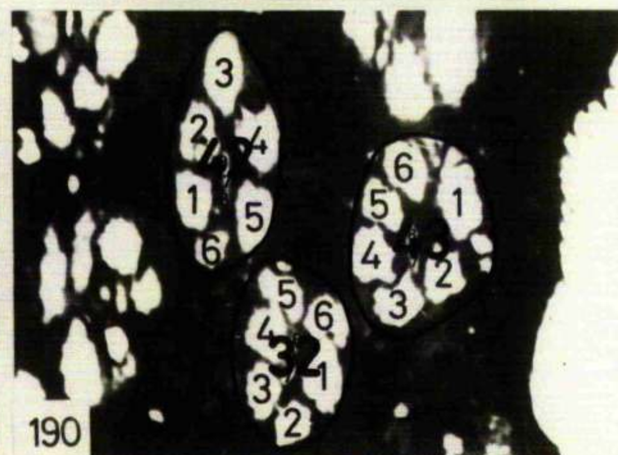


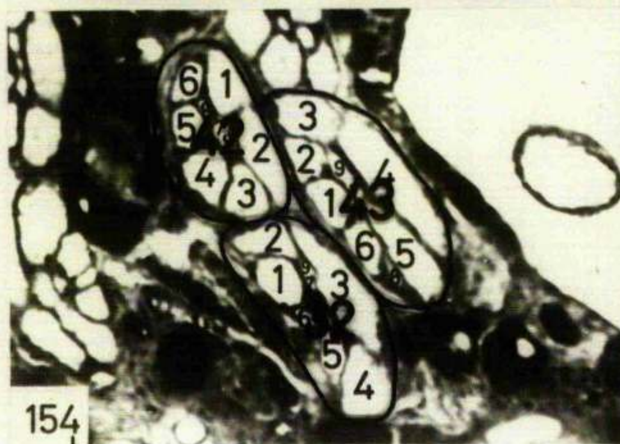


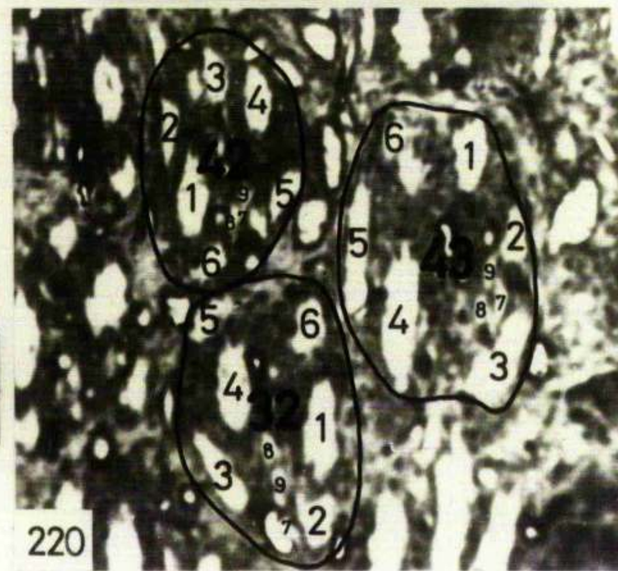






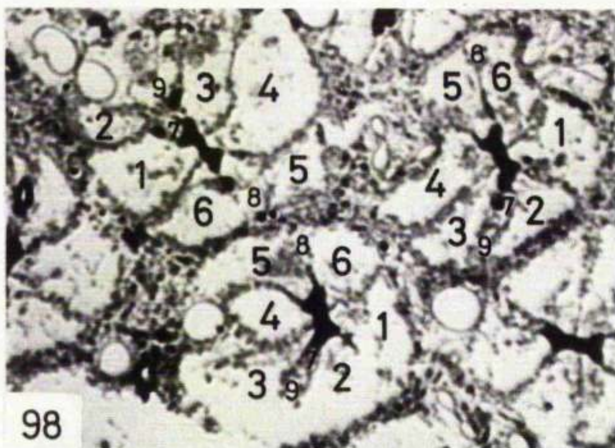


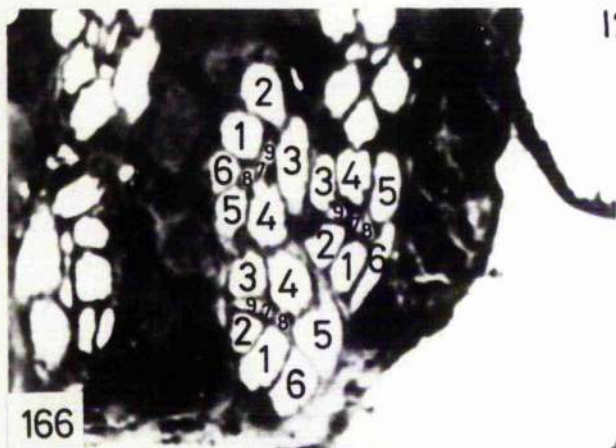


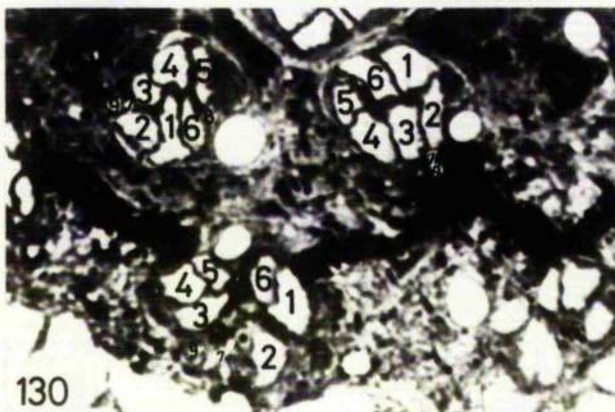


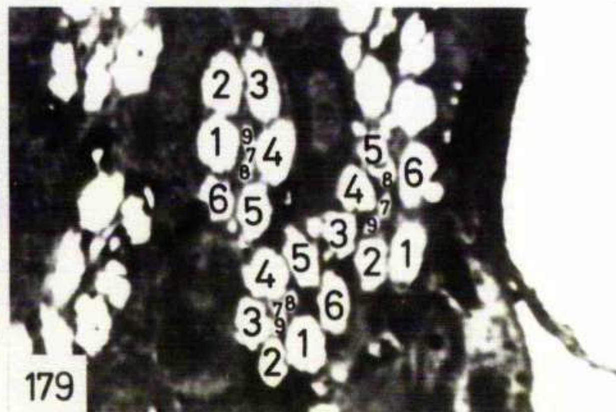
Z

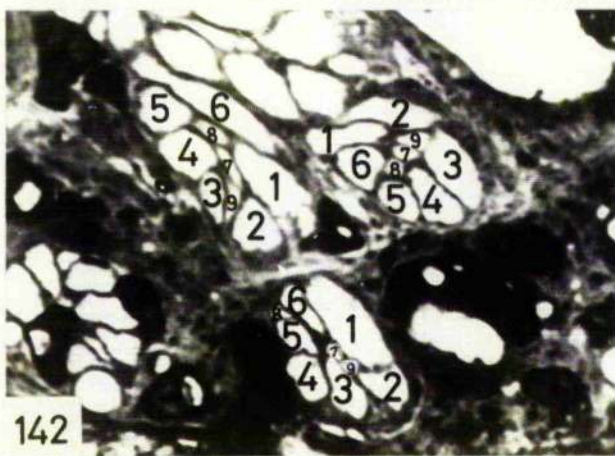
0 10µm.

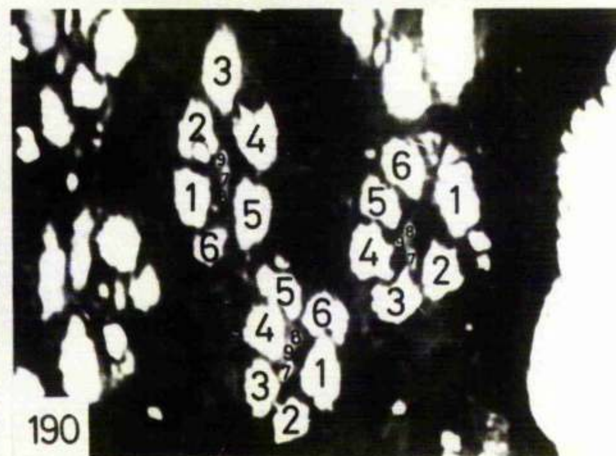


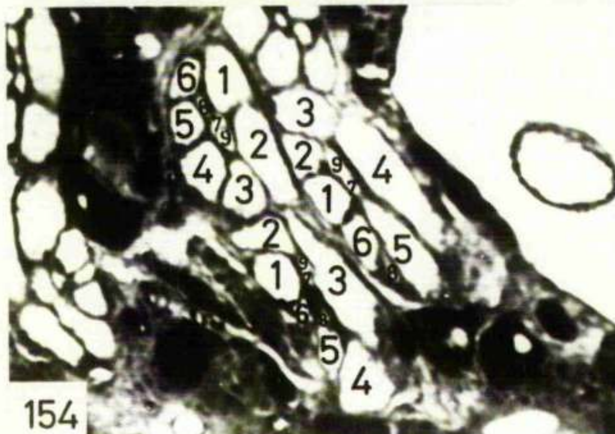


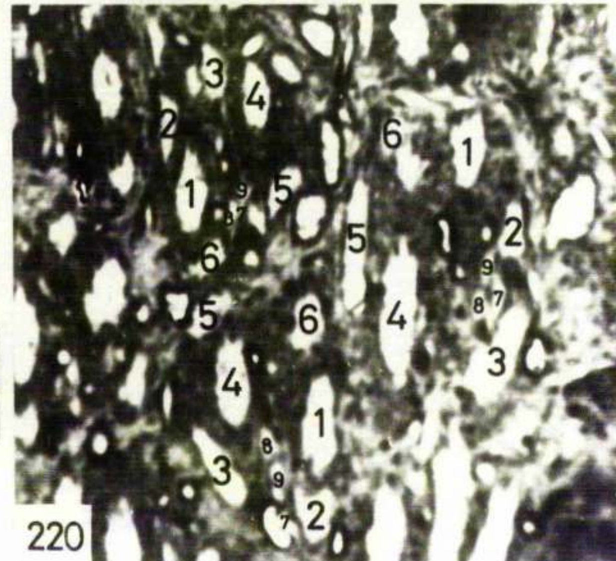












Z

0 10μm.

Similar results have been obtained from one other less extensive series. In all cases the axons of one ommatidium go to one cartridge. The ommatidia examined are shown diagrammatically in the plan of fig. 32.

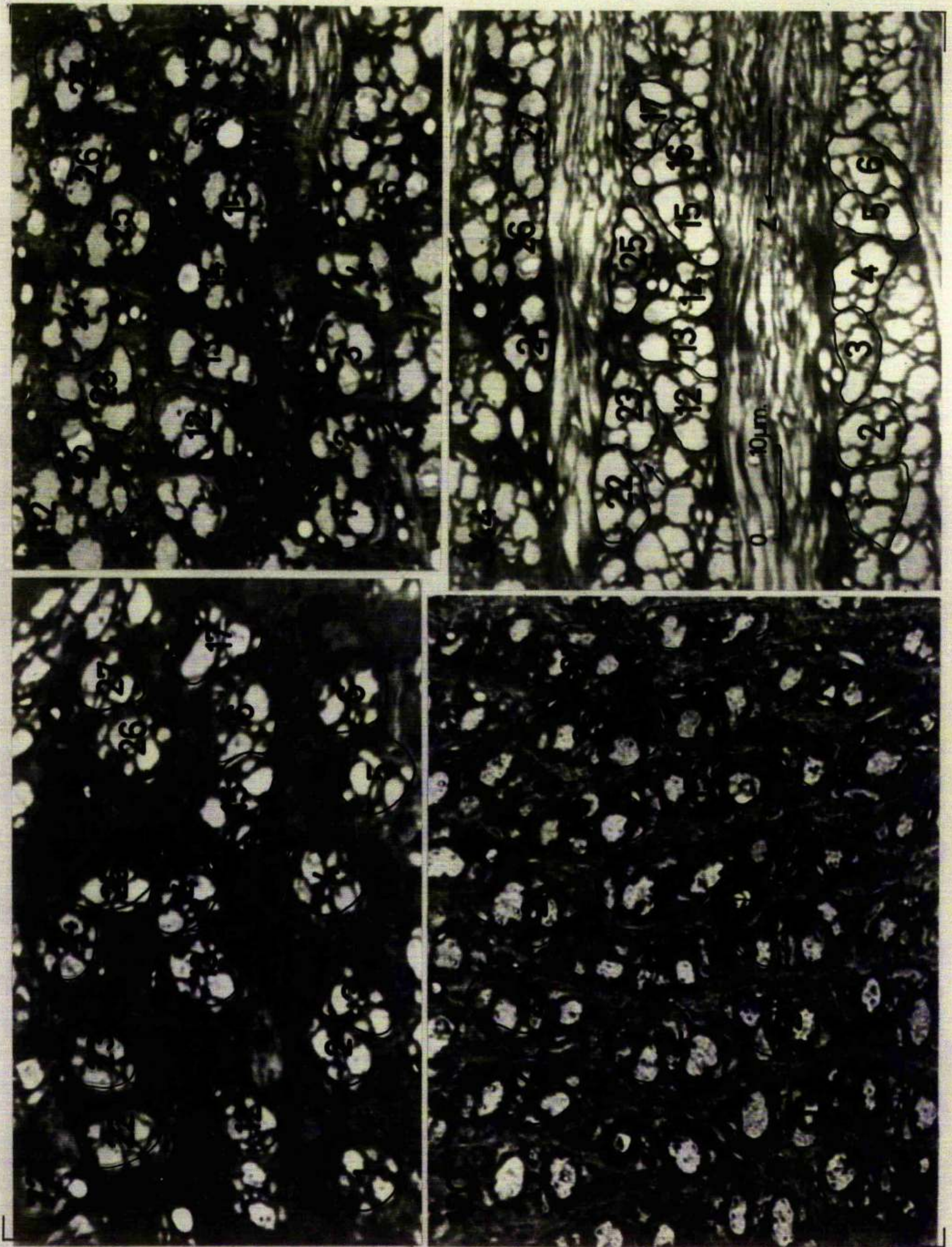
The ommatidial axon bundles twist as they proceed to the lamina (figs. 33 and 35) in either a clockwise or anti-clockwise direction. The consequence of this twist is that at the top of the lamina, the short retinula axon terminals become situated in two horizontal rows of three axons (numbers 6, 1, 2 and 3, 4, 5) which flank the cartridge on either side of the central axons 7, 8, and 9 (fig. 33 and 34). Within each ommatidial bundle axons 1 - 6 are large (approximately 3 µm diameter), with axons 1 and 4 slightly larger, and are easily followed to their termination in the lamina (fig. 35). The long retinula axons 7, 8 and 9 are much more slender (1 µm diameter or less) and shortly after leaving their ommatidium they move to the centre of the bundle encircled by the short retinula axons which preserve their rotational sequence faithfully to the lamina.

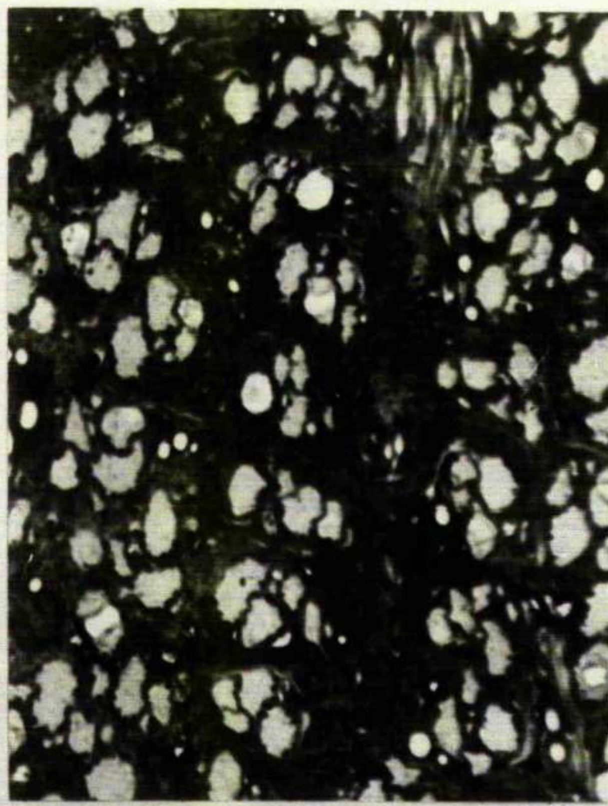
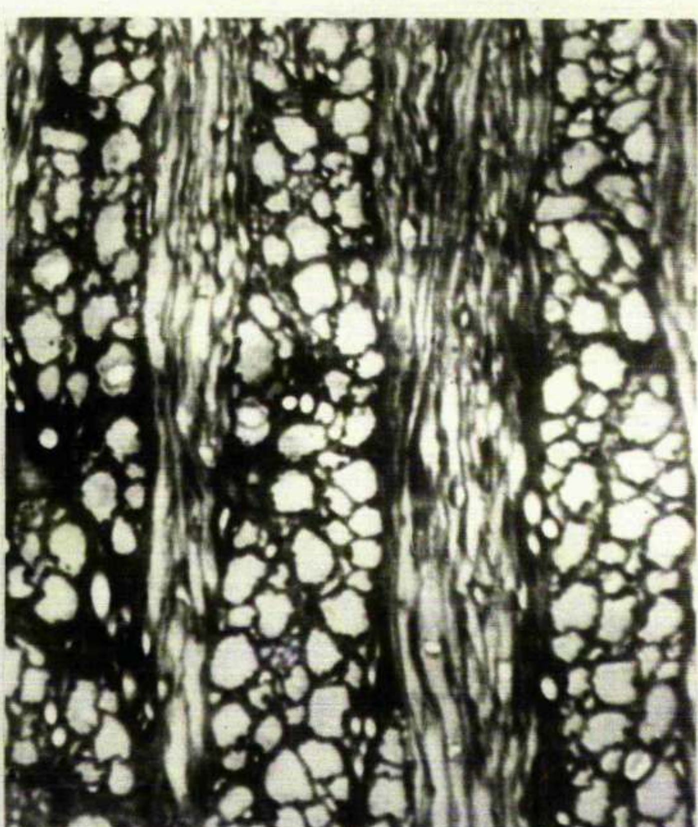
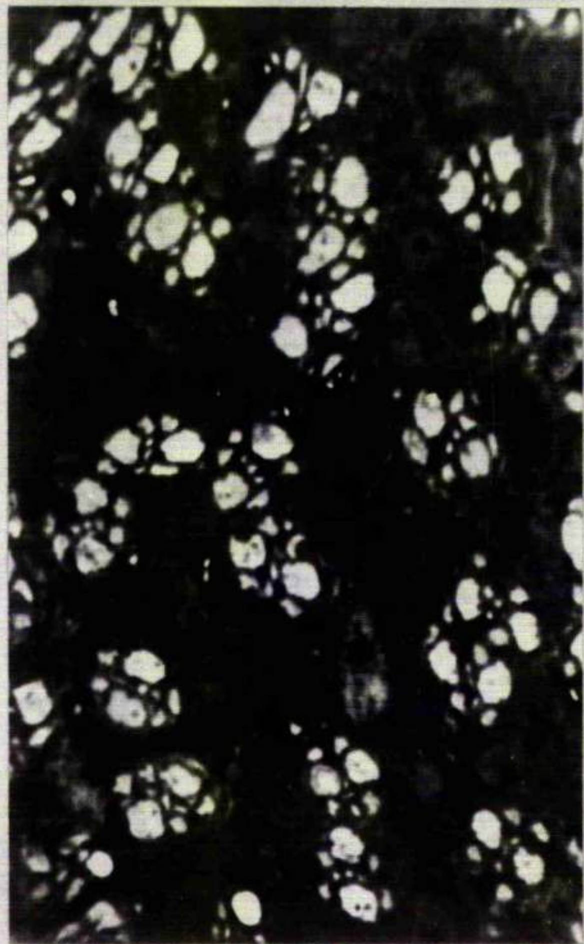
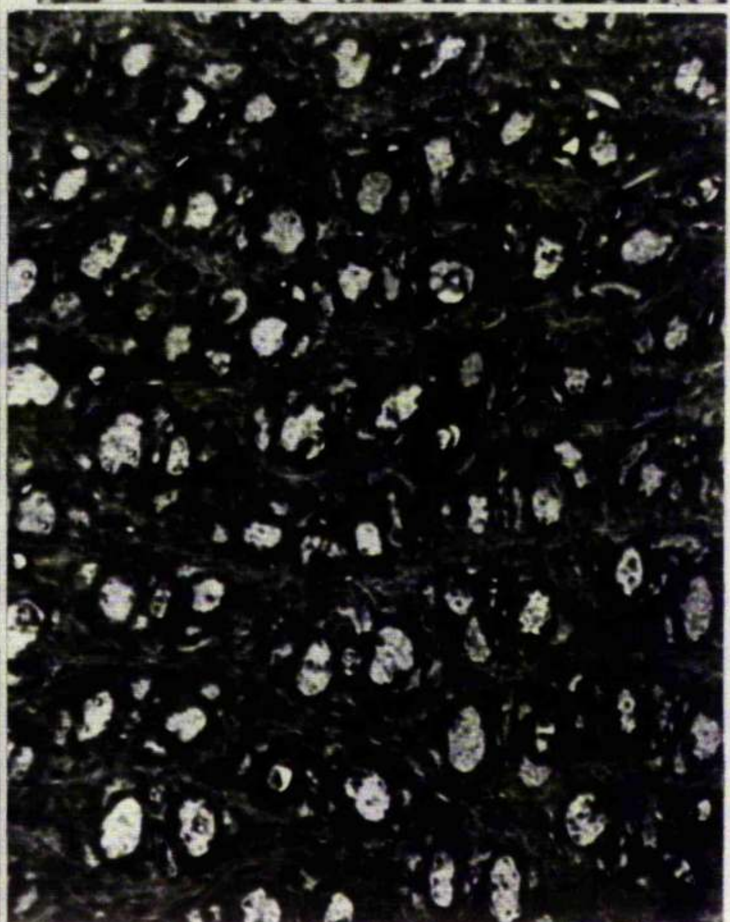
<u>The lamina</u>

The projection through the lamina has been followed from the same series but in a different region to that used for the retina-lamina projection (figs. 31 to 35) and is shown in figs. 36 and 37. The identity of the retinula

Figure 36

Apis Four micrographs showing the axons of eighteen cartridges of the lamina. Figs. 36 and 37 are taken from a different region of the same series as that used for figs. 31 to 35. Section 72; just above the lamina showing ommatidial bundles separated by columns of ganglion cell bodies. Section 120; middle of the lamina showing the cartridge array. Section 312; bottom of the lamina showing a bundle of at least seven axons from each cartridge entering the chiasma. Section 344; top of the chiasma showing chiasmal axons with groups of extremely fine axons (arrowed) scattered about.



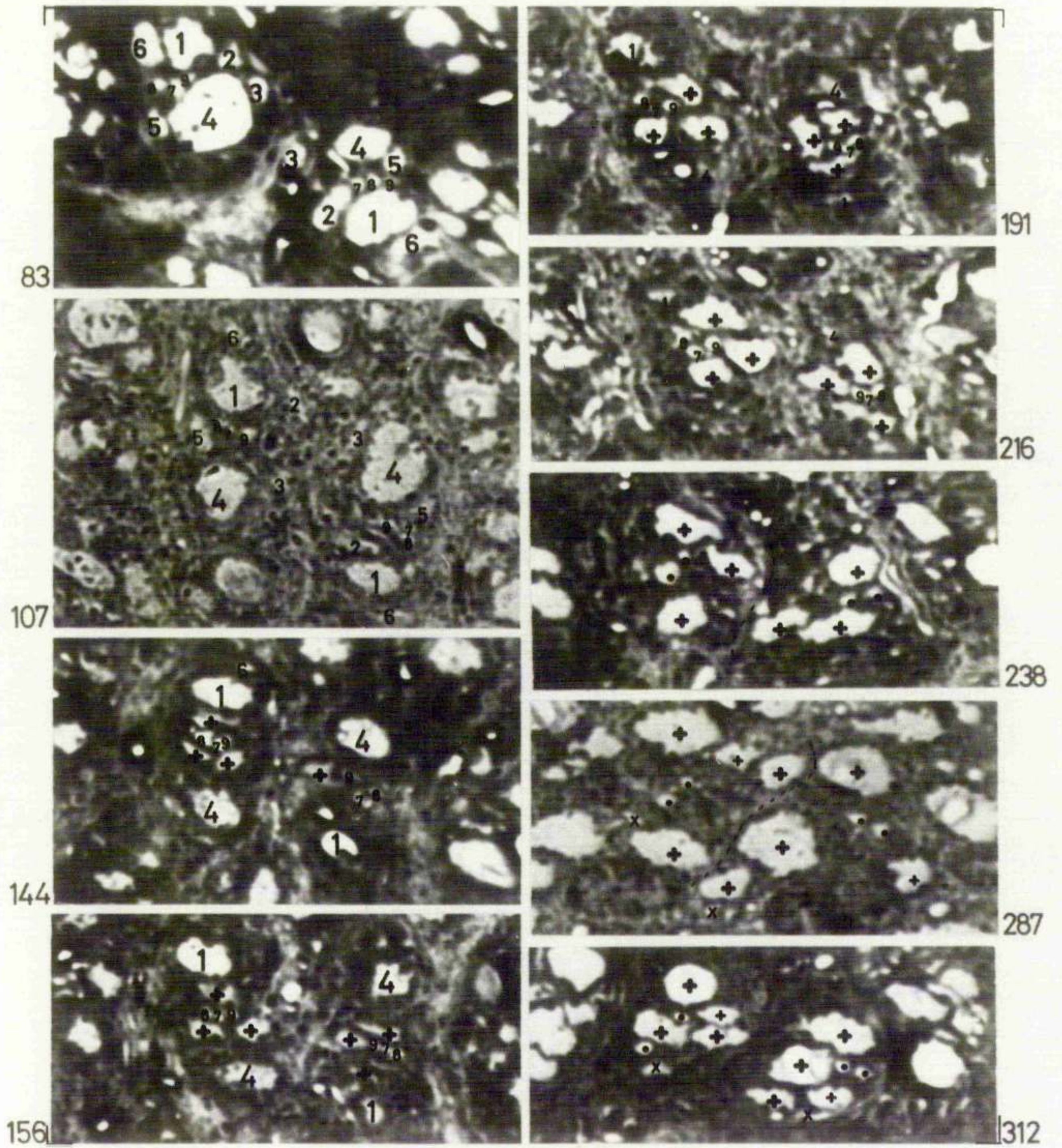





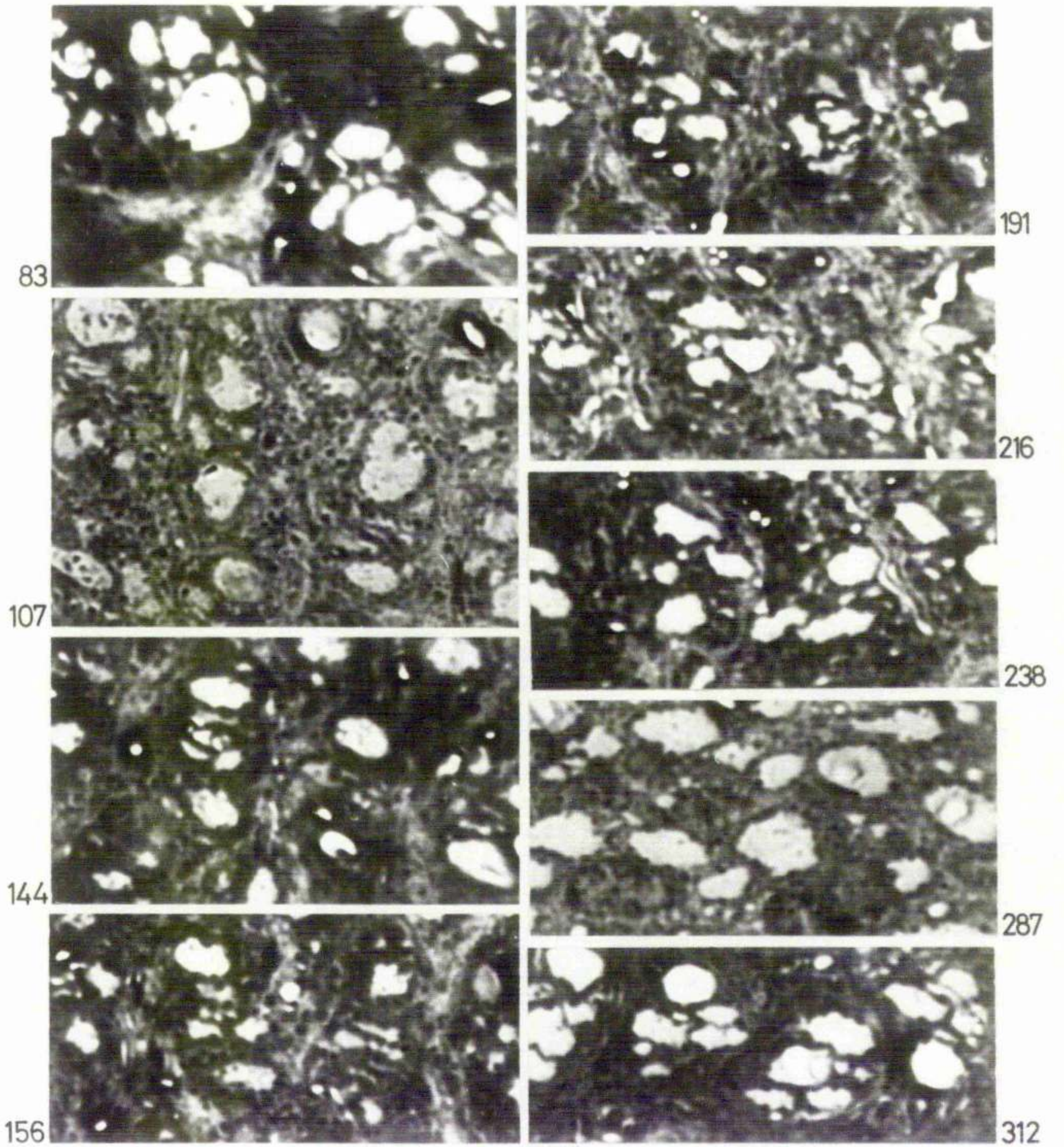
axons was inferred from the symmetry of the axon bundles near the basement membrane. The cartridges form a distinct array and the axon profiles of each are clearly separated from those of neighbouring cartridges (fig. 36). Neuropile regions surround the axons of the cartridges and stain homogenously with toluidine blue. The short retinula axons 2, 6, 5, and 3 rearrange their positions in the distal portion of the cartridge (fig. 37, and shown diagramatically in fig. 38).

