AMINERGIC FLUORESCENCE IN THE CEPHALOPOD BRAIN

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(*Communicated by J. Z. Young, F.R.S. – Received* 11 *February* 1980)

[Plates 1–12]

CONTENTS

A fluorescence histochemical technique with glyoxylic acid has been employed to locate the catecholamines noradrenalin and dopamine and the indolealkylamine 5-hydroxytryptamine (5HT) in the brains of a number of genera of cephalopods. The slow-fading green fluorescence typical of noradrenalin and dopamine was located in the neuropil of most lobes and in some of the cell bodies of the superior and posterior

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buccal lobes, the median basal, magnocellular and vasomotor lobes. Fast-fading fluorescence typical of 5HT was associated with green fluorescence in the neuropil of the peduncle, anterior basal, olfactory, subpedunculate, subvertical, precommissural, superior buccal, optic, pedal and brachial lobes. Only the optic gland displayed yellow fluorescence alone. Photographic evidence is presented to emphasize that in the cephalopod brain the fluorescence is not uniformly distributed. The possibility that noradrenalin, dopamine and 5HT have specific neurotransmitter functions is discussed.

Introduction

There are a number of reports of the presence of possible neurotransmitters in the cephalopod brain (see, for example, Tansey (1979)). Although many of these studies have shown that particular chemicals are present, the anatomical localization has usually been at a gross level. This is particularly unfortunate because the anatomy of the cephalopod central nervous system has been thoroughly investigated (for *Octopus* see Young (1971); for *Loligo* see Young (1974, 1976, 1977, 1979) and Messenger (1979)). Moreover, Gray & Young (1964) have presented ultrastructural evidence that most, if not all, the synapses in the brain of *Octopus* are chemical rather than electrical. It was therefore decided to undertake a study that would relate by means of histochemical techniques the morphology of the brain to its biochemical constituents. These techniques enable us to determine not only which parts of the brain contain certain chemicals, but also the relative distribution within the nerve cell bodies and neuropil of individual lobes.

This paper relates histochemical data to the neural organization of the cephalopod brain at the level of the light microscope. It extends previous histochemical work on the cephalopod c.n.s. (Drukker & Schade 1963, 1964, 1967; Barlow 1971, 1977; Matus 1973). It is also specifically concerned with the direct localization of the putative neurotransmitters dopamine, noradrenalin and 5-hydroxytryptamine (5HT). Matus (1973) used the classic Falck–Hillarp histochemical technique (Falck *et al.* 1962) to demonstrate the presence of these chemicals in the optic lobe and supraoesophageal brain of *Octopus vulgaris*, while Barlow (1971) used the same method to examine the vertical lobe of the same species. However, both these authors reported great difficulties in using this technique with cephalopod brains (Matus, personal communication, 1976; Barlow 1977). The present work reports the use of a modified Falck–Hillarp technique, with glyoxylic acid on cryostat sections, to locate these amines.

Materials and methods

(*a*) *Animals*

Specimens of both sexes of *Sepia officinalis* L., *Eledone cirrosa* (Lamarck) and *Octopus vulgaris* (Cuvier) were obtained from the Marine Laboratory, Plymouth, the Gatty Marine Laboratory, St Andrews, and the Stazione Zoologica, Naples. All animals were dispatched to Sheffield in large polythene bags with a little seawater and with air replaced by oxygen. They were maintained in Sheffield in tanks with recirculating seawater at a temperature of 10–14 °C. They were fed on live prawns and small crabs.

In addition, some specimens of *Sepia officinalis*, *Octopus vulgaris*, *Alloteuthis subulata* (Lamarck), *Loligo pealeii* (Lesueur) and one specimen of *Sepiola atlantica* (Orbigny) were prepared for histochemistry in Plymouth or Naples and were transported to Sheffield in dry ice. At least twelve animals of each species except *Sepiola*, of which only one specimen was available, were examined.

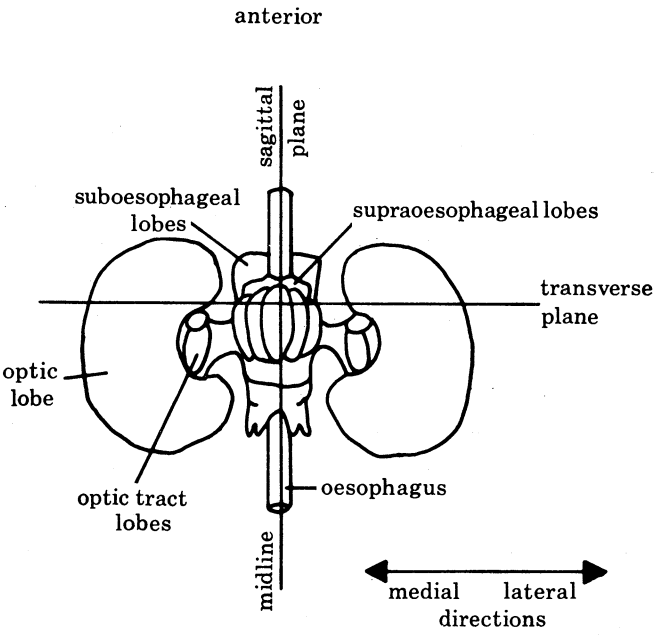


FIGURE 1. Diagram of an octopus brain from the dorsal aspect, defining the terms used in the text. The horizontal plane is in the plane of the page. Adapted from Young (1964).