The short retinula terminals 1 - 6 become indistinct at a superficial level in the lamina although the terminals of axons 1 and 4, which are larger and more conspicuous, are visible throughout the top third of the lamina. The rearrangement of short retinula terminals has also been noted in worker bee by Varela (1970), though the pattern apparently differs slightly from that in drone, and is associated with the establishment of synaptic contacts with monopolar spines and processes of centrifugal neurones. The axons of cells 7, 8 and 9 occupy a central position in the cartridge throughout the lamina but their profiles, which are extremely slender and indistinct in the middle of the lamina, are difficult to follow with certainty. Therefore the axons can be followed through the lamina as a group but they cannot be traced individually with complete confidence. All three combinations of long retinula axon pairs have been observed

Figure 37

Apis Micrographs of the cartridges 14 (left) and 15 (right) from fig. 36. Section 83, showing the nine axons of the two ommatidial bundles identically arranged but rotated by 180° to each other. Section 107, showing the arrangement of these nine axons at the top of their cartridge. Section 144, only axons 1 and 4 are still conspicuous. Section 156, three presumed monopolar cell axons (marked ÷) are present. Two of the long retinula axons pass through to section 238 and are marked with a spot. The sixth large axon (marked +) and seventh fine axon (marked +) of each cartridge appear in Section 287.


83
107
144
156
191
216
238
287
312
0
10μm.
Z



83
107
144
156
191
216
238
287
312
0
10µm.
Z

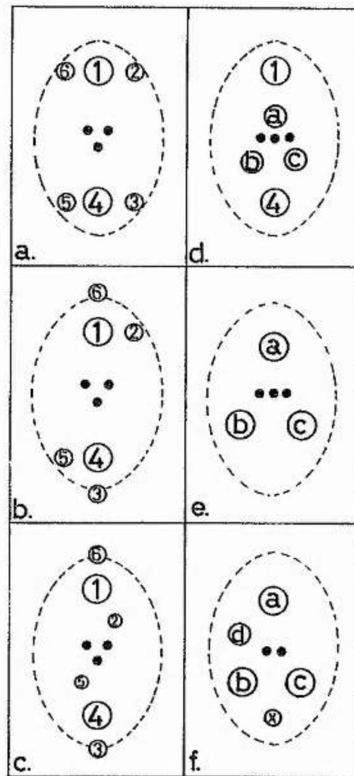


Figure 38

Diagrams of the configuration of axons, shown in fig. 37 in sections a, (83); b, (98); c, (113); d, (163); e, (233); f, (313).

to enter the chiasma though axons 7 and 8 are most common. It can only safely be concluded that two (unspecified) of three axons 7, 8 and 9 pass through the lamina.

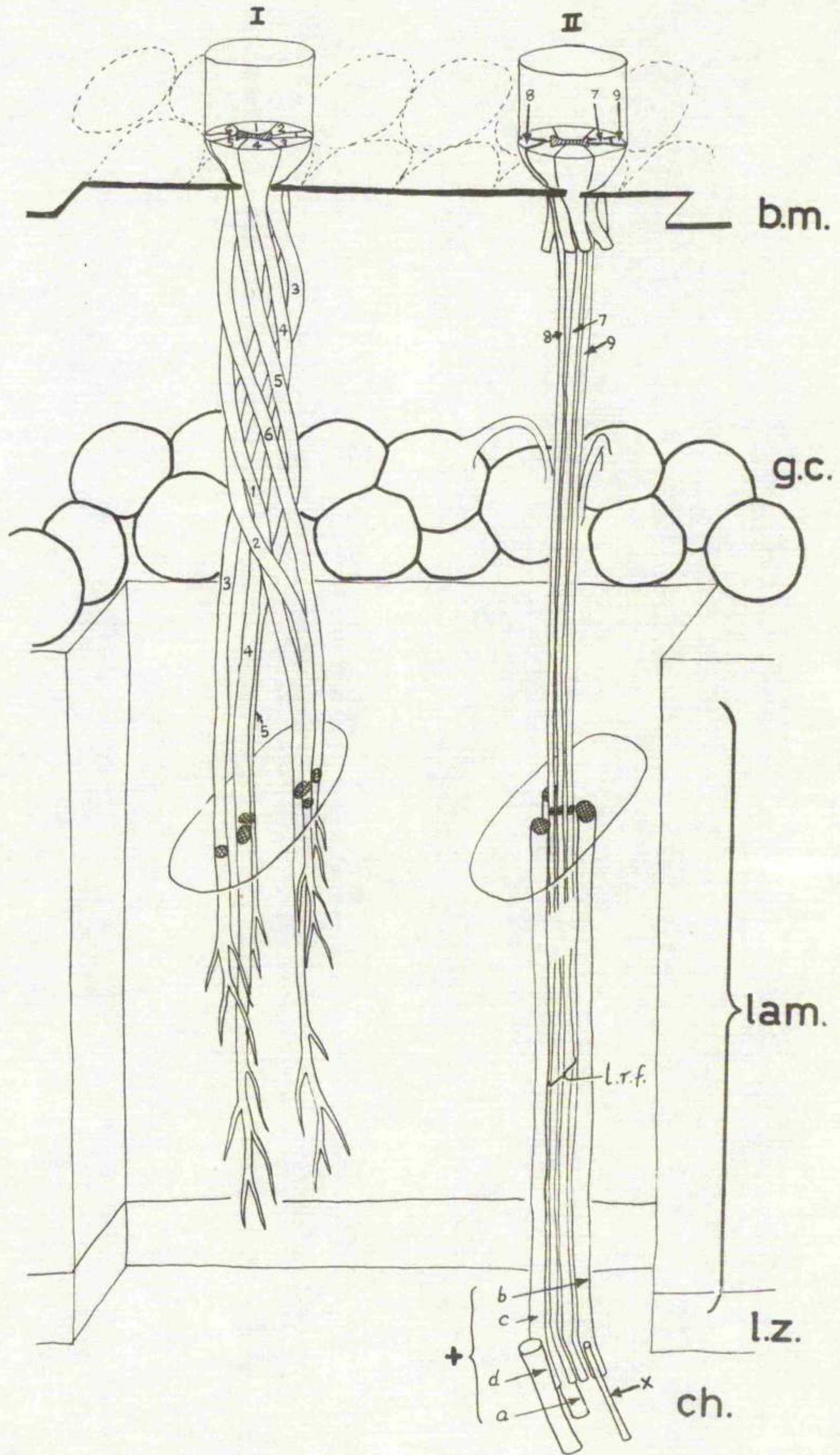
One third of the way through the lamina three axons (labelled a, b and c in fig. 38) appear around the central group of long retinula axons. These are presumed to be the three monopolar axons described for the worker bee by Varela (1970) though they cannot be traced from their cell bodies. Varela (1970) found a consistent size difference between one small and two large monopolar axons but this has not been observed here. In addition the arrangement of the axons within the cartridge is slightly different in worker and drone bee.

In the lamina each cartridge has seven axons, that can be traced into the chiasma, associated with it (figs. 36 and 37). These axons are only those that can be followed by light microscopy; the presence of other fine axons (section 344, fig. 36) which can be seen but not followed indicates that in the bee as in other insects the cartridges contain more neurones than described by these methods.

In the bottom quarter of the lamina another axon of small calibre (labelled d in fig. 37) becomes visible in addition to the three monopolar axons. At the bottom of the lamina it increases in diameter to equal the size of the three monopolar axons. The last remaining axon (labelled

Figure 39

Apis Drawing of the first projections and lamina (lam.) for two ommatidia I and II. In ommatidia I the six short retinula axons pierce the basement membrane (b.m.) rotate and polarize into two groups (1, 2, 6 and 3, 4, 5). The terminations (at two levels) are drawn after Golgi-impregnated silhouettes. In ommatidium II three long retinula fibres (l. r. f.) occupy a central position within their cartridge. Two of these pass through the lamina. They are joined by five more axons and all enter the chiasma as a bundle. L.z = lower zone of lamina, a dense neuropile layer derived from arborizations of the axons of the small monopolar neurones.

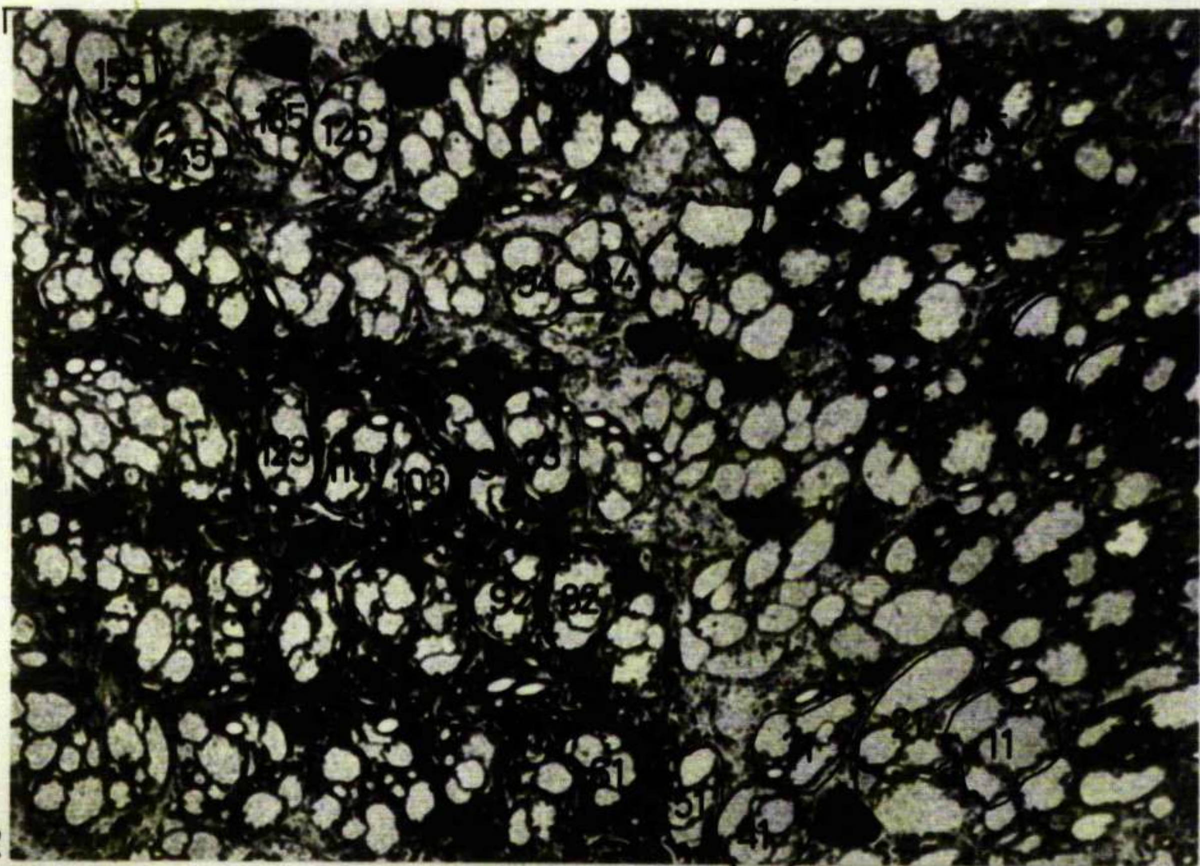

I
II
b.m.
g.c.
lam.
l.z.
ch.
l.r.f.
b
c
d
a
x
+

Figures 40 and 41

Apis Series of four micrographs showing the projection of twenty-seven lamina cartridges through the chiasma (taken from a different series to those of figs. 31 to 37.

Figure 40

Section 492 shows a portion of lamina containing two regions of neuropile, the lower zone containing cartridges 123 - 83, 92 - 82 and 61 - 51 and the middle zone in the remainder of the micrograph. The direction of twist of the seven groups of cartridge axons is indicated by arrows on the right in section 588.


492

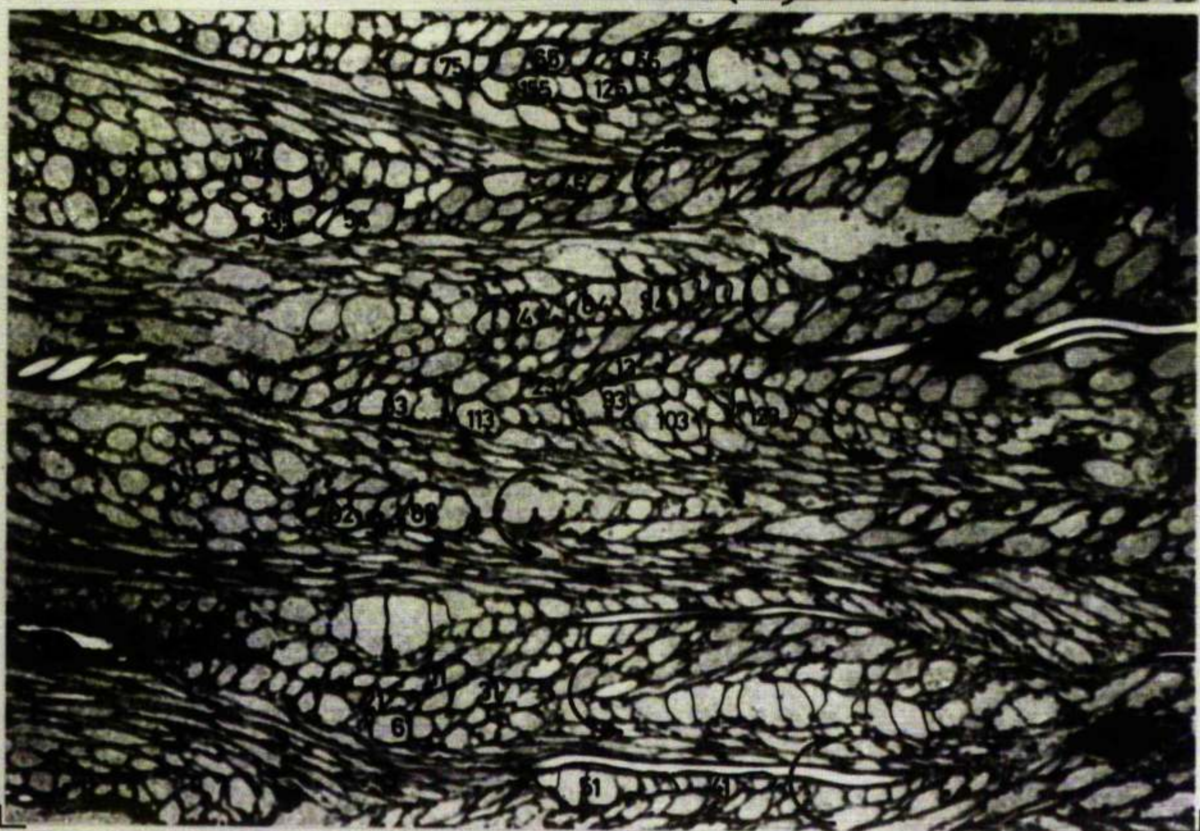
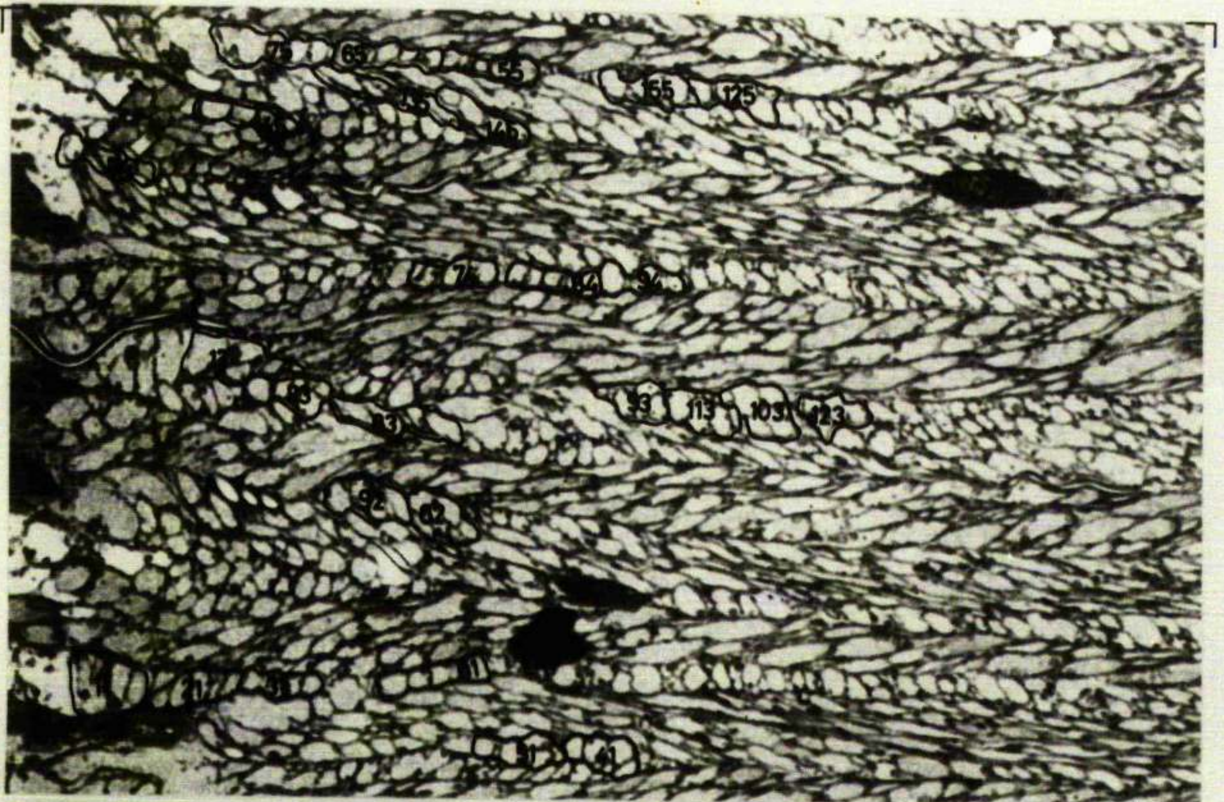
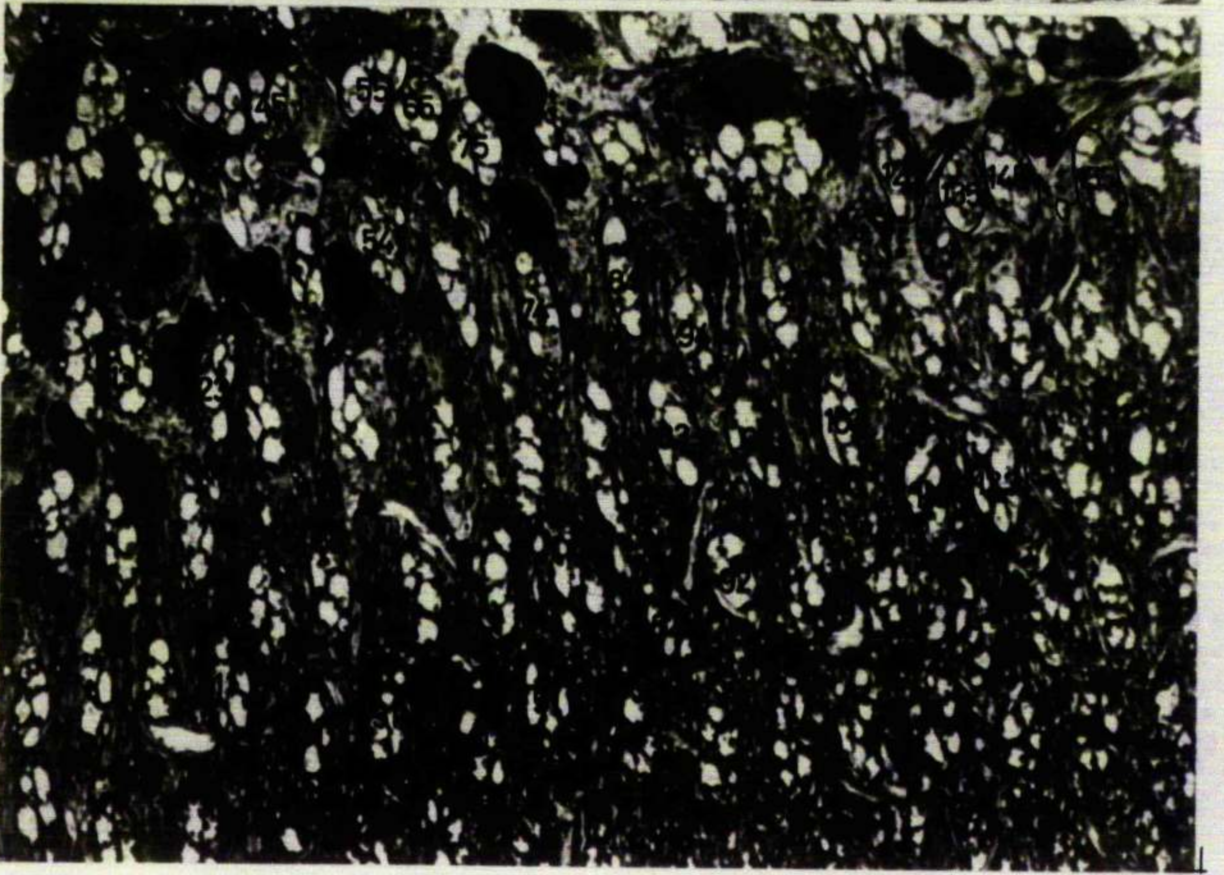

588
0
25µm.
Z

Figure 41

Section 768 shows the corresponding portion of medulla to the lamina of section 492 (fig. 40). The lamina array is repeated in the medulla after an antero-posterior inversion.


660


768
0
25µm.
Z ←

x in fig. 37) to be traced into the chiasma is very fine and can be followed only from a point near the bottom of the lamina. Possibly these last two axons are the two types of centrifugal neurone described by Cajal and Sánchez (1915). Thus seven axons enter the chiasma and connect with the medulla, four larger (2 - 3 µm diameter at the top of the chiasma) and three smaller (1 µm diameter or less).

The overall arrangement of the drone bee first projection and lamina neurones is shown in the diagram of fig. 39.

The lamina-medulla projection

Axons from twenty-seven cartridges from five horizontal rows of the lamina have been followed through the chiasma to the medulla. Their passage through the chiasma is shown in micrographs at four levels in figs. 40 and 41. Comparison of section 492 (fig. 40) and 768 (fig. 41) indicates that the axons which have been followed project homotopically between lamina and medulla; those from one lamina cartridge stay together in the chiasma and enter one medulla cartridge. Leaving the lamina, axon bundles enter the chiasma where they are grouped into strata which relate to horizontal rows of the lamina. The relationship is only approximate however since for example rows 45 - 155 and 11 - 61 are both split between two strata. The bundles invert their horizontal

Figure 42

Apis Section 492, from fig. 40, at greater magnification with the seven axons associated with each lamina cartridge individually marked. Two long retinula axons are marked with a spot, four large axons a, b, c and d are marked + (d is not separately distinguished) and a seventh fine axon is marked (x) in those cartridges in which it appears at this level.

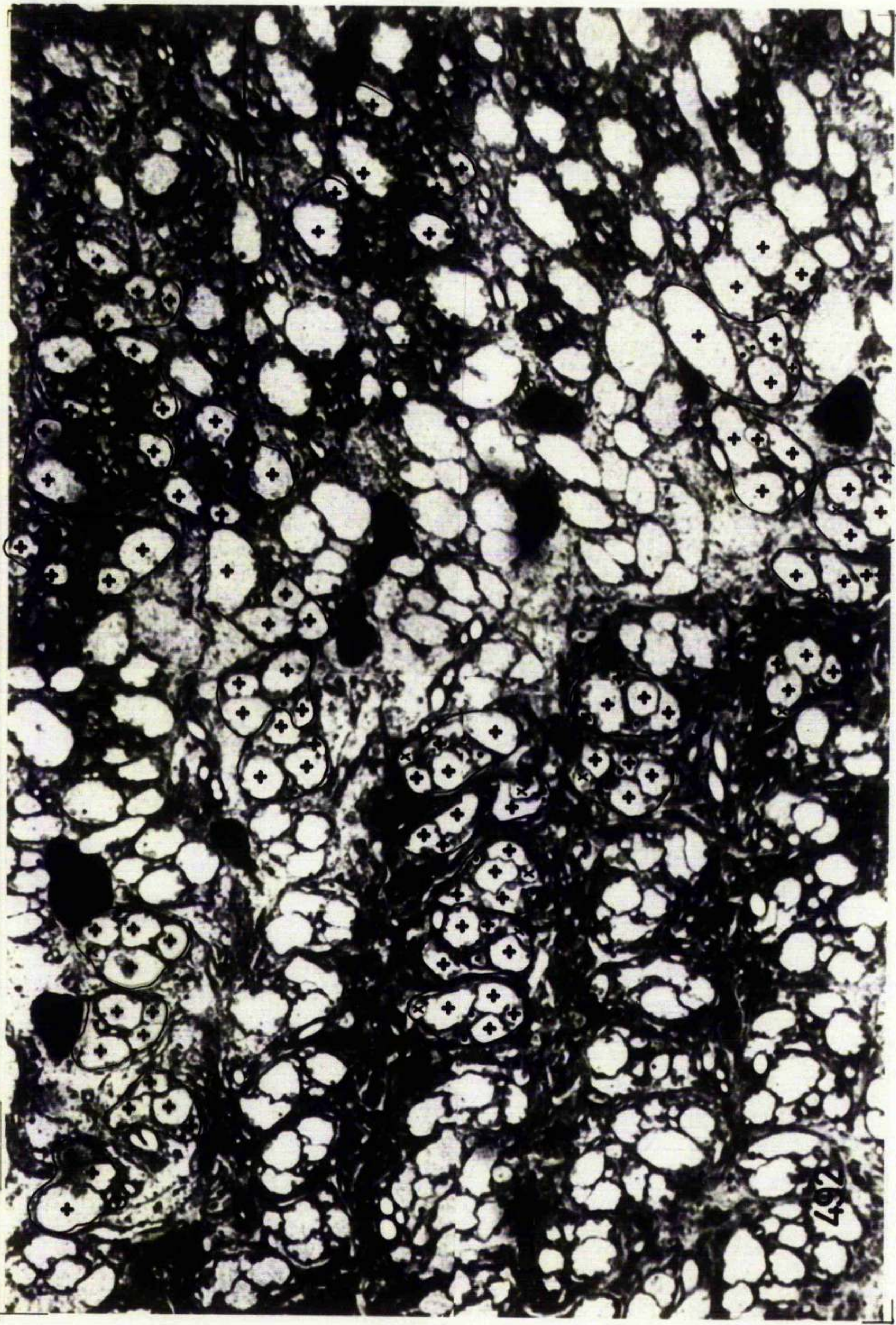
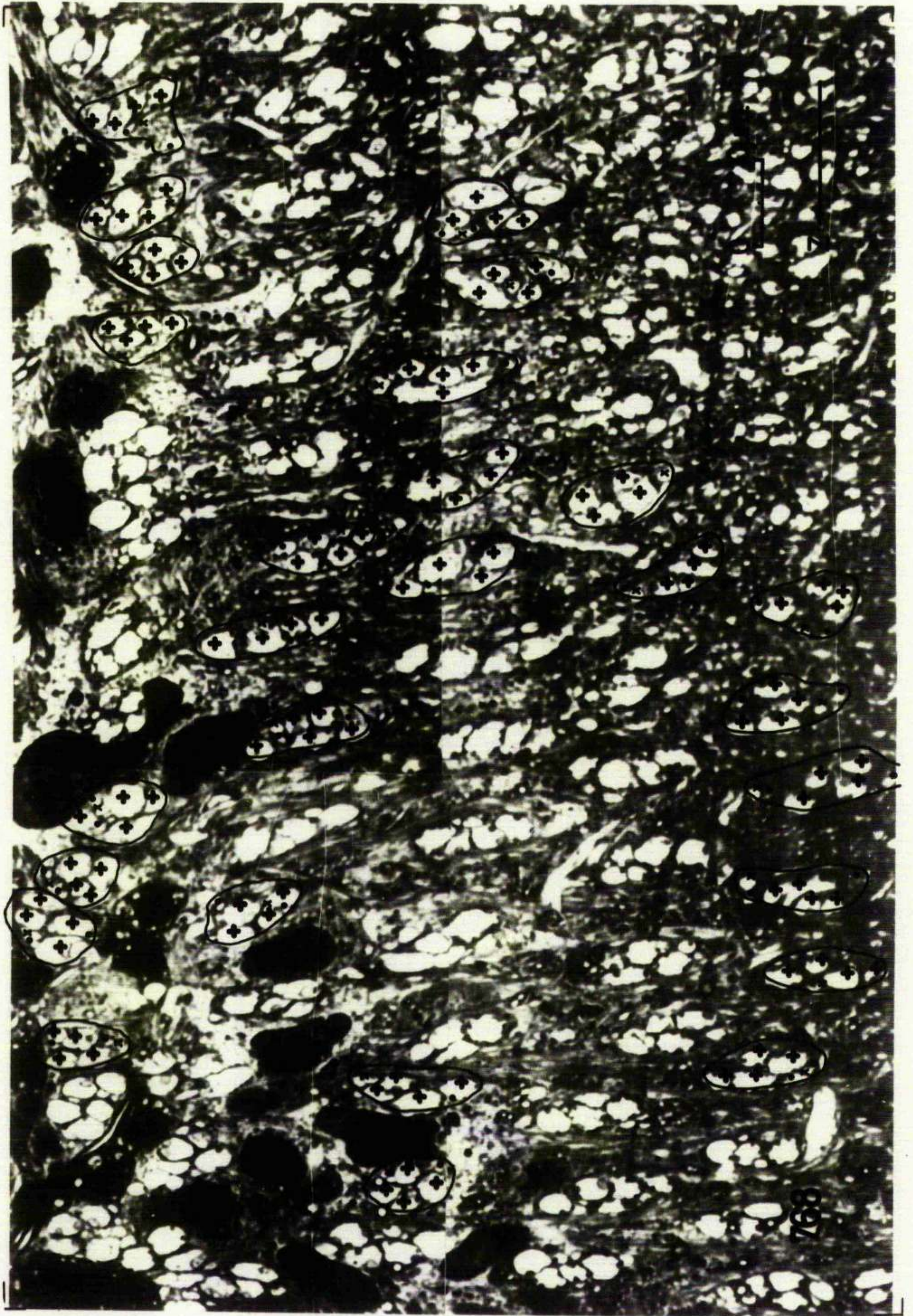


Figure 43

Apis Section 768, from fig. 41, at greater magnification with all those axons that were traced from fig. 42 indivdually marked.



sequence by either a clockwise or an anticlockwise twist of each chiasmal stratum (figs. 40 and 41), but there is no clear pattern of alternation in the directions of twist in successive chiasmal strata, at least in the limited field of axons traced. Both the relationship between horizontal cartridge rows and chiasmal strata, and the direction of twist of the strata need to be studied from a more extensive sample of cartridges before the definitive organization can be revealed.

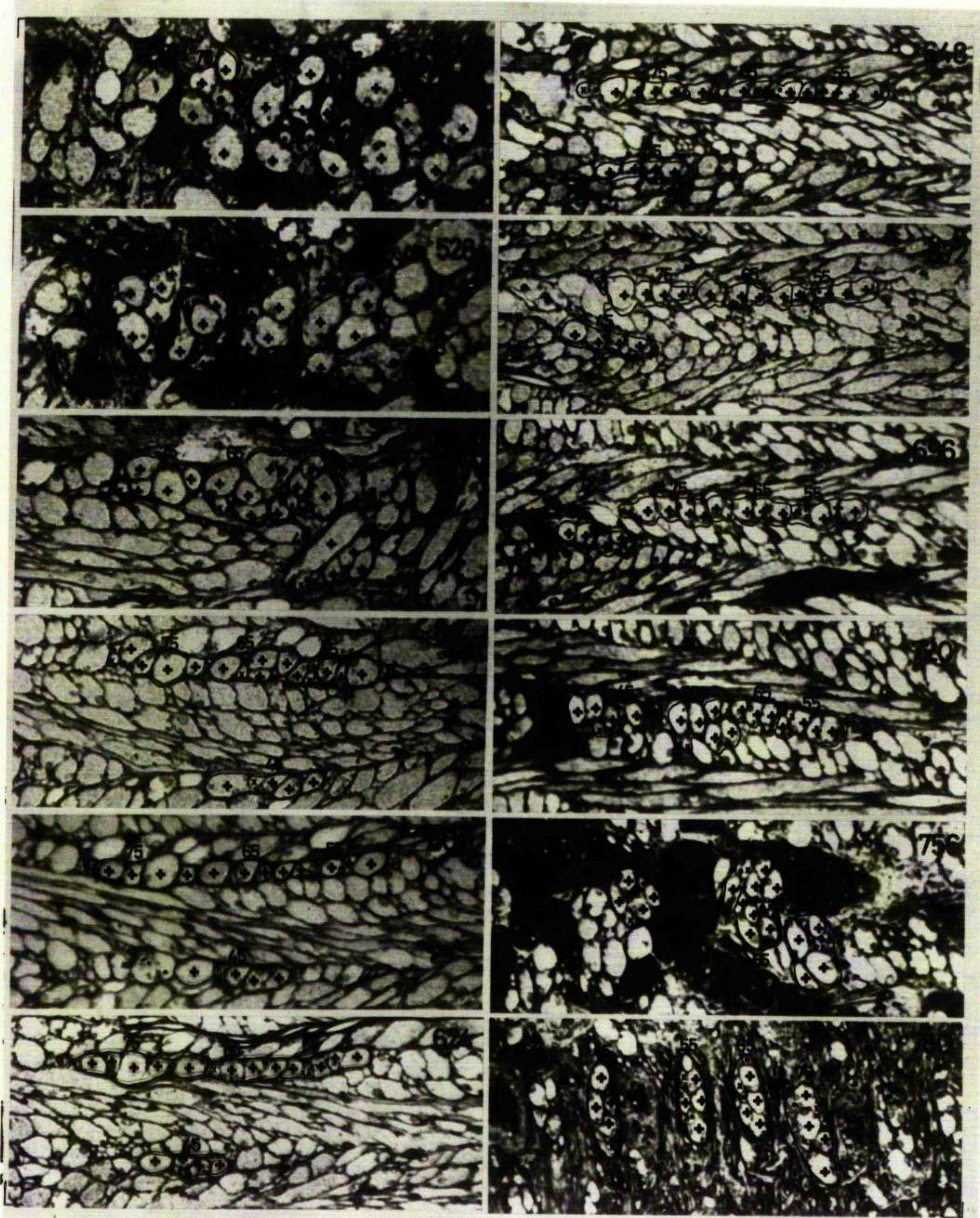
The level of inversion between one cartridge and its neighbour may be anywhere between lamina and medulla (e.g. 74, 84 and 94 invert between sections 492 and 588, fig. 40 ; 55. 65 and 75 invert between sections 660 and 768, fig. 41).

All the axons that have been unambiguously traced through the chiasma are marked in two micrographs of lamina and medulla in figs. 42 and 43. Some of the three small axons of each cartridge i.e. two long retinula axons and one marked x could not be traced right through the chiasma and are therefore not included in their medulla cartridges. All the axons of a single lamina cartridge enter a single medulla cartridge and no divergences were seen.

In addition to the seven axons of each cartridge, groups of extremely fine axons (0.25 - 0.5 µm diameter) are arranged periodically throughout the chiasma at a distal

Figure 44

Apis Series of micrographs of the axons of four lamina cartridges (45 - 75 of figs. 40 to 43) followed through the chiasma. The axons invert their horizontal sequence at section 756.


528
626
696
720
750
0 10μm.
Z

level (fig. 36). Each group contains three or four axons and several groups often form a single bundle. There seems to be approximately one group per cartridge and the axons, though impossible to trace individually, appear to derive from a fine plexus in the lower zone of the lamina. The groups are conspicuous only at the top of the chiasma, and are irresolvable at a deeper level in the chiasmal constriction.

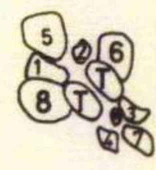
The axons of four cartridges 45 - 75 are shown in fig. 44 with each axon individually labelled. Cartridge 45 separates from the other three, enters a different statum and inverts its position between section 552 and 696 by a clockwise twist. Cartridges 55, 65 and 75 stay as a group and invert their position at section 756 by an anticlockwise twist. In the medulla ganglion cell layer (section 756) the small axon marked x has an irregular outline in some sections. This becomes confluent with a fine process running horizontally in the section. Although the details of such fine axons are difficult to resolve it may represent the T-junction with a linking fibre to a cell body, which would identify this axon as one type of medulla centrifugal neurone. Slightly more centrally, the seven axons contribute to a single cartridge of the medulla, which like that of the lamina is clearly separable from its neighbours.

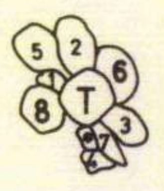
Figure 45

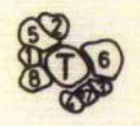
Pieris Micrographs illustrating retinula cell numbering

Left: micrograph of section 1 cutting ommatidia 43, 53, 63 shown in fig. 47 at progressively more distal levels. I, II and III are additional ommatidia of the same row not included in fig. 47.

Right: tracing of the cell profiles of ommatidium 64 showing their relationship to the central tracheole and (in section 1) the rhabdome (Rh). T = tracheole, Trb = Tracheenblase.







1
6
9
14
18

0 5μm.

Z

0 10μm.

Figure 46

<u>Pieris</u> Series of electron micrographs at increasingly more distal levels in the ommatidium. The antero-posterior axis lies between the centre of cells 6 and 8 in all micrographs.

a. A bundle of nine retinula axons surrounding a single tracheole (T) penetrates the basement membrane (b. m.) Scale 1 µm.

b. At the base of the ommatidium the tracheole splits into two halves which are divided by a refractile, cuticular tracheenblase (Trb). Cell 9 has its nucleus (N) at this level. Scale 5 µm.

c. Directly distal to the tracheenblase lies the rhabdome (Rh) which at this level is contributed by cells 1 - 4 only. The nucleus of cell 9 is still at this level (outside the field of the micrograph). x = cell process of unknown origin. Scale 1 µm.

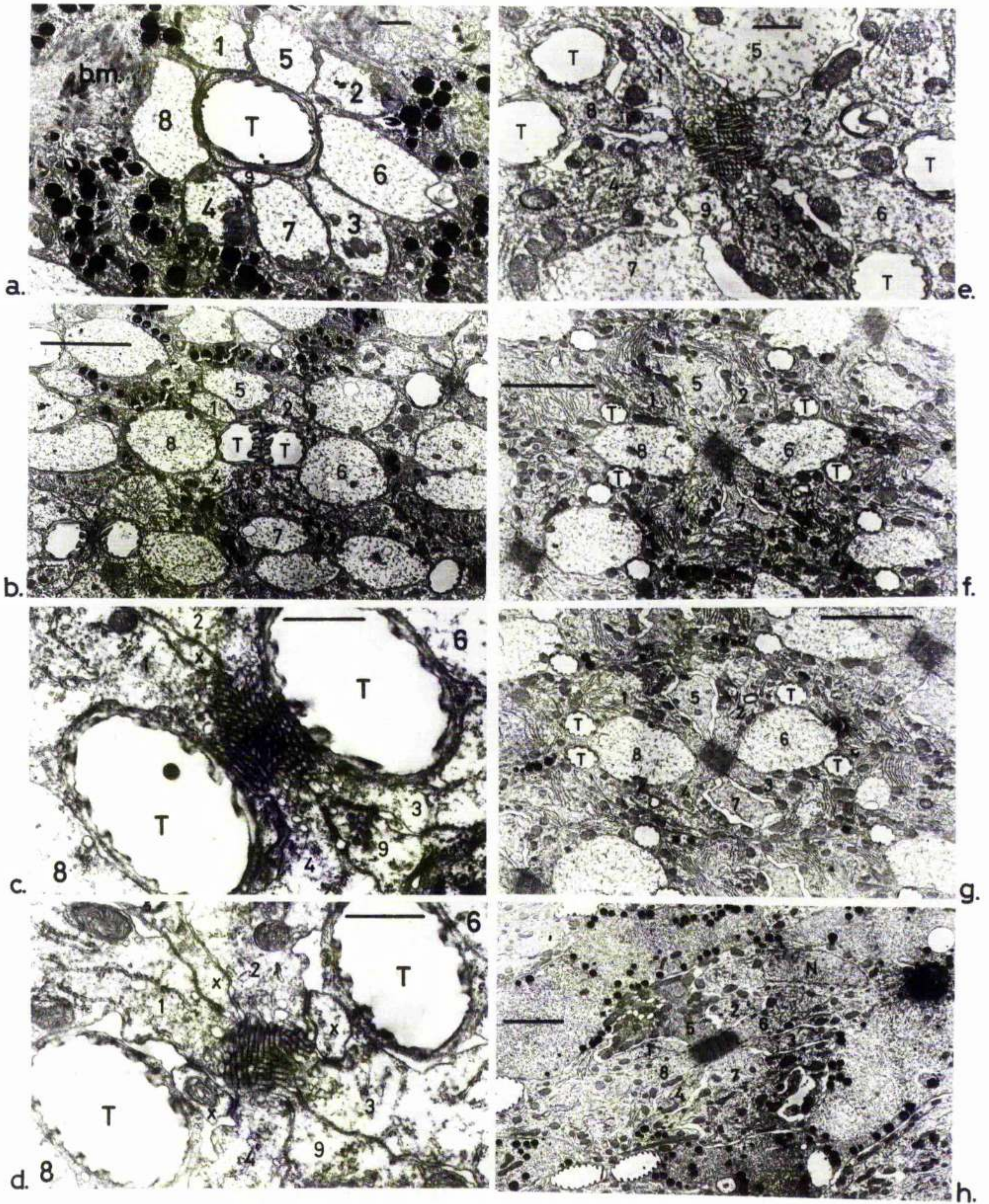
d. At a slightly more distal level cell 9 contributes a few rhabdomeric microvilli. x = cell processes of unknown origin. Scale 1 µm.

e. Cells 6 and 8 pass between the divided tracheoles to abutt (but not contribute to) the rhabdome. Scale 1 µm.

f. At a slightly more distal level cell 9 decreases in size. Scale 5 µm.

g. Cell 9 is no longer present and the rhabdome, which assumes the striated appearance characteristic of its more distal levels, is formed by cells 1 - 4, each cell with microvilli parallel to its own orientation and neighbouring cells with orthogonal orientation. Scale 5 µm.

h. In the more distal portion of the ommatidium the rhabdome is formed mainly by cells 5 - 8. The same orthogonal planes of the microvilli are preserved. Cell 6 has a nucleus (N). Scale 5 µm.

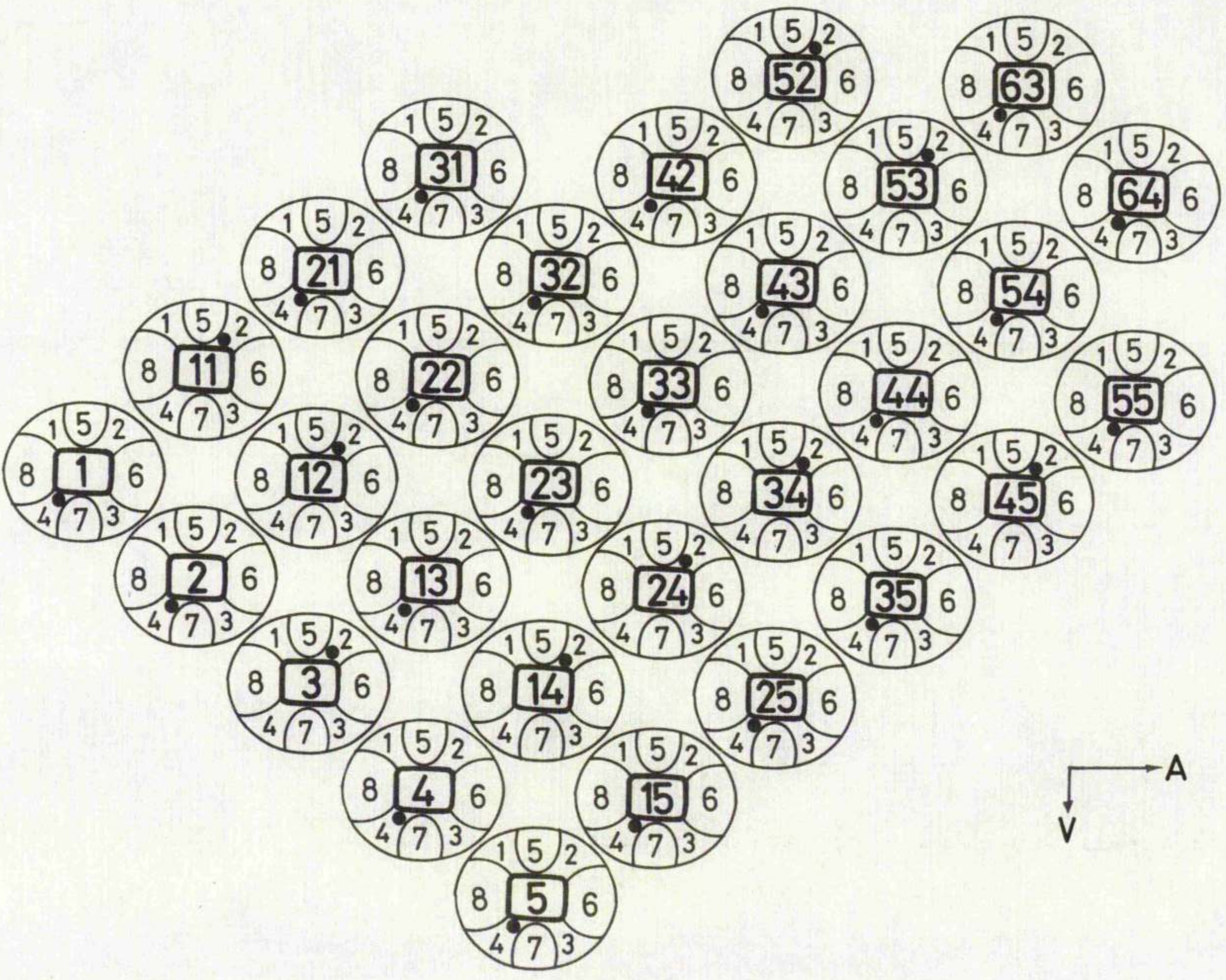

a.
bm.
1
5
2
8
T
9
6
4
7
3
b.
1
5
2
8
T
T
6
4
7
c.
2
x
6
T
T
3
9
4
8
d.
1
x
2
6
T
x
T
x
3
4
9
8
e.
T
5
1
8
T
2
T
4
9
6
7
T
f.
1
5
2
T
8
6
T
T
4
7
g.
1
5
2
T
T
8
6
T
T
4
3
7
h.
N
2
5
6
1
8
7
4

F. Pieris: the small white butterfly

The ommatidium has nine retinula cells and the structure is well described by Nowikoff (1931), whose drawings are exactly comparable with the micrographs in fig. 45. There are eight long retinula cells in each ommatidium divisible into two groups of four, by the positions of their nuclei (Nowikoff, 1931). Cells from the two groups occupy alternate circumferential positions in the ommatidium, cells here called 5 - 8 with distal nuclei lie in the antero-posterior and dorso-ventral quadrants, while cells 1 - 4 have nuclei half way down the ommatidium and occupy positions in the diagonal interstices of the ommatidium between cells 5 - 8 (Nowikoff, 1931). Thus, the rotational sequence of long retinula cells at all levels in the ommatidium is 1, 5, 2, 6, 3, 7, 4 and 8. In addition there is a ninth basal cell with a nucleus at the bottom of the ommatidium (Nowikoff, 1931; fig. 46 b); this has a position in approximately 75% of ommatidia in the ventral quadrant next to cell 7 while in 25% it is next to cell 5. The contribution of the nine cells to the rhabdome is as follows: cell 9 contributes only a very few microvilli over a very small distance in the basal part of the ommatidium (fig. 46 d). In the central part of the ommatidium only the diagonal cells 1 - 4 contribute to the rhabdome while in the distal part most of the rhabdome is contributed by cells 5 - 8 although a little

Figure 47

Pieris Plan of the retina showing thirty ommatidia from which the retinula axons have been followed to the lamina. The central tracheole is represented by a box which bears the ommatidial number. The position of cell 9 in the retinula is marked by a spot.


A
V

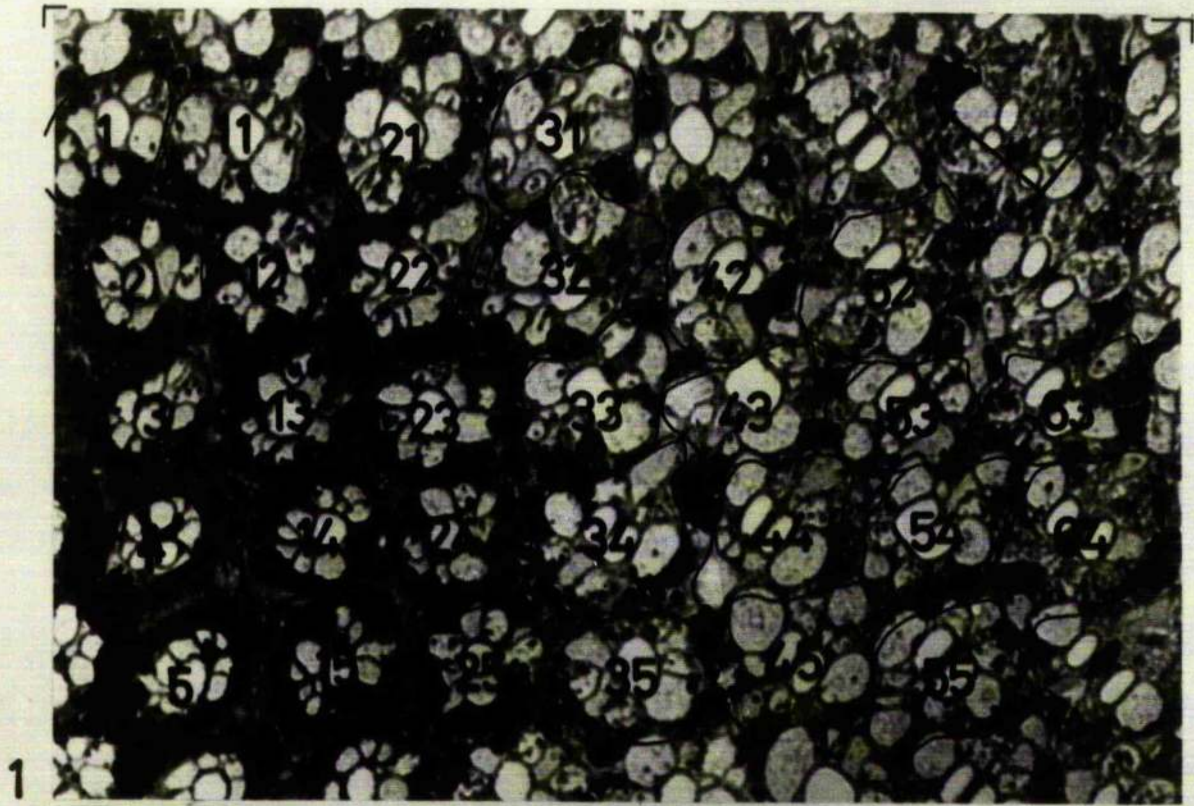
apparently still comes from cells 1 - 4. The rhabdomere microvilli are orientated along the same two (diagonal) orthogonal planes throughout the ommatidium even though they originate essentially from different quartets at different levels (fig. 46). Rhabdomere contributions from neighbouring orthogonal cells within either quartet are layered in an arrangement similar to that described in the lobster by Rutherford and Horridge (1965) (Meyer-Rochow, personal communication). The relationship between the prominent ommatidial tracheoles and the fused rhabdome is described by Nowikoff (1931) and shown in fig. 45. Approximately 15 μm distal to the basement membrane the tracheole at the core of the retinula axon bundle (fig. 46 a) divides, the two tracheolar branches forming between them a refractile cuticular "tracheenblase" (Nowikoff, 1931) the body responsible for eye-glow in butterflies (Miller and Bernard, 1968), (fig. 46 b). Each pair of tracheoles divides again more distally and the two pairs of tracheoles move to a position in the periphery of the ommatidium on either side of the large cells 6 and 8. The axons of the thirty ommatidia shown in the retinal plan (fig. 47) have been traced to cartridges in the lamina from a single series cut from a right eye. Fig. 47 was obtained directly from the micrograph of section 1 in fig. 48. The large retinula cells 6 and 8 (diameter 3 μm) arranged along the antero-posterior axis

Figures 48 and 49

Pieris Four micrographs of the retina-lamina projection of the ommatidia shown in fig. 47.

Figure 48

Section 1. Micrograph of the retina on which fig. 47 was based.

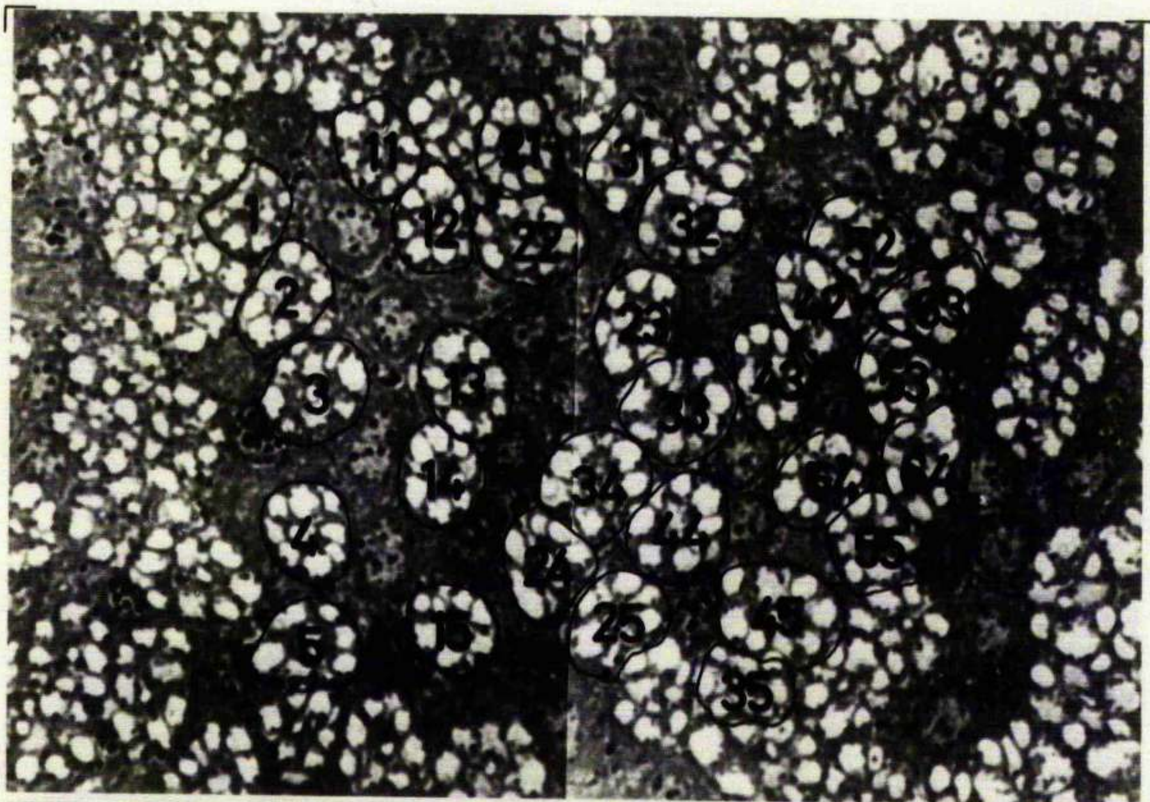


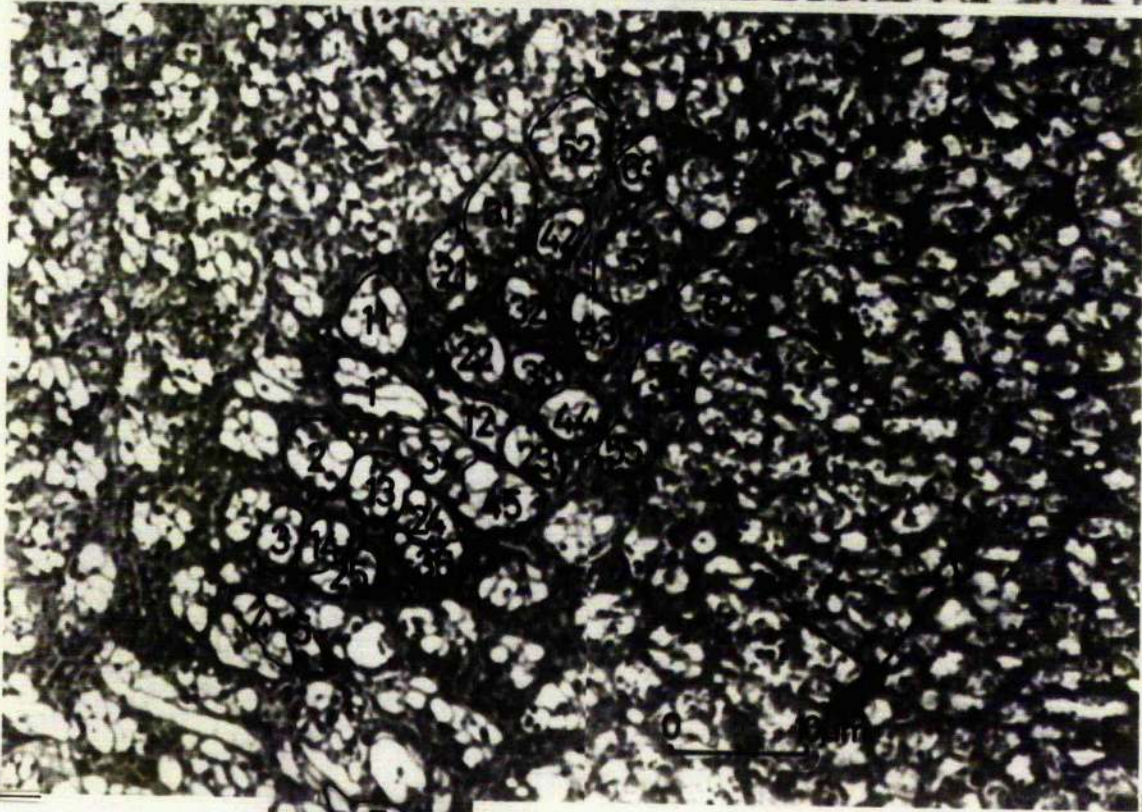
1

48

Figure 49

Section 108. Micrograph of the top of the lamina showing the array of cartridges corresponding to the ommatidial array of section 1 (fig. 48) interspersed amongst lamina monopolar cell bodies. Section 196. Micrograph of the axons entering the lamina-medulla chiasma.


108


196

are obvious in this micrograph. The retinula axon bundles pierce the basement membrane and coalesce into axon tracts separated by tracheae (section 48, fig. 48). The bundles traced underwent a slight lateral displacement (right, section 48, fig. 48).

The axons of one ommatidium stay together as a group within the axon tract and do not separate. In this state they enter the lamina and form cartridges, one cartridge derived from the axons of one ommatidium. The rotational sequence 1, 5, 2, 6, 7, 4 and 8 is preserved nearly perfectly through the entire length of the first projection to the lamina. Axons 8 and 6 maintain their slightly larger diameter for some distance below the basement membrane. Axon 9 is of smaller calibre (1 µm diameter) and usually occupies a central position surrounded by the ring of the other eight retinula axons. At the lamina, axons 5 and 7 usually also come to occupy a central position (fig. 49) and probably two of these three axons are the long visual fibres of the retina (see Discussion, p. 174).

The retinula axon bundles twist during their passage to the lamina but this rotation is inconsistent in direction and magnitude. Neighbouring retinula bundles have been observed to twist in opposite directions and the rotation may be as great as 360°. Some bundles rotate in one direction first, reverse and then rotate in the other. The consequence

Figure 50

Pieris Plan of the lamina derived from section 108 (fig. 49). All retinula axons from one ommatidium go to a single cartridge and the rotational sequence of axons duplicates that of the ommatidia except for axons 6 and 2 in cartridge 42. Cell 9 is marked with a spot.

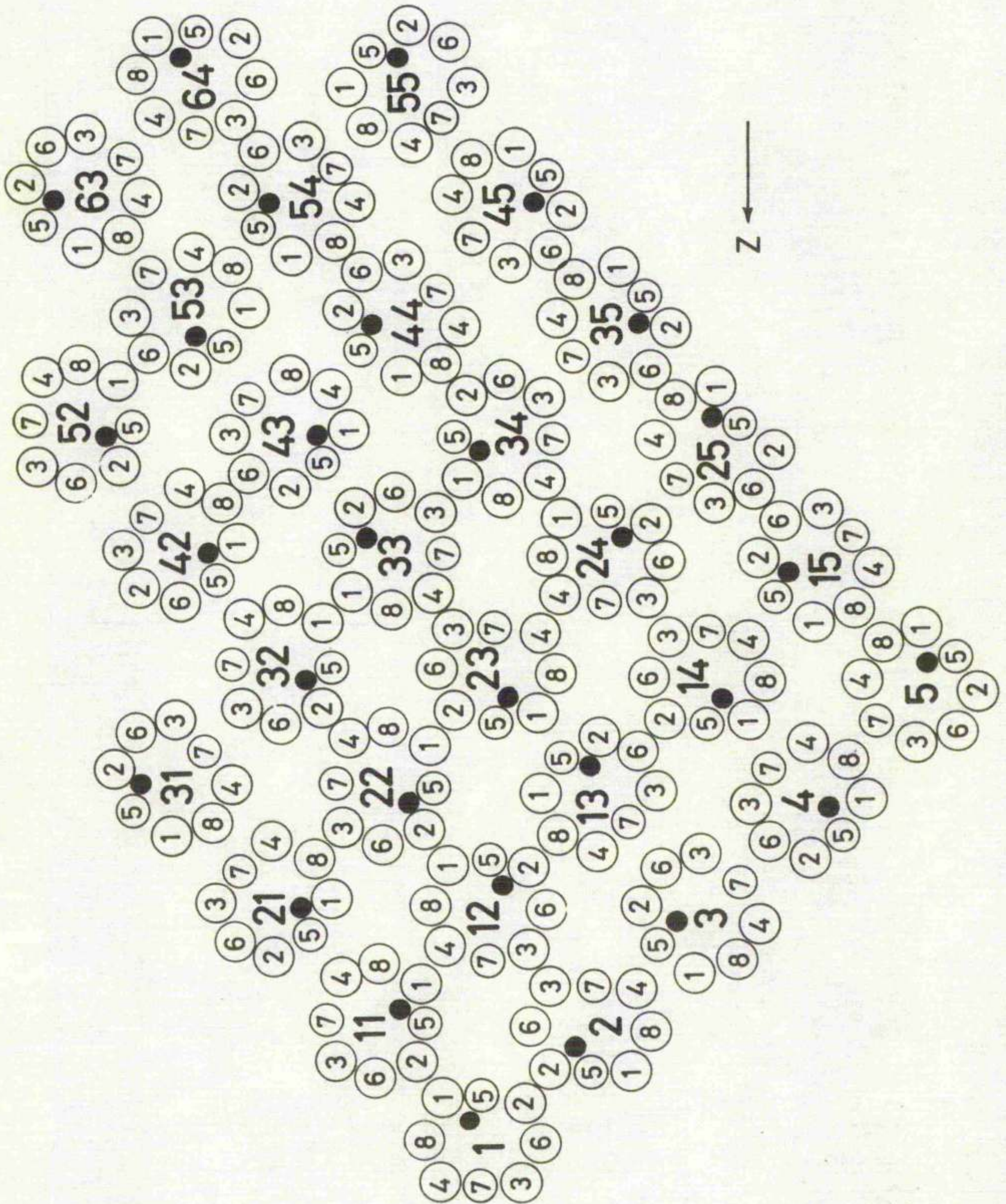
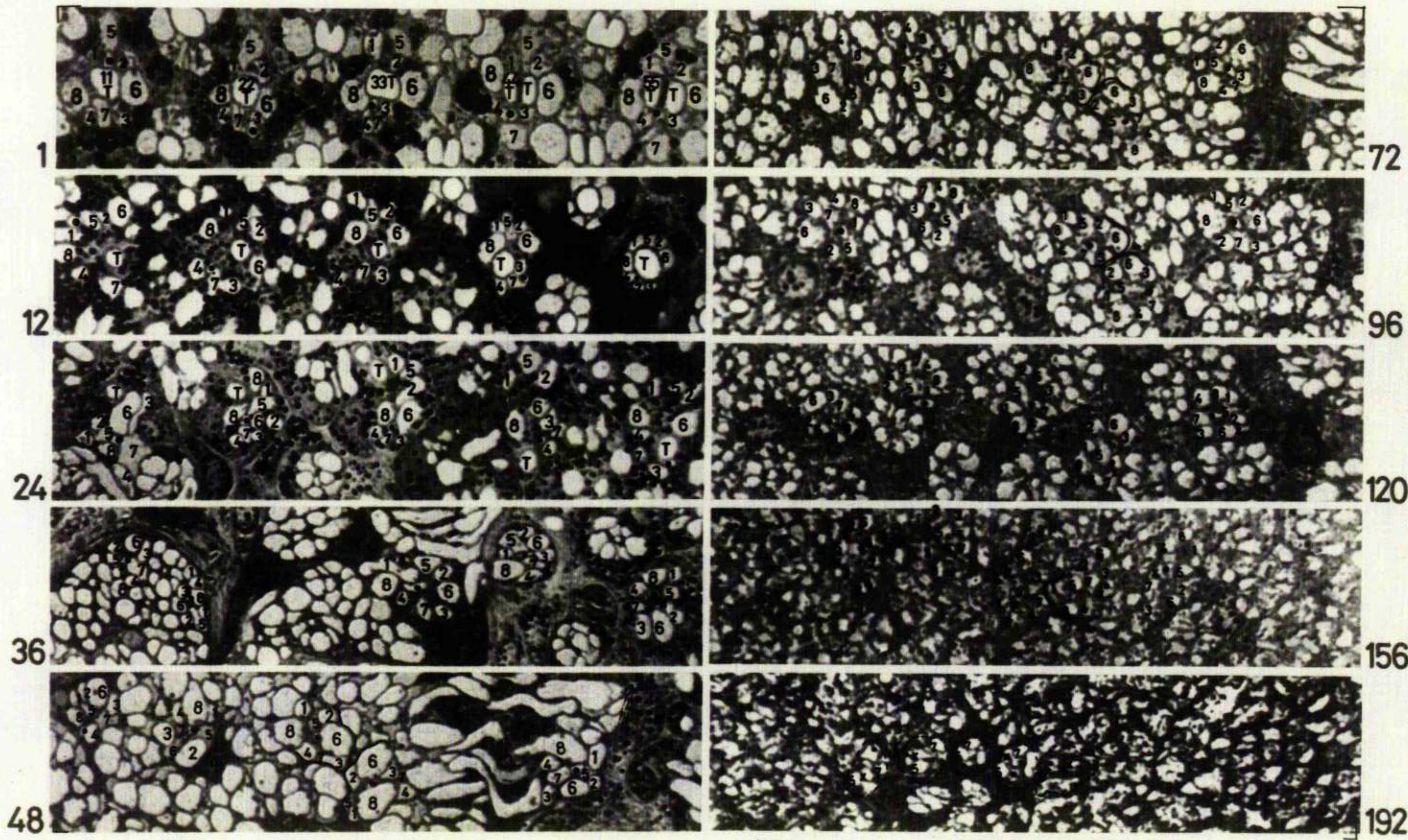

N

Figure 51

Pieris Micrographs of five ommatidial bundles (11, 22, 33, 44 and 55) at different depths between retina and lamina. Section 1 (from fig. 48) shows the base of the ommatidia. Section 12 shows bundles 44 and 45 perforating the basement membrane (BM). Sections 24 to 72 show the progressive coalescence of the axon bundles. At section 48, bundle 55 undergoes a slight lateral displacement. Sections 72 - 120 show the gradual formation of lamina cartridges. Section 192 shows bundles of axons associated with five cartridges entering the chiasma. Each bundle probably contains three long retinula axons, numbers 5, 7 and 9 (marked with a spot), and one other that has a position in the ring of retinula terminals (marked x).


1
12
24
36
48
72
96
120
156
192
0 10µm.
Z

is simply, however, that in the middle of the lamina neuropile axons 6 and 8 are at the dorso-ventral poles of each cartridge.

The cartridges formed by the thirty ommatidia traced are shown in the micrograph of section 108 (fig. 49). At this level the cartridges are quite distinct but the array is distorted to the right. At a deeper level the cartridges are less distinct but the array is more regular. At the bottom of the lamina each cartridge gives rise to a group of axons of possibly up to eight in number, which enters the chiasma (fig. 49). The cartridge array of section 108 (fig. 49) is reproduced in the idealized lamina plan of fig. 50. Each cartridge has the same rotational axon sequence as that of its ommatidium, with one exception (axons 6 and 2 in cartridge 42). The cartridges have not reached their final configuration so that the orientation of axons 6 and 8 is not consistent at this level. Axon 9 usually comes to be next to axon 5.

The projection of five ommatidial bundles between retina and lamina at different levels is shown in fig. 51. Each ommatidial bundle projects to a single cartridge from which emerges a group of axons containing either two or three retinula axons from cells 5, 7 and 9, with one other axon included in the ring of terminals (marked x).

Figure 52

Trapezites Electron micrographs of the eye at various levels. (The micrographs of this figure were kindly prepared by Mrs. C. Giddings).

a. Retinula cells cut distal to the rhabdome at a level where some long retinula cell nuclei (N) are seen. Scale 5 µm.

b. Ommatidia of eight retinula cells with the characteristically shaped fused rhabdome surrounded by a peripheral ring of tapetal tracheoles and pigment granules. The cellular positions are best seen near the rhabdome where they are indicated by the presence of desmosomes. Scale 5 µm.

c. An ommatidium of eight retinula cells cut proximal to those in (b), contains a symmetrical rhabdome with rhabdomeres orientated along four axes at 45° intervals. Scale 2 µm.

d. An ommatidium cut proximal to c. with eight peripheral retinula cells below the level of their rhabdomeres. These cells have the ultrastructural features of axons and surround the cytoplasm and twin rhabdomeres of the ninth basal cell. Scale 2 µm.

e. Section proximal to d. in which the ninth cell is cut through its nucleus (N) below the level of its rhabdomeres. The eight peripheral cells are more axon-like than in d. Scale 2 µm.

f. An ommatidium at the basement membrane. Nine axons (one small one from the basal cell) surround a basal pigment cell with a prominent nucleus which stains darkly in toluidine blue sections and a group of tracheoles. Scale 5µm

g. Ommatidial bundle of nine retinula axons containing two central axons, (one is the small axon of the ninth cell), which are difficult to follow in light microscope sections. Glial nuclei surround the retinula bundles. Scale 5 µm.

h. Mid-lamina. Each cartridge consists of seven pale short retinula axon terminals (with artifactual vacuoles) surrounding a group of at least six axons, two pale and four dark. Scale 1 µm.

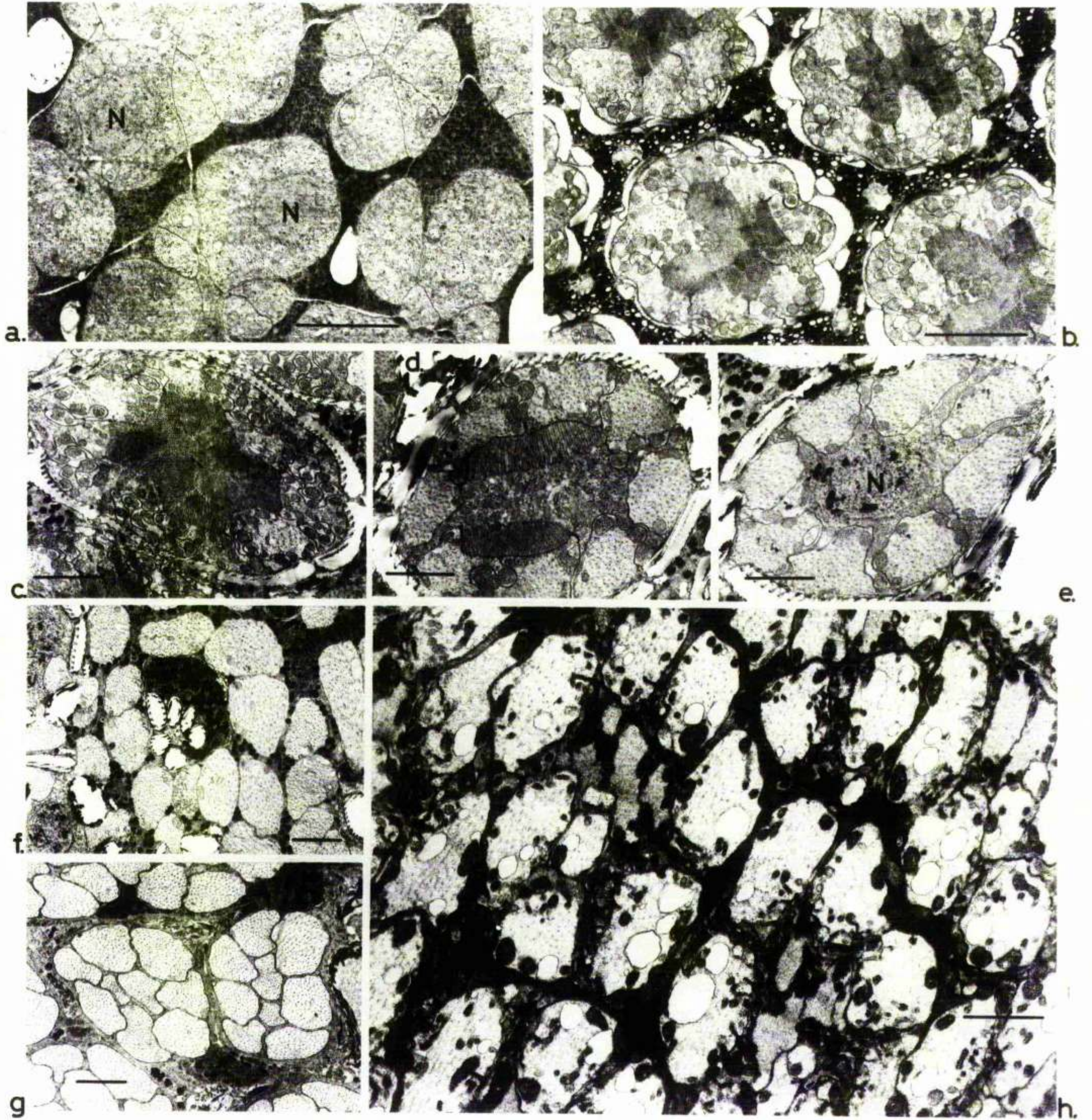
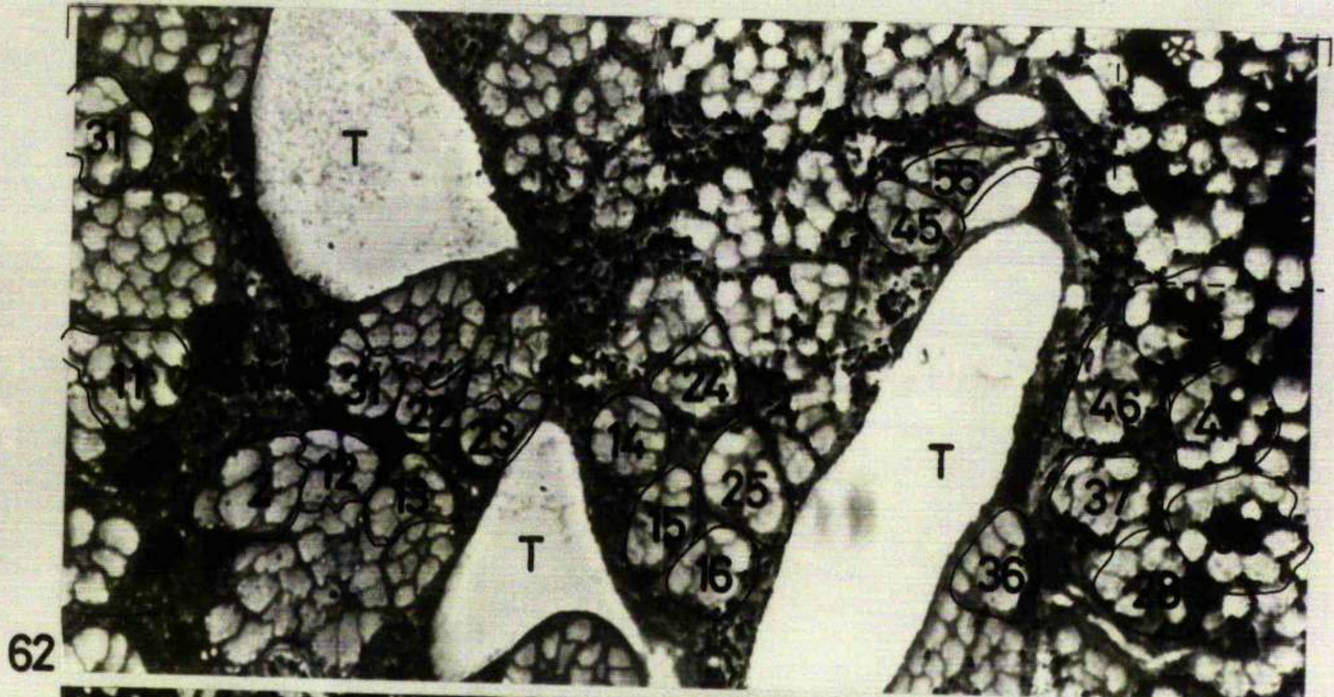

N
N
a.
b.
c.
d
N
e.
f.
g
h.

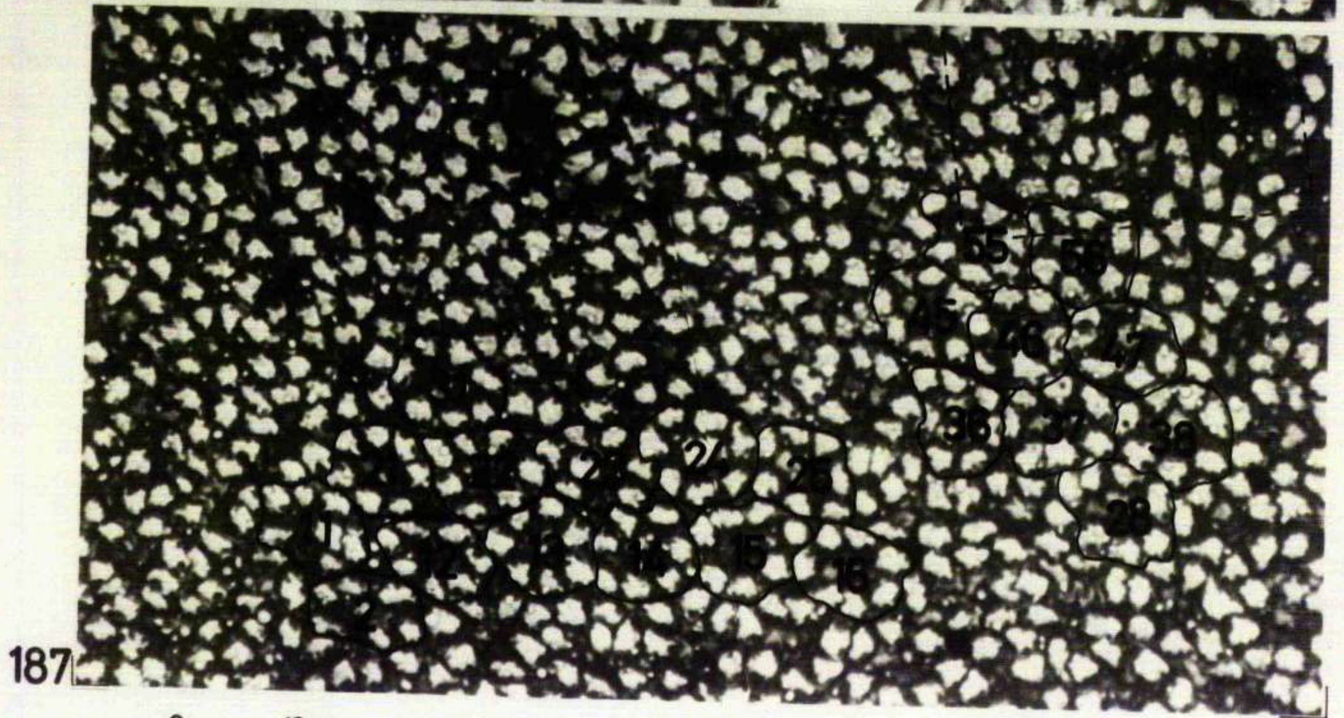
Figure 53

Trapezites Micrographs of the retina-lamina projection of twenty-two ommatidia.

Section 62. Micrograph of region just beneath the retina. The bases of ommatidia are seen in the top right corner. Most of the field shows clearly recognizable axon bundles separated by tracheae.

Section 187. Micrograph of the top of the lamina. Axons derived from one ommatidium are outlined and correspond to an individual cartridge. The area enclosed by a broken line in the top right hand corner of each micrograph is that of fig. 54.





0 10µm.

Z ←

G. The skipper Trapezites

The structure of the ommatidium of the skipper is shown in fig. 52 a - e. Each ommatidium has eight large retinula cells (fig. 52 a - c) and a ninth basal cell with a split rhabdomere (fig. 52 d).

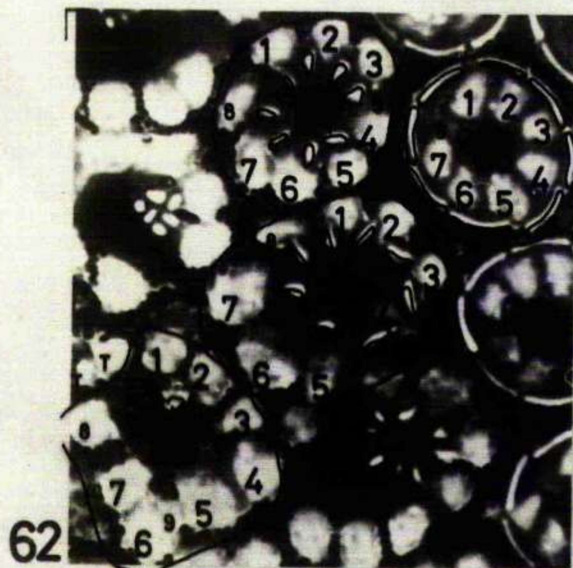
The retina-lamina projection

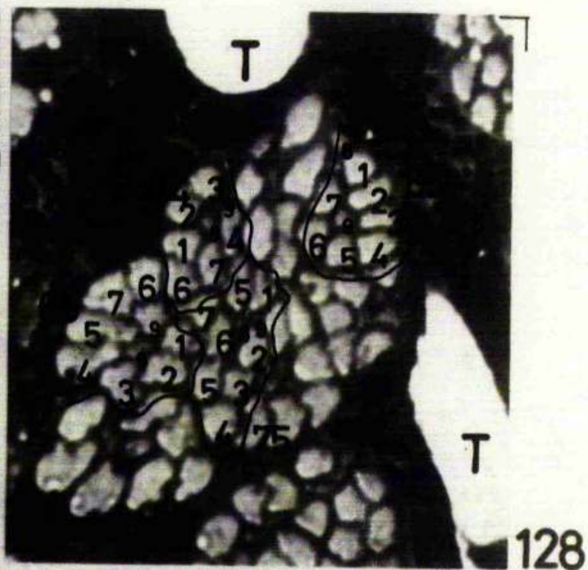
The retina-lamina projections of twenty-two ommatidia have been analysed (figs. 53 to 55). The retinal field is represented by a micrograph of section 62 (fig. 53) which is actually at a level just beneath the basement membrane; at the base of the ommatidia the retina does not photograph well because of the presence of an intensely staining basal pigment cell at the centre and a ring of tracheoles around the periphery of each ommatidium (Swihart, 1969).

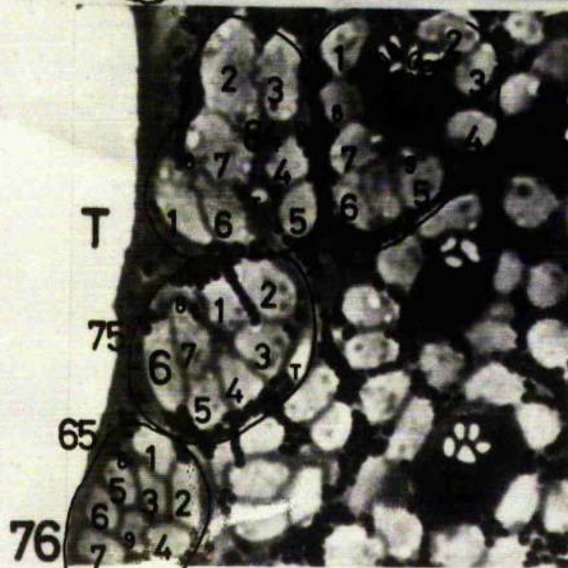
Retinula axon bundles coalesce into wide axon tracts shortly after penetrating the basement membrane and proceed centrally to the lamina. At the top of the lamina (section 187, fig. 53) the array of axon groups corresponds to the ommatidial array, but cartridges are not well separated. At a deeper level the cartridges become more obvious (section 306, fig. 55) but the retinula axon terminals are smaller and less distinct. There are seven conspicuous retinula axon profiles at the periphery of each axon group at the top of the lamina (section 187, fig. 53) together with a further

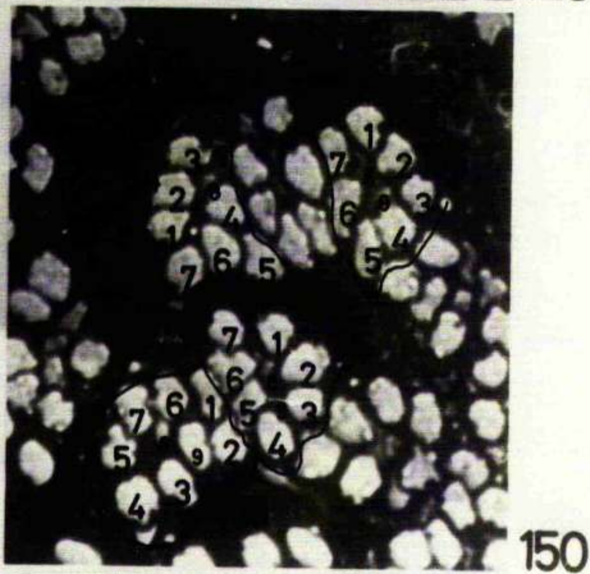
Figure 54

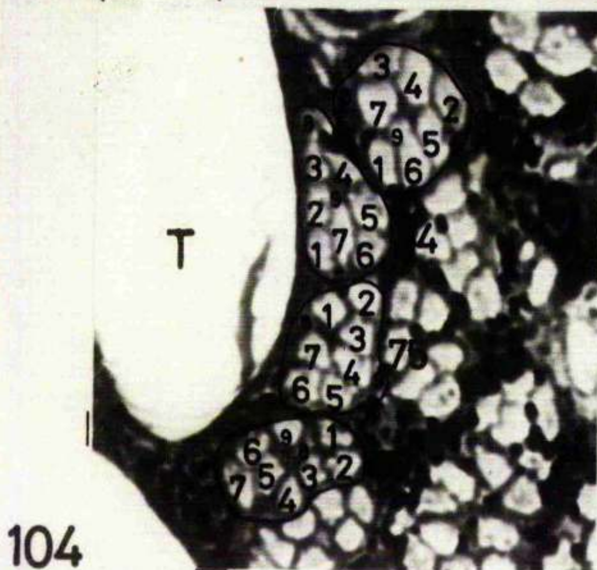
Trapezites The projection of four ommatidia (65 - 68, 84) from the retina enclosed by broken lines in section 62 (fig. 53) to the lamina (section 187, fig. 53). Section 62 shows the bases of the four ommatidia; section 187 shows the formation of indistinct cartridges.

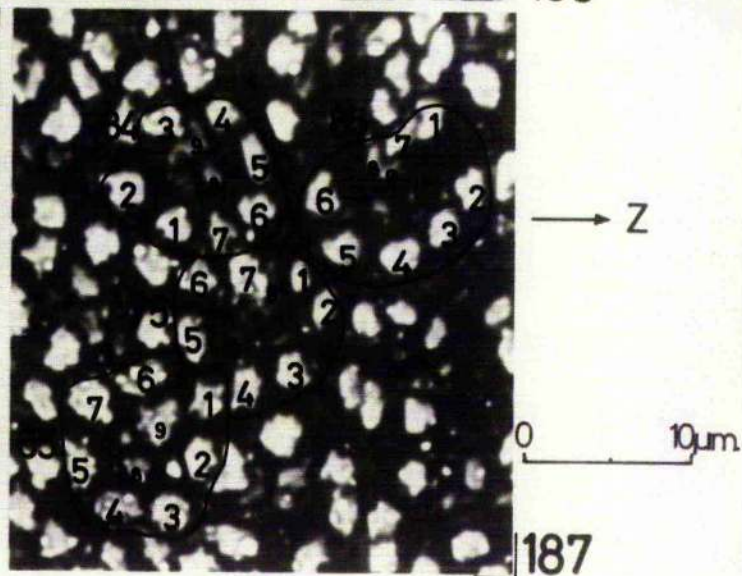

T
62


T
T
75
128


T
75
65
76


150


T
75
104


Z
0
10µm
187

two extremely fine axons which come to lie at the centre of the group (fig. 52 h). All nine axons are derived from the same ommatidium.

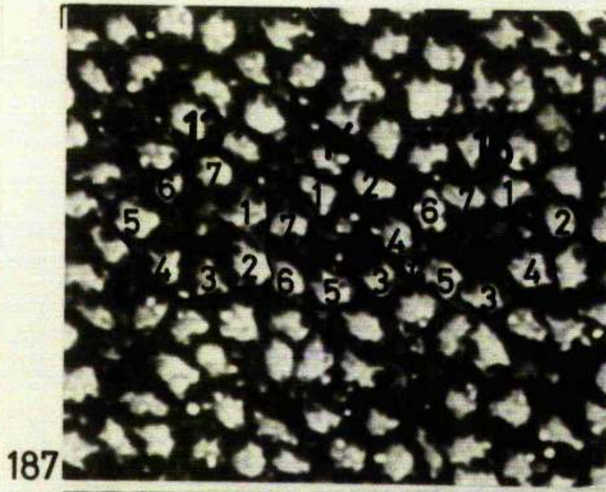
Fig. 54 shows the projection of four ommatidia, 65, 75, 84 and 85, with each axon individually identified. Each ommatidium has n ne retinula cells, seven large ones with axons 2 µm in diameter and arbitrarily numbered 1 - 7, and two indistinct cells 8 and 9 with slender axons (1 µm diameter or less) which are darkly staining and not visible in all sections. The relationship between axon numbering and the arrangement of cells within the ommatidium is not known.

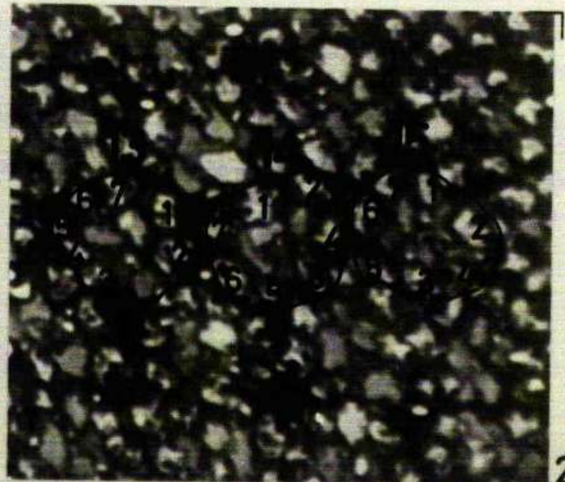
At the base of the ommatidium the peripheral ring of ommatidial tracheoles squeezes between the retinula cell axons and unites to form a common central tracheole which eventually communicates with the tracheae underlying the basement membrane. The arrangement and gradual fusion of these eight tracheoles is illustrated in the sequence contained in fig. 54; ommatidium 85 (section 62), 75 (62), 84 (62), 85 (76), 65 (62) and 84 (76).

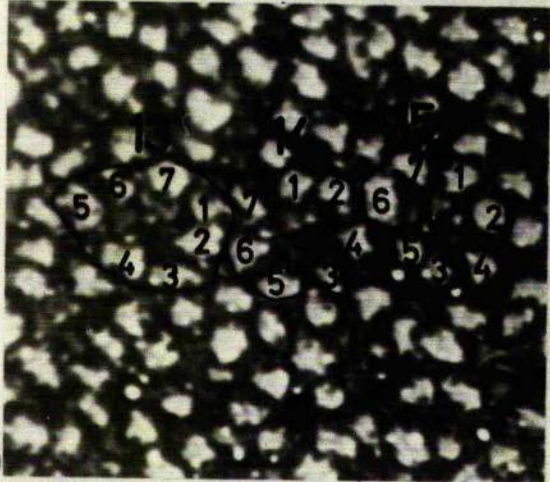
The ommatidial bundles gradually coalesce and without twisting significantly pass through a layer of monopolar cell bodies (sections 128 - 150) then separate to form lamina cartridges. The rotational sequence of axons 1 - 7 is preserved faithfully (section 75 - 150) but the position

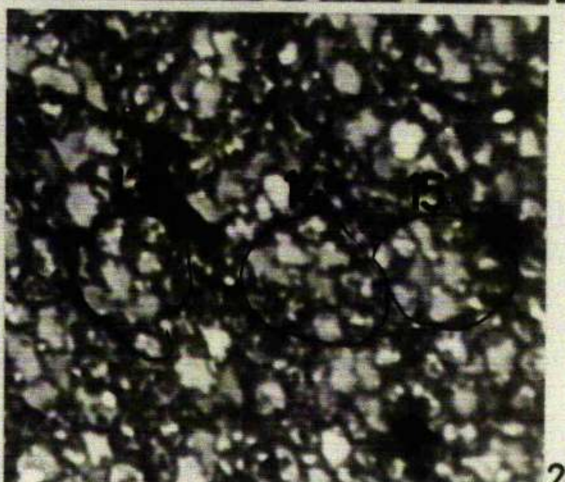
Figure 55

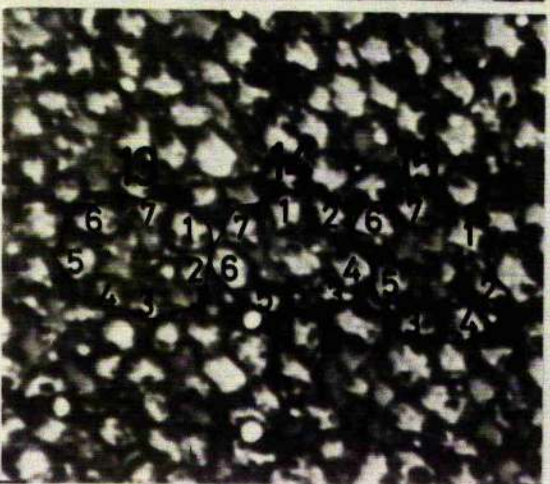
Trapezites A series of micrographs to show the passage of the axons of three cartridges (13, 14 and 15 from fig. 53), through the lamina. Section 187 is a part of the micrograph of fig. 53. The numbering of the short retinula axons is continued to section 258. The series shows the gradual clarification of the cartridges at deeper levels of the lamina and shows that these correspond to the same groups outlined in section 187. Section 306 shows the prominent axons leaving the lamina cartridges for the chiasma.


187


258


210


282


234

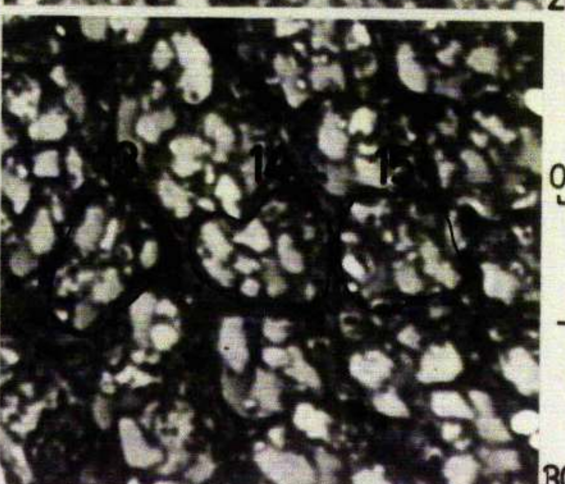
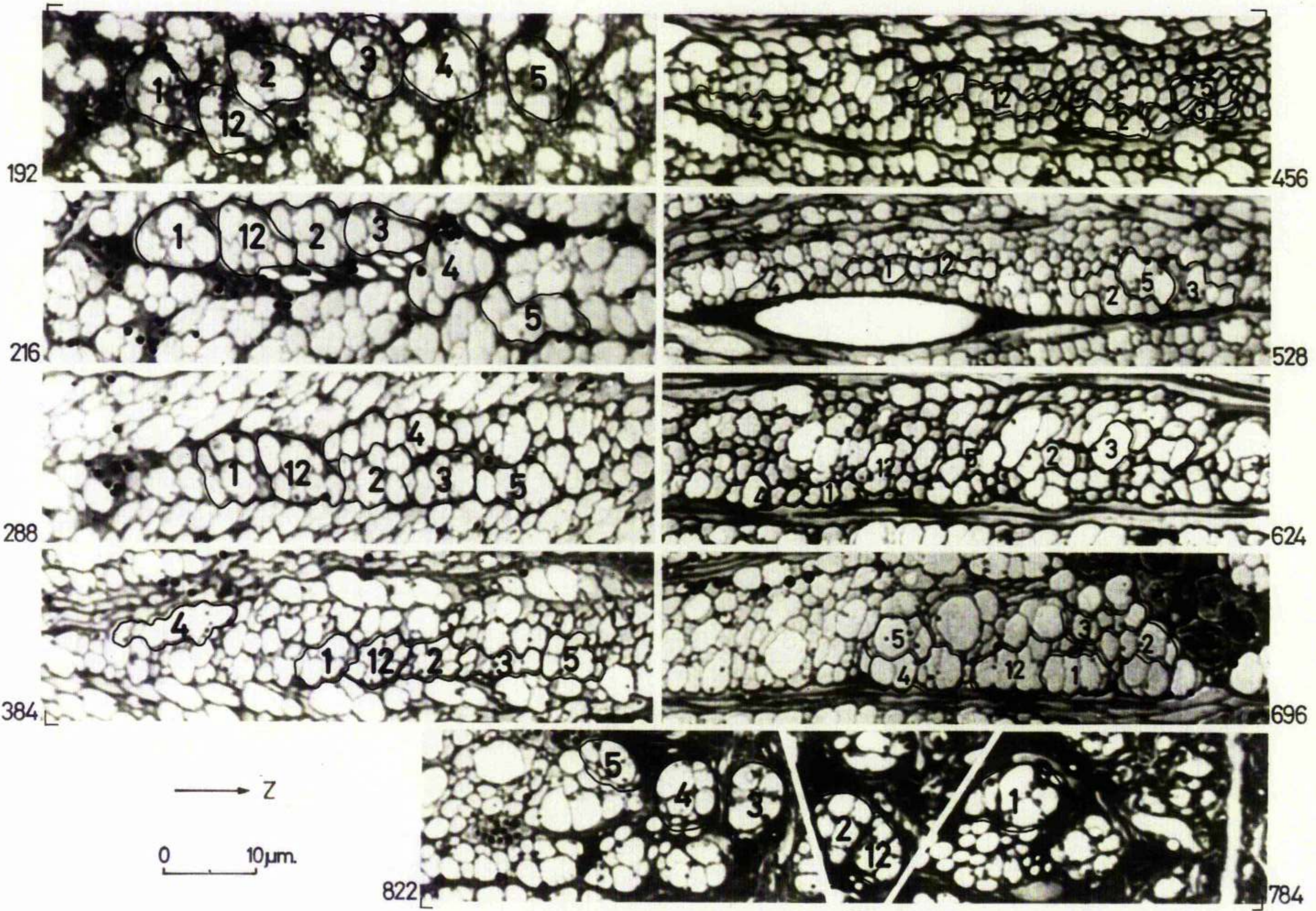

0 5
µm.
Z
306

Figure 56

Trapezites Micrographs of the lamina-medulla projection of the axons of six cartridges. Six axons contained within each profile on the overlay were traced from one lamina cartridge (section 192) to one medulla cartridge (sections 784 - 882). The axons are not individually labelled because their identity is not known. During their passage through the chiasma the axons of cartridge 12 enter the chiasmatal stratum of the other five cartridges (1 - 5) of the next row and the whole group inverts its horizontal sequence by an anticlockwise twist.


192
216
288
384
456
528
624
696
822
804
784
Z
0
10µm.

of axons 8 and 9 is more variable.

Fig. 55 shows the passage of three cartridges 13, 14 and 15 (from section 187, fig. 53) through the lamina. At progressively deeper levels the cartridges become more distinct until at the bottom of the lamina (section 306) they are composed of conspicuous bundles of at least six axons surrounded by a crown of indistinct retinula terminals (1 - 7). Axons 8 and 9 cannot be traced through the centre of the cartridge from top to bottom nor is the identity of the other axons of the cartridge known.

The lamina-medulla projection

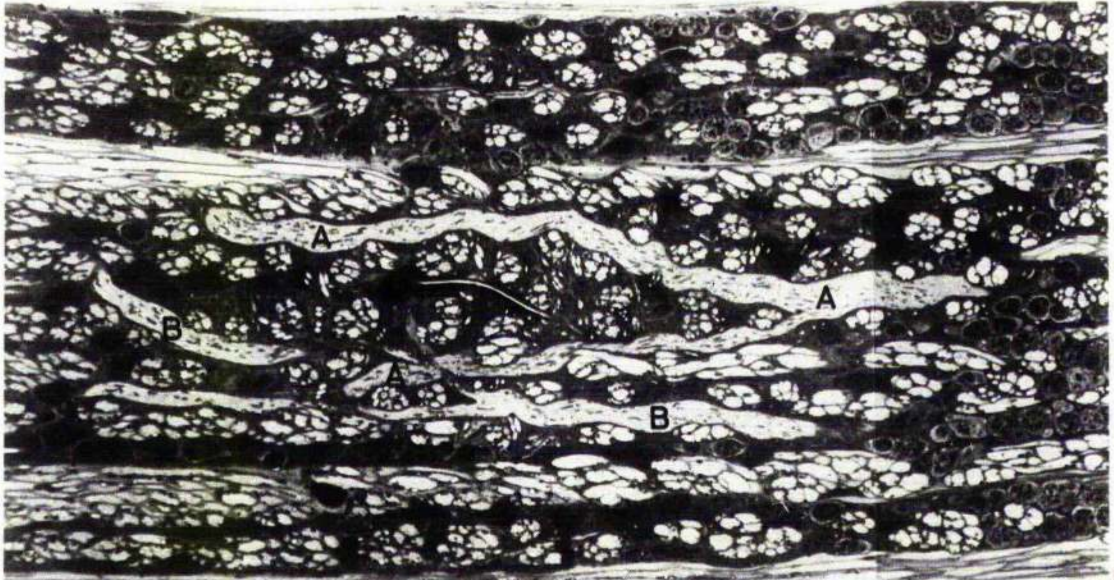
The passage of the axons of six cartridges through the very long chiasma to the medulla is shown in fig. 56 from the same series of sections used for the first projection. The cartridges are in two horizontal rows, the first containing cartridges 1 - 5, the second a single cartridge 12 beneath cartridge 2. The six cartridges invert their horizontal sequence by an anticlockwise twist before projecting upon the medulla. Six axons are traced in each group from a single cartridge but their identities are not known and consequently they are not individually labelled. The two finest axons of the six are difficult to follow above the medulla and only a few cartridges, in which all six axons could be traced, are represented from many that

Figure 57

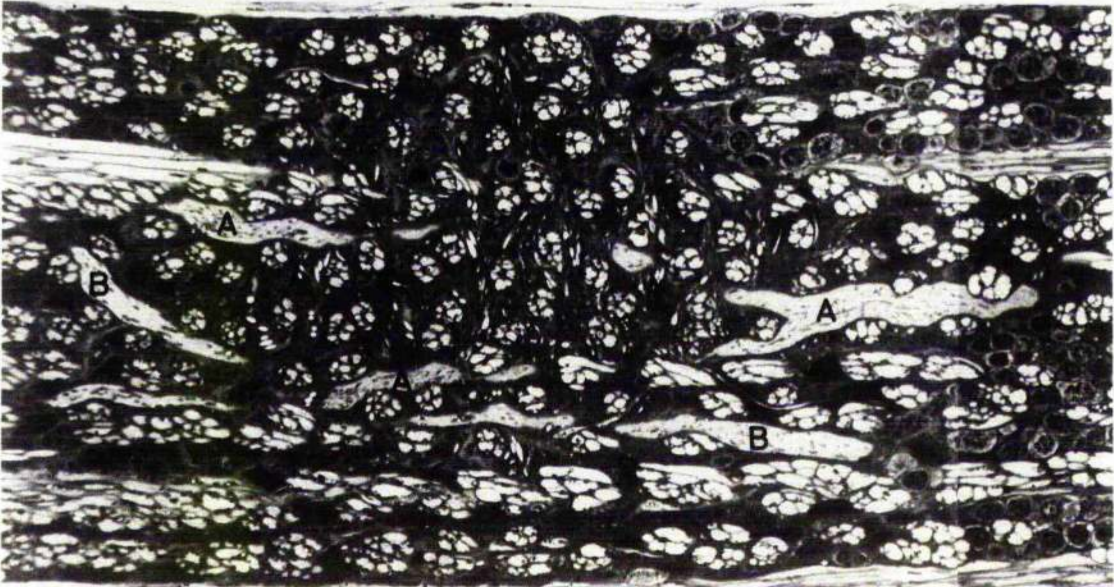
Trapezites Tangential neurones of the medulla, showing the unusually large processes of these neurones of this class in this animal.

a. Top: Micrographs from two sections (661 and 663) from the same area as the plan below showing the large size of the processes (10 µm diameter).

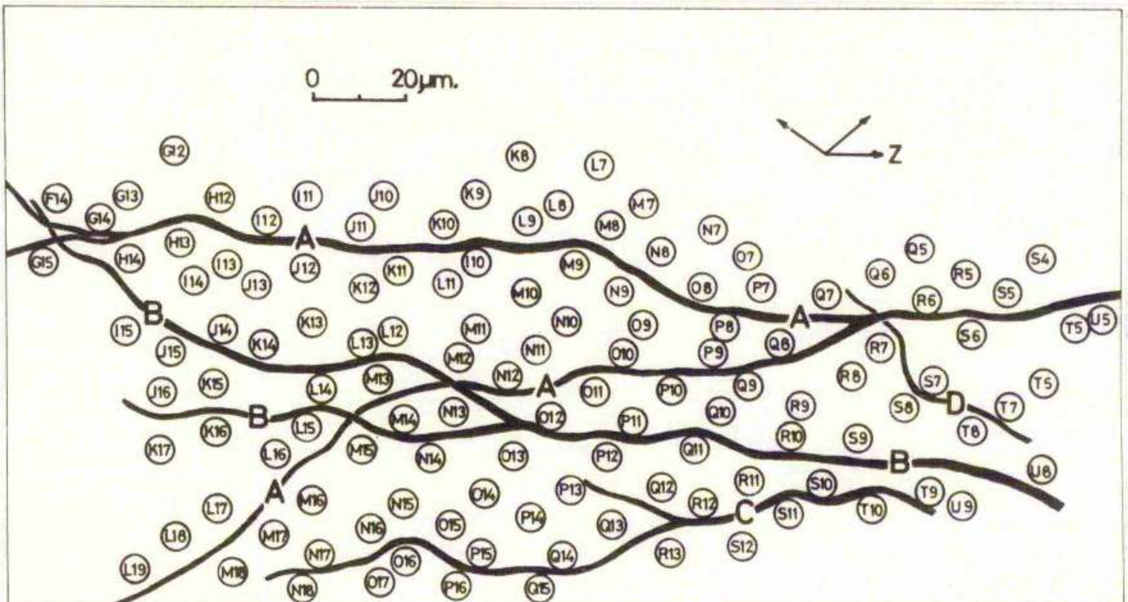
b. Bottom: Plan of the array of medulla cartridges with the paths of four processes (A - D) superimposed.



661



663



were followed incompletely. All six axons from one lamina cartridges contribute to a single cartridge of the medulla.

A conspicuous feature of the medulla of Trapezites is the very large tangential processes running superficially in the neuropile (fig. 57). They are the largest medulla processes seen in this work. Two micrographs of sections 2 µm apart show the profiles of four portions (A - D) of such neurones, which are plotted in a plan (fig. 57) for the area of micrograph available. The total extent of these processes is not known nor the number of neurones they represent. They ramify over the surface of the medulla amongst the cartridges apparently without a fixed pattern.

H. The dragonfly Aeschna

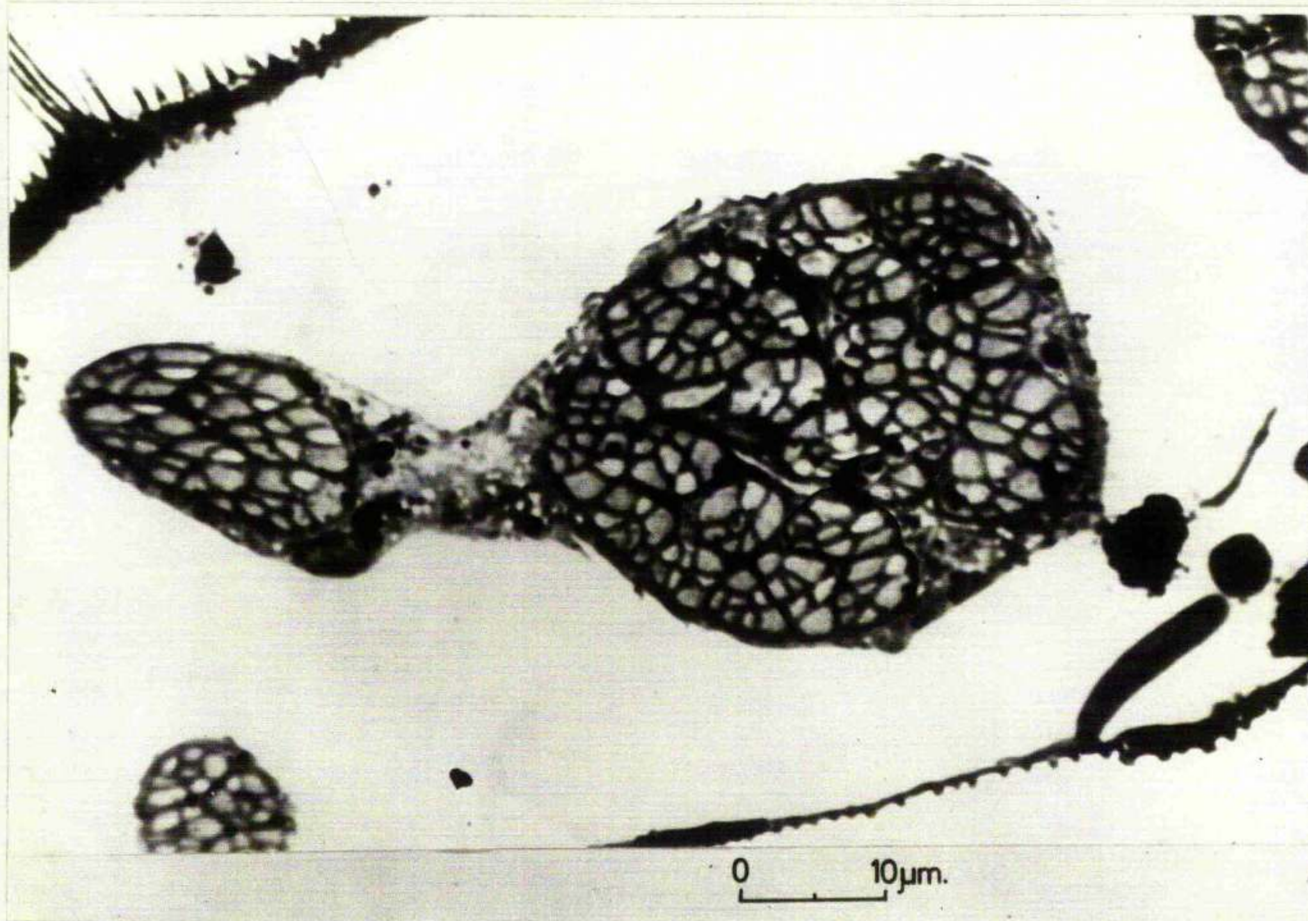
Some preliminary results are available for the retina-lamina projection of dragonfly ommatidia. Each ommatidium gives rise to a bundle of eight retinula axons but one of the eight axons of each bundle is so slender that it is hardly resolvable by light microscopy (fig. 58). The axon however can be seen unequivocally in electron micrographs (Eguchi, 1971). It has a minimum diameter of 0.3 µm, compared with 2 µm for the other seven axons.

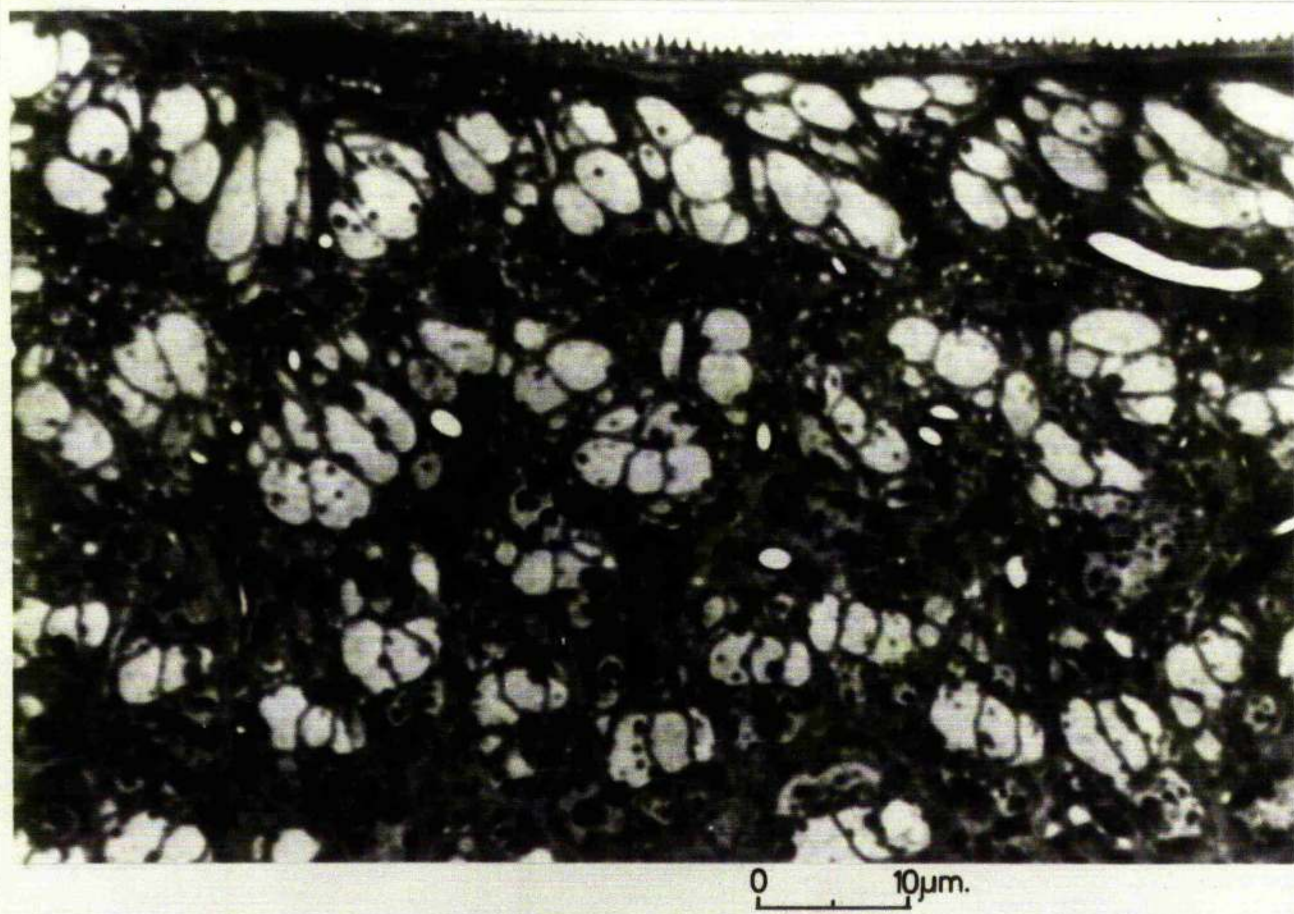
A few bundles of retinula axons have been followed to the lamina in Libellula and each bundle projects to a single

Figure 58

Libellula a. Top. Group of retinula axon bundles between the retina and lamina. Each ommatidial axon bundle is individually recognizable and consists of seven conspicuous axons and one other not obvious in light micrographs. Four such bundles are circled in the micrograph.

b. Bottom. Group of axon bundles at the base of the lamina. Three axons are conspicuous in each bundle and additional ones in those bundles (next to the trachea) that are cut at a deeper level. There seem to be about eight axons visible in each bundle.


0
10µm.


0
10µm.

cartridge. The axons stay together until they reach the lamina where they descend through the ganglion cell layer spreading around the two large cell bodies of the monopolar neurones of their own cartridge. Each monopolar cell body connects perpendicularly through a wide isthmus to its axon (3 - 4 μm diameter). At the bottom of the lamina a group of approximately eight axons is visible emerging from each cartridge (fig. 58) but their identities are not known.

DISCUSSION

A. The projection of the retina upon the medulla

In the following section the projection patterns described earlier will be discussed and where possible the identity of the axons traced will be given. The overall patterns of neural projection in the various insects are remarkably uniform but, until this work, were (with the exception of the first projection of the fly optic lobe) completely unknown. It is necessary however to discuss briefly the quality of evidence upon which the results are based, because of the existence of several contradictory accounts, notably those of Strausfeld (1970), which appeared whilst this work was in progress.

The most decisive factor in the quality of the evidence is the tracing technique itself. There is always a small chance that the profiles of axons are carried over inaccurately between two micrographs, but there is one good reason for discounting this criticism. Where the profiles were identified incorrectly between consecutive micrographs then the overall projection patterns would lack order, because although the axons of one ommatidium (or lamina cartridge) generally stay together they are not separated from those of other ommatidia (or cartridges). Since, especially in the chiasma, bundles of axons with neighbouring positions

at one level often come to have widely separated final positions, mistakes in tracing at this more peripheral level would tend to destroy the order which is in fact observed. Although it is impossible to present sufficient micrographs by which others may assess the reliability of this procedure, an effort to do this has been made in fig. 14, for part of the fly first projection.

The second main factor in the quality of the evidence presented here is the number of axons traced. Most of the projections described are based upon results from the axons of twenty or more ommatidia or cartridges, and many upon results from more than one series. Nevertheless the final number of eyes examined is extremely small and of these only a minute proportion of their neurones is sampled, although within that population the method is total. By comparison, the projection patterns described by Strausfeld are based upon studies of far larger numbers of animals but by methods which involve specific and sometimes selective impregnation which are taken to be less reliable. Thus providing the neurones studied are representative, it is believed that the results presented here are intrinsically more reliable.

The retina-lamina projection

In all cases so far examined the visual field of the retina is exactly duplicated in the lamina and is represented

by an array of cartridges with angular sensitivities determined by the rhabdome(re) admission function of the receptors. In fused-rhabdomere eyes the angular difference between cartridges is determined by the interommatidial angular separation of rhabdome optical axes, while in open-rhabdomere eyes the angular separation between cartridges is determined by the divergence angle between the optical axes of neighbouring intraommatidial rhabdomeres. In the fly it happens that this interrhabdomere angle is equivalent to the interommatidial angle. In the case of the water-bug there is no evidence for cartridge angular sensitivity but some mixing between retinula inputs of different optical axes may occur during light-adaptation (see Discussion, p.). It is assumed that short retinula axon terminals make synaptic contact with a group of lamina monopolar neurones, as has been shown to occur between retinula terminals and the central monopolar neurones in fly and worker bee.

Two very early observations on the retina-lamina projections of fused-rhabdomere ommatidia of the worker bee (Phillips, 1905) and the lepidopteran, Adela (Johnas, 1911) anticipate this work. Both authors report that there appeared to be bundles of retinula axons from single ommatidia travelling to the lamina without intermingling with other bundles, but neither author traced these axons in sections between the two regions.

The projection patterns described here for the fly and locust confirm electrophysiological recordings from second-order units of their laminae. In the fly, neural convergence first described morphologically by Trujillo-Cenóz (1966) and Braitenberg (1967) was later demonstrated by Scholes (1969) from records of lamina units giving depolarizations. These units have subsequently been shown by Järvilehto and Zettler (1970) to be summed presynaptic responses from the retinula terminals of one cartridge, whereas postsynaptic recordings from the axons of lamina monopolar neurones (almost certainly L1 or L2) show hyperpolarizing responses (Autrum et al., 1970), with narrow but as yet unmeasured fields (Ioannides, personal communication). Hyperpolarizations have also been recorded from locust lamina (Shaw, 1968) with angular sensitivities equivalent to that of the acceptance angle of one ommatidium. These responses were predicted on electrophysiological evidence to be from a post-synaptic (monopolar ?) neurone at the lamina and the ommatidial projection reported here supports this interpretation.

One observation reported, which is at variance with the projection, generalized for fused-rhabdomere eyes, of one ommatidium upon one lamina cartridge is to be found in Strausfeld and Blest (1970). In *Pieris brassicae* these authors report four axons from one ommatidium going to one lamina cartridge, while another two entering that cartridge

are derived from nearby ommatidia. In view of these authors' own caution in the interpretation of their preparations there seems little doubt that the projection of Pieris is like that of all fused-rhabdomere eyes described by Horridge and Meinertzhagen (1970 b); species differences are possible but seem unlikely. In locust Golgi preparations it has been observed that one of the short retinula terminals also diverges to a neighbouring cartridge (Horridge, personal communication). These two observations illustrate the hazards in following axons individually and unambiguously in longitudinal sections, even in Golgi preparations with only a few stained elements; the local divergences and lateral displacements as the axon tracts move around tracheae etc. can give the impression that the projection is not homotopic.

One other account (Ninomiya, Tominaga and Kuwabara, 1969) mentions the retina-lamina projection in the damselflies, Ishunura and Cersion in which the retinula axons are said to interweave in an unspecified pattern before reaching the lamina, but it is extremely doubtful whether this can be stated reliably from the random electron micrographs which seem to form the basis of their evidence. Preliminary observations on the related libellulid dragonfly (see Results) do not reveal a diverging projection of the retinula axons. For the same reasons the description of interweaving amongst

retinula axons of the lobster optic lobe (Hámori and Horridge, 1966 a) must be held in doubt until these axons have been properly traced through their entire length.

In summary, the precedent is now established for a variety of insects with fused-rhabdomere eyes that the visual field is exactly projected upon the lamina. The quality of existing evidence which is at variance with this description is in general poorer than that presented here and evidence of this sort which may be presented in the future cannot be accepted without question. In particular it is inadequate to describe the projection patterns of retinula axons from random electron micrographs and probably inadequate in most cases to do so from silver stained preparations. Nonetheless, differing projection patterns must be anticipated in other animals until a wider sample of eyes has been examined, and the generalization ought ideally to be extended to include the eyes of Crustacea.

1. The retinula cell composition of the ommatidium

The ommatidial composition of the insects studied in this work may be summarized as follows (Tables 3 and 4). Open-rhabdomere ommatidia e.g. fly and water-bug, have six peripheral and two central retinula cells. The two central cells have rhabdomeres with orthogonal microvilli and have long retinula axons in fly and probably also in water-bug.

Table 3

The long visual fibres and their cells of origin.

Insect	Retinula Cells of Origin	Axon diameter (at specified level)	Axon length	Twist in chiasmal strata (lamina to medulla)	Retinula cell length	Rhabdo
Calliphora	7 + 8	1 µm (lamina), 0.6 µm (bottom of chiasma)	475 µm	Clockwise in one half; anticlockwise in other. Dorsal left ≡ ventral right	7; top two thirds of ommatidium 8; bottom third of ommatidium	7; ve 8; ho
Notonecta	not known	not known	not known	Both clockwise and anticlockwise in one eye		
Lethocerus	possibly 7 and 8	8; 4 µm 7; 8 µm (both above lamina)	>1 mm		7 and 8 extend full length of ommatidium	7; ap 8; ap
Apis (drone)	Two of 7, 8 and 9. Most likely 7 and 8.	1 µm (lamina) 0.5 µm (bottom of chiasma)	600 µm	Both clockwise and anticlockwise in one eye	7 and 8; full length of ommatidium 9; basal only	7 and plar both neig 9; m of t neig
Trapezites	possibly 8 and 9	Both axons extremely slender in lamina (approx. 0.5 µm)	1 mm	Only one twist (anticlockwise) observed	8; full length of rhabdome 9; basal only	not kn
Pieris	Two of 5, 7, 9; most likely 5, 7	9; 0.5 µm 5, 7; 1 µm (at top of lamina)	not known	not known	5 and 7; top half of rhabdome 9; basal only	not kn

The fused-rhabdomere ommatidia have large retinula cells and either or both small retinula cells and basal cells. The rhabdome is contributed mainly by the large retinula cells (usually six or more) with smaller contributions from the less numerous small retinula cells (one or two) and basal cells (one or two). The two small retinula cells are cells 7 and 8 (drone bee) and their equivalents (worker bee) and cell 3 (locust). There is a single basal cell 9 in drone bee, *Pieris* and the skipper. The arrangement in tiered retinae (*Pieris* and the dragonfly) is more complicated; in *Pieris* cells 5 and 7 of the top tier of four cells (5 - 8) are smaller than cells 6 and 8. Most likely, in the bee and *Pieris* the two small retinula cells 7, 8 and 5, 7 respectively have long visual fibres that go to the medulla. This summary is not meant to be a definitive classification but presents baldly the picture that emerges from the discussion to follow.

2. The long visual fibres

In general, this work suggests that all insect ommatidia have a pair of long visual fibres which belong either to the small basal or central retinula cells (Table 3), but this arrangement is known definitely only for fly. For the other insects the following is known.

In the case of the water-bug the central cells 7 and 8 most likely give rise to long visual fibres (see Results),

and though the cells are represented throughout the full length of the ommatidium their position is clearly analogous to that of the two central cells of the fly ommatidium.

In Pieris the long visual fibres are from two or three of cells 5, 7 and 9. Of these three only cell 9 is a basal cell whereas cells 5 and 7, in common with their partners 6 and 8, have rhabdomeric microvilli in the distal part of the ommatidium. Although axons of all three cells have been traced through the lamina their courses are not absolutely certain and quite possibly only two of the cells have long visual fibres as reported by Strausfeld and Blest (1970) who saw in reduced-silver preparations only pairs of such axons in the chiasma. However the fact they describe three types of long visual fibre from Golgi preparations with each observed pair consisting of some of the possible combinations of the three types raises three possibilities. Either (1) there are three long visual fibres from all ommatidia but only pairs have ever been seen in reduced-silver preparations, or (2) the same two cells in each ommatidium have long visual fibres and the three forms of ending described by Strausfeld and Blest cannot be meaningfully related to cell of origin; or (3) there is variation between ommatidia as to which two of three cells have long visual fibres. At the present the second alternative seems most likely, i.e. that there are pairs of long visual fibres and

the shapes of terminals as revealed by Golgi impregnation are not meaningfully related to cell of origin. In support of this idea, Strausfeld (1970) reports that in dipterans the same combinations of long visual fibre types are seen in Golgi preparations as in Pieris, and in flies it is known definitely that there are only two such axons. In addition there is evidence that cell 9 in Pieris has a short retinula axon (see p. 174).

This is a typical dilemma; Golgi impregnation distinguishes neuronal silhouettes with a subtlety not attainable by other methods and supplementary Golgi/EM studies are required to correlate these silhouettes with their cells of origin before the finer classifications of a Golgi study become meaningful. Mercifully, Cajal and Sánchez (1915) describe only one type of long visual fibre in worker bee.

The problem in drone bee is to know which two of three cells (i.e. 7, 8 and 9) have long visual fibres. At the moment the evidence rests on which one of the three has a short retinula axon and reasons are presented in the next section (p. 173) for the belief that this is cell 9.

The long visual fibres are not known for either the skipper or locust. In the case of the skipper no axons could be traced through the lamina although apparently only the fine retinula axons 8 and 9, at the centre of the

cartridge, enter the chiasma. One of these probably originates from the basal cell 9, suggesting that the basal cell and the cell giving rise to axon 8 both have long visual fibres.

In locust, there is no direct evidence to suggest which cells have long visual fibres. At the moment one type of long visual fibre is shown incompletely impregnated in Golgi preparations (Horridge, 1968 b) and this has a smaller diameter than the short retinula axons. The fine calibre axons of cells 3, 7 and 8 suggests that these include the long visual fibre(s) while the preferred positions of axons 7 and 8 at the centre of the cartridge is reminiscent of the arrangement of long visual fibres in cartridges of other fused-rhabdomere eyes. The identification of the cells of origin of these three axons requires some reinterpretation of the accounts of Horridge and Barnard (1965) and Horridge (1966). Axons 7 and 8 arise from the two cells described by Horridge and Barnard (1965) as eccentric cells. These cells have the following characteristics: positions in the ventral and dorsal quadrants of the ommatidium respectively; nuclei in the basal third of the ommatidium; presence in the basal half of the ommatidium only; they are thus properly called basal cells. Axon 3 arises from another cell described by Horridge in fig. 9 of his 1966 paper as a basal cell. Apparently it is distinguishable

from other long retinula cells only by its smaller diameter cell and axon and its position in the posterior quadrant of the ommatidium. The presence and extent of the contribution of each of the three cells to the rhabdome is still not clear; none has more than a few rhabdomeric microvilli and some cells may have none.

With the exception of the fly, the long visual fibres in the various insects studied here lie at the centre of or within the compass of their own cartridge. The satallite position of long visual fibres in fly is well known but Strausfeld also describe a similar arrangement for Pieris (Strausfeld and Blest, 1970; Strausfeld, 1970) which is at variance with the results presented in this work. Apart from species differences, there is no obvious reason why different results have been obtained.

Only in larval Aeschna (Zawarzin, 1913) and in various rhynchotan bugs (Pflugfelder, 1937) are the long visual fibres not reported. In addition this type of neurone was not seen in the dragonflies Libellula and Agrion (Cajal and Sánchez, 1915). These improbable but much quoted ommissions cannot be taken at face value without further studies. Though there is evidence from Golgi impregnation (Horridge, personal communication) that this class of neurone is in fact present in Notonecta, no observations have been reported for dragonfly since the original descriptions.

Table 4

The short retinula axons and their cells of origin.

Insect	Retinula cells of origin	Axon diameter (at specified level)	Axon length (basement membrane to lamina)	Direction of twist (retina to lamina)	Preservation of rotational sequence	Length of retinula cell of origin	Rhabdomere microvilli orientation
Calliphora	1 - 6	2 µm (above lamina)	120 µm	Clockwise in one half; anticlockwise in other. Dorsal right ≡ ventral left.	good	Length of ommatidium	Three classes with approximate orientation along horizontal (2, 5) x (1, 4) and y (3, 6) axes.
Lethocerus	most likely 1 - 6	4 µm (200 µm beneath basement membrane)	>1 mm	not known	not known	Length of ommatidium	Two orthogonal classes (1, 3, 4, 6 and 2, 5) approximately horizontal and vertically respectively.
Schistocerca	possibly 1, 2, 4, 5, 6 and one other	2.5 µm 1 µm (both 50 µm below basement membrane)	330 µm	none	fairly good	Length of ommatidium	Three classes, each at 120° to the next. a. 1, 8; b. 7, 6, 4; c. 2, 5. Microvilli of cell 3 indistinct.
Apis (drone)	1 - 6 and one other of 7, 8 and 9	3 µm (sometimes 2, 3, 5 and 6 smaller, 2 µm) 0.7 - 1 µm (both 40 µm below basement membrane)	140 µm	Clockwise and anticlockwise. Variable extent 180° - 360°. No apparent pattern.	good	Length of ommatidium	Two classes 1, 2, 4, 5 and 3, 6 with approximately orthogonal microvilli, but absolute orientation variable.
Pieris	1 - 4, 6, 8 and probably one of 5, 7, 9	Variable 1.5 - 3 µm (6 + 8 larger) 0.5 - 1 µm	125 µm	Variable; can be both directions in one bundle.	good	1 - 4; length of ommatidium but major contribution to rhabdome in basal half only. 6, 8; length of ommatidium but no rhabdomere in basal half.	Two orthogonal classes (1, 3, 6 and 2, 4, 8) 45° to vertical and horizontal.
Trapezites	possibly 1 - 7	2 µm (above lamina)	125 µm	none	good	Length of rhabdome	not known

3. The short retinula axons

In general the long retinula cells have short axons that terminate in the lamina (Table 4). This is certainly well established for fly and requires no further comment for this group.

In bee, this work shows the generalization to be true for cells 1 - 6, but one of the cells 7, 8 and 9 must also have a short axon since only two have long visual fibres entering the chiasma. The lamina terminals of the short retinula axons of worker bee have already been classified into two types (Cajal and Sánchez, 1915 ; see Introduction, p. 42). These are the short lamina terminals which end in the centre of the middle lamina zone and the long lamina terminals which end at the bottom of the middle lamina zone. The long lamina terminals are presumably either from the one of the cells 7, 8 or 9 which has a short retinula axon; or from cells 1 and 4. In favour of the former alternative, Cajal draws these terminals in worker bee (Cajal and Sánchez, 1915 fig. 15A) arising from axons as thin as the long visual fibres, while in favour of the latter, axons 1 and 4 are distinguishable down to a deeper level in the lamina (see Results). The first alternative is probably based on the best evidence, since the micrographs used here do not show the position of the terminal brush itself, but only the depth to which the main axon trunk may be followed.

In the water-bug there is no additional evidence for the identity of the cells of origin of short retinula axons than that given for the long visual fibres (p. 193). If the central cells give rise to the pairs of central axons in the lamina cartridges, which are long visual fibres, then the remaining six axons are those of cells 1 - 6. The division of the six into two groups of three is appropriate for such an interpretation.

In *Pieris*, short retinula axons come from cells 1 - 4, 6 and 8. Strausfeld and Blest (1970) describe three types of terminal; combinations of these three types occur in groups in the lamina, probably one group in each cartridge. The types distinguished have axons of two diameters; type 2 has a slender axon 0.6 μm in diameter, which could correspond only to axon 9 seen in this work (see Table 4), i.e. the long visual fibres in *Pieris* must be from cells 5 and 7 (see p. 169). Types 1 and 3 have axons of the same diameter but are distinguishable on the basis of their lateral processes. Since Strausfeld and Blest (1970) describe two type 3 and three or four type 1 endings occurring in a group, it might be anticipated for reasons of symmetry that the type 3 endings are of the remaining two cells of the distal quartet (i.e. 6 and 8), while the type 1 endings belong to the basal quartet (cells 1 - 4). All the retinula terminals are in fact very similar and the only reason that

their cell of origin needs to be known is that at the outer margin of the lamina neuropile the type 3 endings have two lateral dendrites long enough (3 - 7 μm) to interact with neighbouring cartridges.

No more evidence is available for the identity of the cells of origin of the short retinula axons of the ommatidia of the skipper or locust than that presented for the long visual fibres of these animals. Further work is necessary to clarify these points.

The lamina-medulla projection

In those eyes in which the projection of some of the perpendicular elements has successfully been traced between lamina and medulla, these axons of one lamina cartridge all enter a single cartridge of the medulla and no exceptions have been found. This is known for six neurones of the lamina cartridge of fly, the skipper and *Notonecta*, and for seven of the lamina cartridge neurones of drone bee (see Table 1). These numbers of axons do not represent all the perpendicular neurones crossing the chiasma since electron micrographs at the base of the lamina cartridges reveal other fine axons that cannot be followed by light microscopy. In the skipper and *Notonecta* the groups of axons traced may include the long visual fibres but this is not known certainly. However, in the fly and bee the groups of axons traced are known to include the pair of

long visual fibres from each ommatidium. Thus for the fly and bee at least, activity derived from a single visual axis is, in the first instance, carried by perpendicular neurones to one medulla cartridge i.e. the visual field of the retina is projected exactly across the array of medulla cartridges (Horridge and Meinertzhagen, 1970 b).

This account of the activity arriving at a single medulla cartridge is only a first approximation, for activity may be modified by other forms of interaction. Firstly, lateral dendrites of lamina monopolar neurones may have synaptic contact with the elements of neighbouring cartridges. Secondly, recurrent activity from presumed centrifugal neurones, which may be derived from the amacrines of the medulla, could modify activity in the lamina cartridge. Thirdly, amacrine neurones of the lamina probably have connections with adjacent cartridges.

For all these four insects the axons cross in the chiasma so that the horizontal lamina sequence is projected on to the medulla in exactly reversed order. The sequence of horizontal cartridges is reestablished in the medulla as it was in the lamina and there is no cross-over in the vertical plane. Consequently the ommatidial lattice is repeated in the lamina and, through the chiasma, in the medulla. The uncrossed projection of chiasma fibres in the intermediate regions of the eye, originally observed by Cajal and

Sánchez (1915), has been sought in this work but not found and, with the exception of some lamina aperiodic tangential neurones in dipterans, they have not been seen by other workers (see Introduction). Although it is extremely difficult to rule out the existence of small areas of uncrossed axons in the chiasma they almost certainly do not exist for the small field elements studied in this work. Thus the proposal of Trujillo-Cenóz (1969) that the intermediate optic tract (the central region of the horizontal width of the chiasma)might be the region of the uncrossed axons may be discounted,as may be the pattern of medulla excitation that would result from such a projection in response to objects moving across the visual field (Braitenberg, 1968).

There are two observations at variance with the lamina-medulla projections found this work. Firstly, in Calliphora, Strausfeld (1970) describes medulla cartridges (or columns), as seen in reduced silver-stained preparations, with a width half that of the lamina cartridges. More reliable toluidine blue stained plastic sections however fail to reveal such an organization, the unit structure of the medulla having a repeat period the same as that of the lamina. In addition the large monopolar axons L1 and L2 which are easy to follow through the chiasma are never observed to separate by 10 μm or so as described by Strausfeld; they always travel as a pair into one quadrant of their medulla cartridge. This

result is also found by Trujillo-Cenóz (1969) from combined light and electron microscopy. At the moment there seems to be no way in which the results may be reconciled but there remains the strong possibility of false observations from reduced silver-stained preparations.

The second point of variance described by Strausfeld (1970) in Pieris and also possibly in Sphinx, has greater functional consequences. It concerns the reported divergence between the pairs of long visual fibres and of monopolar axons from the same lamina cartridge, which go to different medulla cartridges. Since the projection of perpendicular neurones through the chiasma in Pieris has not been studied in this work because the axons are too fine to trace in P. rapae, either the lamina-medulla projections are different in Pieris and other fused-rhabdomere eyes, or the chiasmata of all fused-rhabdomere eyes contain both types of projection, or what is perhaps most likely one of the observations is at fault. The methods used in this work have produced more reliable results in the studies on first optic projections than the few that have been obtained by metallic impregnation but the results obtained here for the second projections are probably not quite as reliable because the axons are generally finer and travel greater distances. The methods adopted here ought intrinsically to be more reliable in plotting the spatial distribution of axons between neuropile

layers, but Strausfeld's micrograph (Strausfeld, 1970 fig. 110) seems convincing. Of course, it is still possible that the displacement of lateral relationships between long visual and lamina monopolar axons, which in Sphinx takes place very close to the surface of the medulla, could occur in the bee in the superficial layers of medulla neuropile, although diverging pathways of this sort would probably have been observed by Cajal in Golgi preparations.

The identification of the axons which have been followed through the chiasma to the medulla in the four insects mentioned above is not complete. Most information is available for the lamina monopolar neurones of fly.

The correlation between monopolar neurones known from Golgi impregnation (Strausfeld, 1970) and reduced silver (Braitenberg, 1967; Strausfeld and Braitenberg, 1970) has not yet been made but is to be the subject of a forthcoming publication (Strausfeld, personal communication). At present, L4 seems to correspond most closely to the bistratified midget monopolar (Strausfeld's type M:m); L1 and L2 are usually depicted as either diffuse bilateral or radial monopolar neurones, and as the cell body of L2 is lower than that of L1, possibly L1 is Strausfeld's type M2:1 and L2 his type M2:2 (Strausfeld, 1970, fig. 34); the identity of L3 remains uncertain. The identity of a fifth monopolar neurone with a peripheral position in the cartridge (Trujillo-Cenóz and

Melamed, 1970) is not known nor, apart from differences between species, is the reason for the different numbers of monopolar neurones found by these workers. It is important however to bear in mind the observations of Cajal and Sanchez (1915) from reduced silver preparations that the composition of the cartridges varies in a consistent way across the lamina. These variations may be in addition to those at the lamina edge where cartridges with reduced numbers of short retinula axon terminals occur (see Results). One of the axons to be traced through the chiasma in fly is probably that of a medulla centrifugal neurone (see Results). It is perhaps not surprising that its cell body linking fibre has not been observed because such a fine process cut in longitudinal section would not easily be resolved and would rapidly move out of the field of view. There is some evidence that the comparable T-junction has been seen in drone bee (p. 44).

In the drone bee only the identity of the long visual fibres and of the three large calibre monopolar axons is known although none of these five axons is uniquely identifiable. There are three candidates for the cell of origin of the two long visual fibres (p. 36); while the types of each of the three monopolar neurones cannot be predicted from the positions of their axons in the cartridge. The two other axons traced may be either additional monopolars or medulla centrifugal axons or one of each, but one is probably that

of a T-shaped centrifugal neurone (p. 44). The other axon is most likely that of an additional monopolar neurone because it has a large diameter in the chiasma (the fourth large calibre axon of each bundle); if so then it is most likely to be one of the small monopolars described by Cajal and Sánchez (1915) with wide-field lateral dendrites in the lower zone of lamina neuropile and a fine axon to a cell body in the lamina ganglion cell layer (e.g. fig. 10 c, d of their paper) because in toluidine blue stained sections the axon is visible only in the lower zone of the lamina. The other prominent neurone with a single straight axon that may have been traced through the lamina is another centrifugal ending (Cajal and Sánchez, 1915 fig. 20 c) with a wide-field plexus of processes in the upper zone of lamina neuropile, but the axon that traverses the lamina neuropile is so fine that it is doubtful if it would be conspicuous in toluidine blue stained sections.

In Notonecta and Trapezites the identities of the six axons in each bundle traced are not available. Since in other insects the lamina monopolar neurones have the largest calibre axons, presumably a representative sample of the axons of this type of neurone has been followed. Further work including a thorough Golgi study together with Golgi/EM correlations of appropriate cell types will be necessary to establish which neurones have been studied. Nevertheless

the results obtained suggest that similar projection patterns exist in these two insects as in fly and bee.

Thus the overall generalization that arises from this work is that the projection of the visual field on the medulla through perpendicular neurones of the lamina is the same for both open- and fused-rhabdomere eyes. It is quite obvious from the preceding discussion that much more information from a greater range of animals is necessary before this important principle can become firmly established.

B. Functional consequences of the projection patterns
Visual information available at the medulla cartridges

The visual inputs to the medulla cartridges are provided largely by the long visual fibres and the lamina monopolar terminals. Of the activity of long visual and lamina monopolar neurones generally only that directly derived from their retinal inputs is known or inferrable, whilst modification of this activity from centrifugal or amacrine connections or from lateral monopolar connections within the lamina is quite unknown. The projection of the various types of lamina tangential neurone upon the medulla is not known in detail nor is the sensitivity of these units.

An early suggestion that the locust basal cells may have spike responses (Scholes, 1965) is attractive for the following reason. The combination of large lengths and

small diameters found in these axons would attenuate retinal electronic potentials severely before they reach the medulla. If the basal cells have long visual fibres and if these have spiking responses then the problem of signal attenuation (particularly at low light intensities) would be overcome. The following objections are however applicable to the idea. There is no evidence that electrotonically conducted retinal responses are inadequate to convey information through these axons; it is merely that, assuming the same electrophysiological constants to exist in long visual fibres as in short retinula axons, the attenuation would be more severe. Examples of the attenuation actually observed suggest that there is some margin of safety e.g. attenuation by approximately 50% is seen in the retinal responses in water-bug retinula axons when recorded at sites at least 2 mm central to the retina (Ioannides, personal communication). Shaw (1968 a) proposed an alternative mechanism for the origin of retinal spike responses, that they arose from retinula cells with injured axons. In addition, in the fly it is known that the central retinula cells do not have spiking responses (Scholes, 1969), whilst in the water-bug the axon of the large central cell 7 does not have spiking responses in or just distal to the lamina (Ioannides, personal communication). In both these animals, however, the central cells have well-developed rhabdomeres,

unlike the basal cells of locust which have little or none, and which presumably therefore do not generate large responses of their own.

The sensitivity of the long visual fibres is known only for the fly where the separation of the rhabdomeres of cells 7 and 8 has greatly facilitated analyses of the properties of these cells. From work on Drosophila (Stephens, Fingerman and Brown, 1953) and Sarcophaga (Wellington, 1953) it is known that some dipterans are able to perceive the plane of polarization of light and the central cells 7 and 8 are favourably situated to act as a polarization-plane analyser (Melamed and Trujillo-Cenóz, 1968) because their rhabdomeric microvilli are arranged in orthogonal planes. The observation, however, that the E-vector maximally absorbed in cell 7 is perpendicular and not parallel to the axes of the microvilli as expected (Kirschfeld, 1969), requires that one must be cautious in predicting polarization sensitivity for these (or any other) cells, on morphological criteria.

Spectral sensitivity also is known for fly photoreceptors (Burkhardt, 1962). All cells are sensitive in the UV but are separable by their sensitivity to visible wavelengths. Three types of spectral sensitivity curve are described with peaks at the following wavelengths, green (486 nm), blue (470 nm) and yellow-green (521 nm), in the ratios 5 : 1 : 1. The types are not well separated and intermediates

exist. Spectral absorption measurements of single rhabdomeres in retinal slices indicate that the rhabdomeres of the peripheral retinula cells 1 - 6 absorb maximally in green wavelengths (Burkhardt's most frequently found type), whilst the central rhabdome of cells 7 and 8 absorbs maximally in blue wavelengths. Possibly the rhabdomere of cell 7 (the major part of the central rhabdome) absorbs maximally in blue, while that of cell 8 may have a different peak absorption. It is difficult to imagine Burkhardt sampling from cells 7 and 8 with the same probability as from cells 1 - 6 by intracellular methods, so that the location of all the spectral types (apart from green-sensitive ones) still remains obscure and the possibility of intraommatidial variation exists. Since the inputs from cells 1 - 6 are mixed at the first synapse (Scholes, 1969; Autrum et al., 1970) the spectral sensitivity of monopolar cell axons is presumably also green and in the medulla information on the colour of incident light may be obtained by comparison between the long visual and monopolar axons or between long visual fibres alone if their spectral sensitivities differ.

The lamina monopolar cell input responses are known from one account only in fly (Autrum et al., 1970). Although the cell was stained intracellularly by dye injection, Autrum does not attempt to identify its type. Its appearance corresponds most closely to L1 or L2; it

might possibly be L3 but it is very unlikely that the fine axon of L4 could be penetrated in the lamina. The responses consist of two components, a hyperpolarization and a depolarization. The hyperpolarization is considered to be an active postsynaptic response which decays with time after penetration. It shows bumps at low light intensities which are interpreted as quantal transmitter release. The depolarization which is not labile is interpreted as electrotonic spead from the retinula terminals. If this interpretation is correct only hyperpolarizations are conducted to the medulla. The responses should be maximally sensitive to green wavelengths but be insensitive to the plane of polarization as a result of the mixing of the retinula inputs with differing maximal sensitivities to polarized light (Scholes, 1969).

The lamina monopolar responses of fly lend support to the interpretation of responses previously recorded from the locust lamina (Shaw, 1968 b) suggesting that these responses too are those of monopolar neurones. The responses are in any case identified as a second-order because of the short latency between the mean times of receptor depolarizations and of onset of lamina hyperpolarizations, at equivalent light intensities. The lamina responses show either no or a very poor polarization sensitivity, consistent with the convergence of inputs from

retinula cells with well developed but differing planes of maximal sensitivity to polarized light (Shaw, 1967). The few colour responses recorded at lamina hyperpolarization sites (Shaw, 1969) were inconclusive. The spectral sensitivities of such units cannot be predicted for, although retinula cell responses with various spectral sensitivities are described in locust (Bennett et al., 1967), every cell is different and their intraommatidial distribution is unknown.

From an analysis of the components of the ERG in dragonfly dorsal ocellus, Ruck (1961) concluded that dendritic terminals of the ocellar nerve fibres which are postsynaptic to receptor cells similarly have hyperpolarizing responses which inhibit ongoing activity in the ocellar nerve fibres.

In drone, the sensitivity of the long visual fibres is not known. Attempted penetrations of the small retinula cells (7 - 9) of the drone bee ommatidium have proved unsuccessful, as judged from cell marking techniques (Bertrand and Perrelet, 1969). Cell 9 is least likely to have a pronounced spectral sensitivity peak because its basal rhabdomere, though quite well-developed, is presumably affected most by pigment self-absorption in the rhabdome above (providing absorption occurs at the peak wavelength of the basal rhabdomere). The microvilli of the basal rhabdomere are orientated at

45° to those of the other microvilli of the rhabdome (Perrelet and Baumann, 1969 a). The possibility exists that the responses of the basal cell are largely determined by the responses of the larger cells around it; certainly there is electrical coupling between the long retinula cells (Shaw, 1969 a). Cells 7 and 8 could have a pronounced spectral sensitivity peak because their rhabdomeres extend to the cone like those of the large retinula cells. The same arguments by which Shaw (1969 a) predicts that the intraommatidial distribution of spectral sensitivities of the large retinula cells is monochromatic can apply for the sensitivies of cells 7 and 8. These would therefore be expected to have maximal sensitivity to UV light and a secondary sensitivity in blue wavelengths, providing that interreceptor coupling exists between all retinula cells as has been shown to exist between the presumed cells 1 - 6 only (Shaw, 1969a). It seems unlikely that cells 7 and 8 are maximally sensitive to light polarized in only one plane because their rhabdomere tubules are orientated in two directions parallel to those of the two neighbouring long retinula cells (Perrelet and Baumann, 1969 b). Since the lamina monopolar neurones receive their retinula input from cells 1 - 6 the spectral sensitivity of these units would be anticipated to be in the UV and blue wavelengths. Although polarization plane sensitivity must be small,

because receptor responses are small, the weakly modulated responses of four cells (1, 2, 4, 5) with similar rhabdomere orientation could dominate over the responses of the other two (3, 6).

In worker bee, the retinal arrangement, at least, differs from that of drone. The two small retinal cells (the eccentric and eighth cells of Varela and Porter, 1969) are in fact little different in size from the large retinula cells and in the distal region of the ommatidium have large rhabdomeres, although in the basal region of the ommatidium they become smaller and give rise to small axons. The rhabdomere microvilli of the two cells are parallel to each other and thus presumably both are maximally sensitive to light polarized in the same plane. The question of interreceptor coupling in worker bee ommatidia awaits two channel intracellular recording experiments but preliminary observations (Laughlin, personal communication) indicate that the response modulation caused by E-vector plane is small, in the central region of the eye, suggesting coupling. Tight junctions between rhabdomere microvilli, which are one of the presumed sites of intercellular coupling, are seen in drone rhabdome (Perrelet, 1970) but not, however, in worker (Varela and Porter, 1969). The intraommatidial distribution of spectral sensitivity peaks is known only from rather inadequate evidence (Gribakin, 1969) using

selective adaptation by light of different wavelengths. This potentially valuable method of demonstrating spectral sensitivity requires the use of electrophysiological response measuring and marking techniques as an independant standard to establish its validity. Gribakin proposes that the three main spectral types described by Autrum and von Zwehl (1964) are distributed within the ommatidium as follows: 2 yellow sensitive (530 nm), 4 blue sensitive (430 nm) and two ultraviolet (340 nm) in the cyclic order 530, 430, 340, 430, 530, 430, 340, 430. The two ultraviolet receptors have large axons and may correspond to cells 1 and 4 of the drone ommatidium. If this were so then the small retinula cells of worker would be maximally sensitive at wavelengths around 530 nm. It is known that bees orientate by means of the polarization plane of light from the sun but only to light of UV wavelengths striking the dorsal half of the eye (von Frisch, 1967), therefore there must somewhere be integration of spectral and polarization sensitivities so that wide-field units are presumed to exist, sensitive to polarization plane and wavelengths around 340 nm.

The retinula cell input to the cartridges of the water-bug lamina

Preliminary recordings from retinula cells allow some speculation concerning the sensitivities of the cartridges of

the water-bug lamina (Ioannides, personal communication). Records indicate that for the dark-adapted retina, the retinula cell acceptance function and the divergence angles of interrhabdomere axes are both wider than the interommatidial angle. The dark-adapted retinula cell acceptance angle and interrhabdomere angle are both 9°; the interommatidial angle is approximately 3°; consequently there is an overlap of retinula cell acceptance angles between neighbouring ommatidia. The rhabdomeric microvilli are orientated in two orthogonal planes which, when the eye is held in its normal postural orientation, are horizontal (the microvilli of cells 2, 5 and 7) and vertical (the microvilli of cells 1, 3, 4, 6 and 8). Polarization sensitivity presumably corresponds to these orientations and intracellularly recorded sensitivities (Ioannides, personal communication) show two classes, horizontal and vertical.

In the light-adapted retina the rhabdomeres become separated from the cone by a crystalline thread (Walcott, 1969) and the optical axes of the retinula cells within a single ommatidium appear to merge (Ioannides, personal communication) i.e. the ommatidium behaves as a fused rhabdomere type. At the same time retinula cell angular sensitivity narrows and the acceptance angle decreases to about the same value as the interommatidial angle (i.e.

approximately 3°). Changes in angular sensitivity on light-adaptation are accompanied by changes in absolute sensitivity to light (which decreases up to 1000-fold). There is a correlation between this absolute sensitivity and the anatomical state of adaptation (Walcott, 1971 b).

The distribution of excitation on the lamina cannot be considered until the first projection is known in detail, although preliminary observations (see Results) suggest a system of neural recombination analagous to that of fly. If such a divergent system exists then the transition between dark- and light-adaptation would produce a broadening of angular sensitivity of the cartridges, providing all retinula cells received light fluxes of comparable magnitude. At the moment it seems most likely that in the light-adapted state the central pair (cells 7 and 8) situated axially behind the crystalline thread receive most light. Consequently, they might be the major determinant of angular sensitivity of the second-order neurones of the cartridges in the light-adapted state. There is some evidence however that cells 7 and 8 have long visual fibres which by comparison with other insects exclude the likelihood of their synaptic interaction with lamina monopolar neurones. Long visual fibres have been seen in Golgi preparations of the related genus Notonecta (Horridge, personal communication). In water-bug, the occurrence of groups of eight axons at the

top of the lamina and the deeper subdivision of these groups into two bundles of three and a central bundle of two axons suggests that each group is an ommatidial bundle of retinula axons. Furthermore the correlation between retinula axon diameters at the top of the lamina and retinula cell diameters at the base of the ommatidia suggests that the central pair of axons (one large, one small) are those of cells 7 and 8. These axons which are such a conspicuous periodic feature at a peripheral level in the lamina appear from longitudinal sections to descend vertically through the lamina, and enter the chiasma. In other words, the central cells 7 and 8 probably have long visual fibres. If so, this interpretation would be at variance with the mechanism proposed above for improving acuity in the light-adapted lamina cartridges.

The significance of the open-rhabdomere retina

It is important to ask what improvement in performance has been made by the aquisition of the morphologically complex open-rhabdomere retinal organization and first visual projection of the fly. The system does not improve acuity (Kirschfeld, 1967; Scholes, 1969) as might be thought possible with the subdivision of intraommatidial optical axes, because of the neural recombination at the lamina (Braitenberg, 1967); all behavioural evidence for

example points to the neurommatidium (i.e. the collection of short retinula axons with common visual axes) as being the functional unit of the movement perception apparatus (Reichardt, 1969). The fly visual system therefore offers no improvement in acuity over the apposition type of fused-rhabdomere eye, for which the ommatidium is the functional unit. The advantage of the fly system seems to be in its enhanced sensitivity; the effective aperture of each neurommatidium is increased because light is accepted through six facets and not through only one as is the case for the cartridges of apposition type fused- rhabdomere eyes (Scholes, 1969). An additional advantage of the open-rhabdomere system is that injury to or occlusion of one or a few facets of the retina would not lead to some silent cartridges (because of convergence of excitation) as would be the case in fused-rhabdomere eyes.

A disadvantage of the open-rhabdomere organization is its curious susceptibility to mechanical deformation or internal pressure of the head capsule. Because the visual axis of the retinula cells depends on the spatial relationship between rhabdomere and cone, pressures which disturb this relationship destroy the complementarity between neighbouring retinula cell axes and therefore diminish acuity. Mid-air collisions are examples of such mechanical forces that must be very much a part of the life of a fly.

Strong antidromic illumination for long periods has been shown to alter the pattern of rhabdomeres with coincident visual axes (Gemperlein and Järvilehto, 1969) but the reason for this modification is unclear. It is unlikely to have physiological significance since it is not obtained with orthodromic illumination nor if the head capsule is intact (Franceschini and Kirschfeld, 1971).

The significance of neurone-glia synapses in the fly lamina

The description of synaptic contacts of retinula terminal and centrifugal neurone on to the epithelial glial cells of the fly lamina is an anomaly, apparently without precedent. It requires a modification of our ideas either of the function of glia as cells without fast electrical responses or of the function of structurally defined synapses as a region of rapid synaptic transmission.

Current ideas on glial function in the insect central nervous system are derived largely from studies on the movements of potassium ion in cockroach by Treherne (e.g. Treherne, Lane, Moreton and Pichon, 1970). In this system the glial network of an intact unstretched ganglion behaves as a significant barrier to diffusion of potassium ion between haemolymph and the axonal extracellular space, because of the presence of tight junctions and desmosomes at the intercellular clefts (Maddrell and Treherne, 1967).

This barrier is important in isolating the neuropile from the high potassium ion concentration of insect haemolymph. The electrophysiological properties of insect glia are not known, but the optic nerve glia of the amphibian Necturus (Orkand, Nicholls and Kuffler, 1966) respond with slow potentials to ongoing neural activity, probably through local release of potassium ion around active neurones. These slow responses are conducted through the glial network by low resistance contacts. Similar depolarizations, generated locally at a focal point in response to neural activity, are seen in the Müller cells of Necturus retina which extend through the entire retinal depth (Miller and Dowling, 1970). Membrane properties of leech glial cells (Kuffler and Potter, 1964) are similar to those of vertebrate glia. In all these examples the glia receive some measure of ongoing neural activity, perhaps related to the long claimed view of the metabolic dependence of neurones upon glia. The long-term maintenance and axonal growth of embryonic cockroach neurones in glia-free cultures (Chen and Levi-Montalcini, 1970) apparently questions the importance of this metabolic dependence, but there is not yet any evidence that there is electrophysiological interaction in the cultured neurones; in the leech, neurones can in any case maintain short-term activity after destruction of their surrounding glia (Kuffler and Potter, 1964).

The synaptic contacts upon the glia of fly lamina suggest that the glial cells similarly have a function requiring information concerning the level of activity of individual cartridges, that is distinct from the generalized ionic shifts resulting from activity. It would be interesting to know if the glia show electrical responses to presynaptic activation and if so, the time course of these responses. Even if they have fast neurone-like responses, the absence of elements postsynaptic to the glial cell suggests that these cells do not have neurone-like effects on the electrophysiological interaction of the cartridge.

Lateral interaction and movement perception

All arthropods so far tested behaviourally exhibit evidence of movement perception and this quality has been a preoccupation of arthropod visual physiologists. A movement perception system functions by temporal correlations between two parallel receptor inputs and therefore has two main requirements, lateral connections between the two parallel inputs and the appropriate temporal comparison between the converged inputs in at least one neurone. Some morphological candidates for lateral interaction are known and some physiological correlates of a motion perception system have been identified. However, most evidence for the behaviour

of individual neuronal components comes from the overall performance of the system in intact animals.

1. Behavioural performance of movement perception systems in arthropods

The movement perception systems of arthropods show great diversity in their ranges of performance. Most analysis has been carried out on the results of optomotor experiments using intact animals, but this method has been supplemented by recording from movement sensitive units in the deep optic neuropiles in response to moving patterns in the visual field. Maximal sensitivity to motion has been shown to lie in two orthogonal planes, horizontal and vertical, each sensitivity resulting from a separate visual subsystem. This has been most elegantly shown for *Drosophila* and *Musca*, in which the torque and thrust control systems of flight are separately controlled by two systems which are maximally sensitive to horizontal and vertical stimuli respectively e.g. the optomotor torque responses caused by a rotating moving stimulus are maximal when the direction of movement is along the horizontal axis of the fly, while the optomotor thrust responses caused by bilateral antero-posterior stimulus movements are nil when the direction of movement is horizontal (Götz, 1968). In locust too, movement is perceived in both horizontal (Burtt and Catton, 1954;

Horridge, 1966) and vertical planes (Thorson, 1966 a, b) but the analysis of optomotor responses to oblique movements is difficult and has not been made. This evidence suggests that two independent subsystems exist, which may nevertheless share part of their neuronal circuitry, and that these have orthogonal sensitivities. This is confirmed by recordings from motion sensitive units of the optic lobe. It is surprising that the two orthogonal planes are vertical and horizontal because tangential neurones are not obviously organized in either of these two planes but rather along the two oblique vertical axes x and y. The sensitivity of the motion perception apparatus to small movements of the visual field is very great. In locust, tested with a stimulus moving across the whole visual field, movements of only 20 seconds of arc produce optomotor responses (Thorson, 1966 a). The spatial acuity of an individual ommatidium on the other hand is limited by its acceptance function which is at least an order of magnitude greater. The speeds of effective moving stimuli vary over a very large range e.g. in locust responses to sinusoidally oscillating stimuli were obtained over 3.5 log units of frequency (0.005 to 5 Hz) (Thorson, 1966 b). The performance in response to slow moving stimuli in other arthropods is more remarkable. Two pathways of movement perception exist in parallel in the crab Carcinus, (Horridge, 1966 c).

One is of short time constant and can be adapted out by rapid oscillations of 2 cps. leaving the response to long duration stimuli unchanged. Carcinus can also perceive extremely slow movements; presented with no other moving stimulus it has been shown to be able to follow the excursions of the sun across the sky (Horridge, 1966 e), representing an effective interommatidial stimulus interval greater than ten minutes.

2. Models derived from behavioural performance

Descriptive models have been developed from studies on the performance of motion perception systems in several insects. Generally the neuronal circuits between receptors and either movement sensitive units or motor output are inaccessible and Thorson (1966 b) has indicated the dangers of assessing the worth of a model from input/output studies alone because there are other neural mechanisms in series and parallel with the motion perception system that the model attempts to describe.

Various forms of lateral interaction between receptor input channels have been proposed (Reichardt, 1961; Thorson, 1966 b; Götz, 1968). All models proposed consider that a second channel will be excited by an altered pattern of illumination to that stimulating the first channel and at a slightly later time. Information is passed laterally from the first channel through a delay circuit so that it

arrives at the second channel synchronously with the initial activity in that channel. Although some components of the model may be represented by known neurophysiological mechanisms they are not directly identifiable.

A well known model developed by Reichardt and coworkers (Hassenstein and Reichardt, 1956; Reichardt, 1961) is based upon early behavioural work with the beetle Chlorophanus and consists of a forward multiplicative mechanism in which information is exchanged laterally between two receptor input channels and after a delay is multiplied with the response from the other channel. Thorson (1966 a) found this model inadequate to predict optomotor responses to small vertical sinusoidal stimuli in locust. Moreover he criticised some aspects of the model because the relationship between speed of optomotor stimulus and strength of optomotor response (Reichardt and Varjú, 1959) was probably rate limited at high frequency by the speed of the neuromuscular mechanisms and at low frequency by adaptation in the retinal receptors (Thorson, 1966 b). A more general criticism which applies to all models of movement perception is that Thorson was able to propose five models all of which provide an adequate description of the optomotor behaviour of locusts, but experiments that measure only the output of a motion perception system cannot distinguish between them. To do so requires that the

responses of some of the intermediate components of the network between receptor and output be known. Experience from vertebrates is illustrative of the neural testing of movement perception models. Barlow and Levick (1965) were able to devise experiments which selected between two broad alternative models, lateral excitation and lateral inhibition respectively, for movement perception in rabbit retina. They recorded movement sensitive units and observed spike frequency for two slightly separated, stationary bright spots presented individually or as a pair separated by a short time delay. If the apparent movement between the two spots for a double presentation is in the preferred direction then the number of spikes is approximately equal to the sum of the responses to individually presented stimuli. However the response to paired stimuli in the null direction is much less than the sum of the responses to the individual stimuli, implicating an inhibitory mechanism. From intracellular recordings from cell types of the retina of Necturus by Werblin and Dowling (1969), Dowling (1970) has proposed that the inhibitory locus lies at amacrine - amacrine synapses. It is a requirement of this scheme that the preferred direction of movement of the dependant ganglion cells relies upon asymmetry in their amacrine connections.

3. Components of the models and their possible anatomical substrate

One preoccupation of compound eye neuroanatomists has

been the search in the optic lobe neuropiles for divergent processes between perpendicular neurones which might serve as the basis for the necessary lateral correlation between parallel receptor inputs. The diverging pathways of the fly first projection were likely candidates for such a system until the demonstration by Kirschfeld (1967) and Braitenberg (1967) that they served merely to recombine activity derived from a common optical axis. It was then thought (e.g. Reichardt, 1969; Braitenberg, 1969; Horridge and Meinertzhagen, 1970 b) that because of the simplicity of the fly lamina and the absence of tangential processes in the lamina neuropile, lateral correlation must occur in the medulla or even more centrally, the lamina serving only as a relay station. The situation in other insects has never been clear because many described lamina monopolar neurones have dendrites with a lateral spread greater than one cartridge width (Cajal and Sánchez, 1915). The possibility that the lamina is the site of lateral correlation is however resurrected by the recent description of fine lateral processes of the L4 neurone which connect with either or both L1 or L2 of two adjacent cartridges (Strausfeld and Braitenberg, 1970). The L4 neurone is obviously suitable for the function of a lateral correlation neurone while the connections of its processes implicate either or both L1 or L2 monopolar neurones as the elemental motion detectors. In addition, the synaptic input to L4 is from the medulla via one of the

medulla centrifugal neurones which could provide the delay circuit necessary for the function of lateral correlation. Further, the paired nature of L1 and L2 is reminiscent of the dual subsystems controlling torque and thrust in flight. It may be a general rule for all insects that one class of monopolar neurones with lateral dendrites in the lower zone of the lamina, i.e. the small monopolar neurones of Cajal and Sánchez (1915), have a consistent pattern of connections with other cartridges, appropriate for motion perception. The Spanish authors have already pointed out that in insects with a stratified lamina e.g. bee, the input in the lower lamina zone to the dendrites of the small monopolar neurones cannot be directly from the retinula terminals as these do not extend into this zone, but must be exclusively from the centrifugal neurones, a situation similar to that of the L4 neurone of fly. Interactions of adjacent ommatidia in the direction of motion of the optomotor stimulus dominate the control of flight torque in Calliphora, while there is little interaction from those more than two or three interommatidial spacings apart (McCann and MacGinitie, 1965). In Chlorophanus it is known that the effective interommatidial separation required for movement perception is between adjacent or immediately subadjacent facets only (Hassenstein and Reichardt, 1956). If slits are interposed between the animal and the rotating

striped drum, movement perception occurs only when the slits are separated by a width equivalent to less than two interommatidial angles. These experiments may be criticised because it has been assumed that the acceptance angle is approximately the same as interommatidial angle, nevertheless similar experiments on different insects would allow a comparison between the maximum effective interommatidial separation and the lateral extent of the dendrites of the small monopolar neurones.

If the reported divergence in the projection pattern of pairs of long visual fibres and central monopolar neurones from one lamina cartridge to the medulla in Pieris (Strausfeld, 1970) can be confirmed, it could also represent the horizontal component of a movement perception apparatus providing the complementary vertical component exists at some (deeper) level in the optic lobe.

The responses of the narrow-field elemental motion detectors are presumably integrated over a wide area on to wide-field tangential neurones. Probably elemental detectors sensitive to movements in four directions (i.e. up, down and backward, forward) exist which initially sum their separate sensitivities. Summation of many inputs on to wide-field neurones results in their great sensitivity to minute movements. Wide-field movement sensitive units are now known from a range of recording sites within the

optic lobe and protocerebrum as far peripheral as the distal medulla. They are known from locust (Burtt and Catton, 1960; Horridge et al., 1965), *Sphinx* (Collett and Blest, 1966; Collett, 1970), *Calliphora* and *Musca* (Bishop, Keehn and McCann, 1968; McCann and Dill, 1969) and worker bee (Kaiser and Bishop, 1970). All units are characterized by a background discharge of spikes, either in the dark or without stimulus movement, with a frequency up to about 50 spikes per second. This spike frequency is increased by stimulus movement in the preferred direction, decreased by stimulus movement in the null direction but unaffected by movement in a perpendicular direction. The preferred sensitivity of some units is in one of four directions (horizontally forward or back, vertically up or down) but other units are sensitive to more general movements.

C. The problem of connectivity

The progressive trend toward the analysis of interaction in neuronal networks by the description of single unit activity has produced changes in approach to both neurophysiology and neuroanatomy. It has required the development of intracellular marking techniques (Kaneko and Hashimoto, 1967; Stretton and Kravitz, 1968; Selveston and Remler, 1968) which enable the correlation of electrophysiological response with morphological cell type.

Marking techniques have in many cases made inadequate the study of randomly sampled neurones and the electrophysiological rationalization of their responses by abstract and often devious manipulations of the parameters of stimulation; a transition has begun between the analysis of unit activity and the analysis of the activity of morphologically defined neurones. These refinements in approach and technique have resulted in a requirement for more exact morphological descriptions of nervous networks (Hoyle, 1970).

Coincidently in the last ten or fifteen years there has been an increase in the number of exact anatomical studies of nervous tissue, the refinement and improvement of silver staining methods which had not regularly been used for over thirty years, and a general elevation in respectability of neuroanatomical investigations. Typical of the convergence between anatomical and physiological approaches are recent studies on vertebrate retina (e.g. review by Dowling, 1970) and cerebellum (symposium, Llinás, 1969) and insect compound eye. The organization of these three tissues is particularly favourable to analysis because it consists of many regular channels repeated in parallel with great spatial order.

At its ultimate the goal is towards a complete morphological analysis of the ramifications and connections between all the neurones of a selected piece of nervous tissue with each neurone, its axon and if necessary all its

processes uniquely identified. The anatomical connections of neurones described with precision at single cell level, constitute the connectivity patterns of those neurones. Connectivity patterns are not necessarily identical with generalizations inferred from the restricted connections of a few elements nor are they equivalent to the sort of models derived from physiological analyses that may and have been drawn with excitatory and inhibitory connections indicated to illustrate the flow of activity between neurones. The idea of connectivity which has been stated most explicitly by Horridge (1968 c) has conceptual simplicity and great interpretative value as its major attractions. Its greatest and possibly sole relevance is for the nervous systems of some invertebrates but at the present time, even in these examples, an immediate limitation to the concept is the improbability that it will ever be realized for more than very simple networks.

The function of such detailed wiring diagrams is that they allow anticipation of the conditions under which a neurone will respond and thus facilitate the interpretation of responses actually observed. However such a model of a nervous system cannot be used to predict the responses of component neurones because, in the first place, excitatory and inhibitory connections are not yet predictable from the morphology of their synapses. Secondly, the model

contains no information about the temporal and spatial characteristics of neuronal membrane responses which can only be obtained from electrophysiological records.

The complete description even of a very limited area of nervous tissue in this way is a formidable task. The determinate nervous systems of some invertebrate groups which have small, fixed numbers of neurones of predictable and recognizable location and morphology will certainly be among the first and perhaps will be the only to be so analysed. Some very simple nervous systems have indeed already been analysed fairly completely at the level of light microscopy, notably that of the roundworm *Ascaris* by Goldschmidt (1908, 1909 and 1910), but this analysis involving approximately two hundred large neurones is exceptionally simple and still was a major work. The majority of systems in insects differ in cell number from *Ascaris* by at least one order of magnitude e.g. the terminal ganglion of the cricket *Acheta* has an estimated 2,100 neurones (Gymer and Edwards, 1967) and more usually by a factor of two, three orders or more (e.g. the corpora pedunculata of bees which consist of approximately 300,000 neurones; Witthoft, 1967). The magnitude of the problem posed by morphological complexity of this scale, even in the smallest insect ganglion or neuropile is consistently underestimated by physiologists unfamiliar with the practical

difficulties of neuroanatomy (Bullock, 1969; Hoyle, 1970). The appearance of any neuropile in low magnification electron micrographs ought to serve as a salutory reminder (see e.g. Steiger, 1967).

As a compromise, two alternative approaches are possible using present methods. Either a complete study of the connectivity of all of a small group of uniquely identifiable neurones which form a small part of the whole nervous system e.g. the (relatively) few motoneurones of an insect ventral ganglion, or a study of a representative selection of neurones from a repetitive neuropile. In the case of insect optic lobe, the connections although complex are highly predictable and for at least one class of neurone the concept of a representative sample is valid; these are the short retinula axons of the fly first projection (Horridge and Meinertzhagen, 1970a; and see Results).

D. Some developmental aspects of connectivity in the optic lobe

The study of errors in the fly first projection

The predictable nature both of axon pathways in the lattice and of position of axon termination strongly suggests the existence of a set of developmental sequences that is rigidly adhered to by all cells of the system.

In our previous work on this system (Horridge and

Meinertzhagen, 1970a) it was hoped to find errors in connectivity in the fly first projection that would provide some insight into the rules by which the axons grew. From these rules it might have been possible to adduce something of the mechanisms of growth. The completed connectivity patterns of a large number of terminals, however, pointed only to the remarkable accuracy of the connections and their invariance from the generalized patterns first described by Braitenberg (1967).

In the event, the only inconsistencies in the connection patterns that were seen were a few pairs of terminals that had inverted their rotational sequence within a cartridge. It was obvious that axons at the equator had connections appropriate for the optical axis of their excitation even though this meant that they had to grow across the equator. In other words the equator does not represent a barrier between the two halves of the optic lobe but a line of demarcation between areas of differing spatial relationships. In particular the concepts of growth milieux developed to explain some phenomena of axon growth (e.g. Hibbard, 1965; Van der Loos, 1965) does not seem appropriate to the problem of the equator of the fly first optic projection.

1. The occurrence of errors

In the new connectivity errors found in this work an overall pattern in the errors is again not discernible and

the errors do not appear to be the consequence of, or clearly related to, a single event but rather to a general disturbance around one region of the equator. Such conclusions from these errors that can be made are general in nature and illustrate the difficulty in inferring growth mechanisms from adult structure in the absence of a detailed knowledge of the developmental process.

The spatial relationships of the lattice of axons may be important in the pattern of axon growth because mistakes are associated with areas in which this lattice is interrupted. In the region around cartridges 88 and 89 several aggregations and knots of axons occur through which pass some of the axons that terminate incorrectly. Curiously, the projections of ommatidia 88 and 89 are both correct and it is those of the ommatidia neighbouring these two that have a high proportion of errors. An additional inconsistency in the region of the dislocation is the complete absence of axon 785. This is the only occasion in which an axon was found to be entirely absent and it would be interesting to know whether there is a causal relationship between the absence of this axon and either the dislocation at the equator or the interruption of the axon lattice.

The direction in which the axons first diverge from their ommatidial bundle depends upon the retinula cell class to which they belong. Nevertheless all axons travel

approximately in a dorso-ventral direction and there is no significant antero-posterior component of the lattice. Even many axons destined to terminate in error leave in the expected direction though not the few axons 1, 2 and 5 found in the small sample(fig. 9).

The only mistakes in axons 1, 2 and 5 that terminate in error originate in ommatidia abutting the equator. The two ommatidia 78 and 90 with axons 1 terminating incorrectly are both in the dorsal half of the eye and the axons move to equivalent cartridges relative to their origin.

Errors occur more frequently in those axons travelling furthest i.e. 3 and 4. In these two classes the axons leave in the proper direction but they stop short of the correct distance and terminate in one of the cartridges en route. In so doing, most axons seem to have a second or third "choice", a preferred cartridge occupying the same position relative to their own but in the wrong row. Some axons 3 and 4 terminate in error even though they do not cross the equator. One axon 743 (fig. 8) presents the special case of an axon terminating in error in an otherwise perfectly connected lattice. In doing so it terminates in one of the preferred second choice cartridges, thus strengthening the idea that this is a general phenomenon not restricted to regions of equatorial dislocation.

In all these examples axons which terminate in the

wrong cartridge stop in a position corresponding to their direction of arrival, a conclusion reached earlier for axons that cross the equator and terminate out of sequence in the correct cartridge (Horridge and Meinertzhagen, 1970 a).

In summary, the distribution of observed errors does not confirm the attempts by Braitenberg (1970) to quantitate the accuracy of axonal connections in the fly first visual projection. Thus, an axon 3 apparently does not after all find its place from one of a possible 222 positions but instead enters a period of growth in an appropriate direction and for a length of time that takes it to its correct cartridge. Some axons do not manage to grow to their correct cartridge but stop at another en route. Within a cartridge an axon appears to terminate in a position corresponding to its direction of arrival and in most cartridges, perhaps by a temporal pattern of axon arrival, this results in a predictable rotational sequence of retinula terminals. In the equatorial cartridges however, axons which arrive from across the equator terminate without a very predictable sequence, perhaps because they arrive at these cartridges nearly synchronously.

2. Variability in the composition of cartridges

The composition of the cartridges is more variable than previously described (Trujillo-Cenóz, 1966; Braitenberg, 1967).

The number of retinula axon terminals in any cartridge is usually fixed according to its position; most have six. On either side of the equator ideally there are two rows of eight-terminal , and then one row of seven-terminal cartridges (Horridge and Meinertzhagen, 1970 a). At the edge of the lamina reduced cartridges occur. As a result of connectivity errors and dislocations in the equator some cartridges are produced with nine terminals and some with less than the ideal number. The cartridges are also variable with respect to the cells of origin of their terminals, cartridges commonly may receive more than one axon from one ommatidium e.g. 804, 805 in 90; 1093, 1094 in 90; 80A4, 80A5 in 90A; 70A4, 70A5 in 80A; 892, 894 in 79; 60B3, 60B4 in 80A; 90B2, 90B3 in 100A; 901, 902 in 99 and 743, 744 in 93, though none has been observed to receive more than two, nor does any axon proceed to the cartridge underlying its ommatidium of origin. Variability is also observed in the rotational sequence of terminals within cartridges. This variability mainly results from the terminals of axons that cross the equator (fig. 15).

3. The origin of the equator in the fly eye

The equator of the dipteran retina is a horizontal division between dorsal and ventral halves which are characterized by mirror-image orientation of the asymmetric rhabdomere pattern of individula ommatidia.

Developmental studies on Drosophila are relevant to the problem of the origin of the equator. Drosophila has an open-rhabdomere eye (Franceschini and Kirschfeld, 1971) with the characteristic asymmetric rhabdomere pattern (Waddington and Perry, 1960) and a retina-lamina projection which in longitudinal section resembles that of Musca (from a Bodian-stained preparation made by the late M. E. Power).

The difference between dorsal and ventral halves of Drosophila retina is fundamental and is determined early in development. Becker (1957) produced Drosophila adults with ommatidia which had mosaic spots by X-irradiating eye discs of young larvae and inducing somatic crossover in some cells. The mosaic patterns, which were interpreted in terms of cell lineage patterns of the imaginal disc, were not observed to extend across the equatorial region of the eye. Similarly, adults of a "lobe-like" mutant of Drosophila have retinae with distinctly shaped dorsal and ventral halves (Becker, 1957). Larvae reared under varying environmental conditions give rise to adults in which the relative growth of the two halves of the retina is altered. It is assumed that the dorsal and ventral halves of the retina first observed by Dietrich (1909) correspond to the two halves demonstrated by the mosaic spots and reduced eye mutant. The equator demonstrated by these developmental

experiments, like that determined by rhabdomere arrangement, is not a regular line but has dislocations. A similar dislocation of the equator has also been observed from the pattern of antidromically illuminated rhabdomeres in Drosophila by Franceschini and Kirschfeld (1971) so that dislocations of this sort may occur quite commonly.

The rules of growth and the problem of neuronal specificity are so profoundly challenging in this system that they warrant a very careful developmental study. In the functional morphology of the system there is a reciprocality between the pattern of the retinula cells with coincident visual axes and the pattern of neural reconvergence; consequently developmental studies must be directed at the reciprocality of two developmental processes. These are the pattern of cell division and particularly of the orientation of the plane of cell division by which the characteristic pattern of retinula cells is first produced and the temporal and spatial pattern of axon growth that results in the formation of the neural projections. These two processes are spatially separate, the first occurs in the eye imaginal disc (Ouweneel, 1970) while the second occurs in the outer glomerlulus, derived from a region underlying an epithelium of imaginal cells in the larval brain and which later gives rise to the lamina (Shatoury,

1956). It is not known if the processes are also temporally separate because the retinula cell lineages are still not known in spite of much work on Drosophila and the appearance of cell clusters that are presumably ommatidial precursors may not be the first indication of retinula cell neuroblasts, but of aggregation of previously divided cells. Clusters of four cells are first seen in mid-third instar larvae (Steinberg, 1943), whilst the outer glomerulus begins differentiation soon after its innervation from the eye disc in the late second instar larva (Shatoury, 1956).

The development of optic lobes and the significance of chiasmata

One question that has consistently recurred in the compound eye literature since the early workers (Cajal and Sánchez, 1915; Braitenberg, 1970) concerns the significance of chiasmata in optic lobes. The functional consequence of the insect lamina-medulla chiasma is merely the lateral inversion of the visual field projected on the medulla, a transposition of no apparent value in the processing of visual information.

Studies by Elofsson on reduced silver preparations of a wide variety of Crustacea at different stages of development (Elofsson and Dahl, 1970) confirm original observations by Parker (1895) on the crayfish Astacus, and provide the first key to the origins of chiasmata. They show that the formation of chiasmata

during development of crustacean optic lobe results from the pattern of fibre growth between two proliferating neuropiles. Depending on which side the growing axon enters the neuropile in which it is to terminate, so the overall pattern of fibre connections between the neuropiles forms a crossed (chiasmal) or an uncrossed projection. According to these workers, a common feature of the development of all crustacean optic lobes is the presence of a medial proliferative zone from which cellular rudiments are contributed to each of three zones. Two of these are parallel, and will form the retina and lamina, and the third situated perpendicular to the others, will form the external medulla. In non-malacostracan crustaceans the growing axons of perpendicular neurones of the lamina and external medulla pierce their own neuropile, cross the gap between the two regions and enter the opposite neuropile. This pattern of growth forms an uncrossed projection of lamina monopolar and medulla centrifugal neurones. On the other hand, in malacostracan crustaceans the growing axons having penetrated their own neuropile enter the other neuropile <u>from the opposite side</u> i.e. through the ganglion cell layer. This simple difference in the pattern of axon growth results in a crossed projection in these crustaceans, while the synchronised cell division in the medial proliferation zone could result in the addition to retina, lamina and

external medulla regions simultaneously, of the cellular components necessary for the formation of one ommatidium, one cartridge of the lamina and one cartridge of the external medulla respectively. Establishment of contacts might occur only between cell sets at a particular stage in their temporal pattern of differentiation, but studies which could reveal activity of the proliferation zone synchronized in such a manner have not been made and await the use of more highly refined techniques. These different patterns of fibre growth explain the formation of chiasmata but not of course why some Crustacea should have different fibre growth patterns to others nor how the transition between the two patterns could have ocurred. The cell types found in malacostracan crustaceans are similar to those found in insects and both groups attain a greater degree of complexity in the organization of their visual systems than that found in non-malacostracans; perhaps the developmental processes which result in the formation of chiasmata in malacostracans and insects, allow the development of greater complexity in the organization of the optic lobe neuropiles.

There are however fundamental differences between the development of the insect visual system and that of crustaceans. The development of the retina is separated from that of the optic lobe and there is almost universal

agreement that in a variety of insects connection with the larval brain is not necessary for normal retinal differentiation (e.g. Kopec, 1922; Schoeller, 1964), whereas normal development of the optic lobe neuropiles requires innervation from the developing retina (e.g. Power, 1943; Schoeller, 1964). Nevertheless, a comparable proliferation zone to that of crustaceans is reported by Nordlander and Edwards (1969 a) to exist in the developing optic analgen of the monarch butterfly, *Danaus*. This zone consists of neuroblasts which produce ganglion mother cells of the medulla cortex on one side and on the other, ganglion cells of the lamina cortex. The earliest ganglion mother cell divisions produce medulla tangential neurones whilst later ones produce perpendicular neurones. The medulla tangential neurones send their axons into the growing neuropile of the medulla at right angles to those of the perpendicular neurones. It would not be unreasonable to imagine the medulla monopolar neurones continuously differentiating and sending their axons through the depth of the medulla fibre mass, adding more and more threads to the warp of that neuropile, while the tangential axons form an ever expanding weft with their growing tips contacting the new perpendicular axons as they grow.

Further interpretations are hindered because of the lack of information on fibre growth patterns (which were

not studied by Nordlander and Edwards) and by the difficulty in recognizing potential cell types from the positions of their undifferentiated somata. In particular Nordlander and Edwards did not make the important distinction between medulla centrifugal and medulla monopolar neurones. It might be expected that these cells be produced at different times since they contribute to different chiasmata, although they are produced by a common proliferation zone. One would thus expect, if the sequence described by Elofsson and Dahl applies also for the formation of insect chiasmata, that the products of medullary ganglion cell divisions would be first medulla tangential cells then medulla monopolar neurones and then medulla centrifugal neurones (produced synchronously with the lamina monopolar neurones). So far all that is known is that medulla tangential neurones are produced first and lamina monopolar neurones last (Nordlander and Edwards, 1969 a). Evidence suggestive of fibre growth between cell sets produced at the same time in development, as proposed by Elofsson and Dahl (1970) was seen by Nordlander and Edwards (1969 a, fig. 9). In optic lobes labelled at four times in development, marked cells lie at corresponding positions along the neuropiles of the medulla and lobula complex respectively. However it is necessary to know the neurone type of labelled cells before more conclusions can be made.

Other relevant observations are that the pattern of neuroblast divisions is usually asymmetrical, each neuroblast dividing to produce another neuroblast and a smaller ganglion mother cell (Panov, 1963; Malzacher, 1968; Nordlander and Edwards, 1969 b). The ganglion mother cell then divides once or twice to produce preganglionic cells which later differentiate into ganglion cells and grow axons. The axons of differentiating ganglion cells which are the progeny of a single neuroblast usually enter the neuropile together in a small bundle (Nordlander and Edwards, 1969 b). None of these details are available in an account of optic lobe development in _Drosophila_ (Shatoury, 1956).

The production of a simple medulla-lobula chiasma in insects by a similar mechanism to that proposed for the lamina-medulla chiasma requires synchronized cell division of the neuroblasts of a second proliferation zone but in a vertical plane, as suggested for crustaceans by Elofsson and Dahl (1970). Such zones exist in _Apis_ (Panov, 1960) and in _Danaus_ (Nordlander and Edwards, 1969 a) but their possible vertical organization is not shown.

The developmental sequences during the formation of the chiasmata are not clear from the accounts of Nordlander and Edwards (1969 a) on _Danaus_ nor from the account of Shatoury (1956) on _Drosophila_. Quite clearly the

morphogenetic events are even more complicated in these animals because both Lepidoptera and Diptera have divided lobulae and the complex medulla-lobula chiasma in *Musca*, for example, consists of vertical strata each of four laminations of axon bundles connecting the three neuropiles (medulla, lobula and lobula plate) in varying combinations (Braitenberg, 1970). Two more observations on chiasmata have already been noted by Braitenberg (1970). The first is that dipteran optic lobes contain not only crossed projections but between lobula and lobula plate and between medulla and lobula plate the projections have no chiasmata. The second observation concerns the direction of twist in the strata of the two chiasmata of dipteran optic lobe. In the lamina-medulla chiasma, strata in dorsal and ventral halves of the eye have opposite twists while in the medulla-lobula chiasma alternate layers of fibre bundles which are the projections from alternate vertical medulla cartridge rows have opposite twists. The analysis of the growth of these various projections represents an extremely challenging problem in morphogenesis.

E. The prospects for future work

A more complete understanding of the interactions in the compound eye, even at a peripheral level, requires a great deal more evidence than is currently available. It

seems unlikely with present electrophysiological techniques that the responses of more than a small proportion of the neurones in any of the optic neuropiles can be sampled. Most of the evidence required therefore will have to be provided by anatomical studies. It is desirable that future work be restricted in the main to a few favourable insects, because only by the integration of anatomical and physiological approaches in one animal will an accurate functional model of an insect visual system be arrived at. It therefore becomes important to select insects for anatomical as well as physiological reasons. The problems in neuroanatomy of insect optic lobe are now at least technically as demanding as those of electrophysiology and absence of more complete information on connectivity is one of the greatest obstacles to the understanding of insect vision. The most outstanding omissions in our knowledge of the connectivity of the lamina are given below.

It is still unclear how universal is the occurrence of a pair of long visual fibres from each ommatidium and which are the cells of origin of these axons. In some cases it may be possible to study these problems by the degeneration of retinula axons, where the distribution of retinula nuclei at different peripheral levels would allow this. In addition combined Golgi/EM studies of the base of the ommatidium are necessary to establish the cell of

origin of long visual fibres. The significance of the three types of long visual fibres observed from Golgi impregnations in both Pieris and dipteran species can only be established by this method.

Much additional information is required on connectivity within the lamina. The total number of neurones of each cartridge still remains in doubt even for fly in which the organization is simple. A notable omission is the connections of the lamina amacrine neurones which remain completely unknown apart from a few Golgi impregnations. Knowledge of the connectivity of the cartridge of another simple lamina, e.g. that of Pieris, would provide a valuable comparison with that of the fly. Ultimately the connections within the more complicated lamina of worker bee need to be known if the important analysis of vision in this animal is to proceed further. The complexity of the neuropile, however, particularly of the lower lamina zone, together with the reported absence of highly differentiated morphological synapses (Varela, 1970) present a gloomy prospect. An additional factor to consider in the connectivity of the lamina is the possibility of variation in cartridge composition in different regions.

The existing disagreement in the description of the second projection of fused-rhabdomere eyes needs to be resolved in the insect for which the evidence of the

diverging projection is most convincing i.e. Pieris. This would best be done by using reduced silver stains with thin transverse sections, which would allow a direct comparison between the methods used by Strausfeld (1970) and those used here.

The analysis of the unit structure of the medulla cartridge cannot be undertaken until these problems are clarified.

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