(*b*) *Normal histology*

Animals for histology were killed by severing the head from the body. Their brains were dissected and immediately fixed in cold 10% (by volume) formaldehyde, previously neutralized with excess magnesium carbonate, made up in filtered seawater.

Serial sections were stained with a trichrome method or a Cajal silver preparation by the method of Stephens (1971). The terminology employed for parts of the brain is that of Young (1971) based on the classification of Dietl (1878). The planes and directions used in descriptions of the brain are shown in figure 1, and typical sections are shown in figures 2 and 3. Sections were examined and photographed on a Zeiss Ultraphot microscope and either Ilford R10 glass plates or Ilford FP4 cut film was used.

(*c*) *Fluorescence histochemistry*

To obtain fluorescence specific for catecholamines, the technique of de la Torre & Surgeon (1976) was followed. Brains were removed rapidly from animals and slices 6–8 mm thick were slowly cooled until frozen (for 6–9 min) in a cryostat chamber at −30 °C. Cryostat sections 12–20 μm thick were cut, mounted onto glass slides and dipped three times into the following solution, titrated to pH 7.4 with normal sodium hydroxide: sucrose (0.2 M), potassium dihydrogen phosphate (0.236 M), and glyoxylic acid (10 g/l).

The slides were then air-dried and reacted in an 80 °C oven for 5 min. Sections were viewed directly in a Zeiss Ultraphot microscope fitted with a mercury vapour lamp emitting u.v. illumination. A BG 12 excitation filter and barrier filters of 470–530 nm were used. Black and white photographs were taken on Ilford FP4 film, and colour transparencies were taken on Agfachrome 50S film and then rephotographed on Ilford FP4 to obtain black-and-white prints.

After viewing and photographing, the sections were stained with haematoxylin and eosin to confirm the localization of any fluorescence observed.

(*d*) *Histochemical controls*

1. The time that the fluorescence lasted was noted, as fading is characteristic of specific aminergic fluorescence.

2. Animals were injected with reserpine (BDH) to suppress specific aminergic fluorescence. The drug was given in a dose of 4 mg/kg (Juorio 1971) dissolved in 0.1 ml of glacial acetic acid, diluted to 0.3–0.5 ml with saline and injected into the base of the first left arm. The animal was killed after 48 h, and the brain was processed as above. This procedure eliminated the aminergic fluorescence.

3. Pargyline hydrochloride (Abbott Laboratories) was given in a dose of 100 mg/kg (Juorio & Killick 1972) to inhibit monoamine oxidase and therefore increase the endogenous monoamines. The salt was dissolved in 0.3 ml of saline and injected into the base of the first left arm. After 3 h the animals were killed and processed as above.

Description of plate 1

Unless otherwise stated scale bars represent 100 μm.

Figure 2. Midline sagittal section of the brain of *Octopus vulgaris*. Cajal stain. Scale bar, 1 mm.

Figure 3. Transverse section through the brain of *Octopus vulgaris*. Cajal stain. Scale bar, 1 mm.

Figure 4. Fluorescent neuropil of the olfactory lobe of *Eledone cirrosa*. Sagittal section.

Figure 5. Section through the cell layer of the superior buccal lobe of *Eledone cirrosa*. Sagittal section.

Figure 6. Fluorescence associated with the blood vessels of the hilum of the optic lobe of *Eledone cirrosa*. Transverse section.

Figure 7. Fluorescence in the ventral optic commissure of *Octopus vulgaris*; showing the typical pattern of fluorescence often found in nerve tracts. Transverse section.

Figure 8. Sagittal section of the anterior suboesophageal brain of *Octopus vulgaris*. Cajal stain. Insets show the approximate locations of figures 9 and 10. Scale bar, 1 mm.

Figure 9. Fluorescence in the neuropil of the postbrachial lobe of *Octopus vulgaris*. Sagittal section.

Figure 10. Fluorescence in the neuropil of the prebrachial lobe of *Octopus vulgaris*. Sagittal section.

Description of plate 2

Unless otherwise stated scale bars represent 100 μm.

Figure 11. Cajal preparation of the prebrachial lobe of *Eledone cirrosa*. Transverse section. Inset shows the approximate location of figure 12. Scale bar, 500 μm.

Figure 12. Fluorescence around the lateral root of a brachial nerve in the prebrachial lobe of *Eledone cirrosa*. Transverse section.

Figure 13. Cajal preparation of the anterior part of the middle suboesophageal mass of *Eledone cirrosa*. Transverse section. Inset shows the approximate location of figure 14. Scale bar, 500 μm.

Figure 14. Fluorescence in the neuropil of part of the anterior pedal lobe of *Eledone cirrosa*. No fluorescence was noted in the anterior chromatophore lobe. Transverse section.

Figure 15. Cajal preparation of the middle suboesophageal mass of *Octopus vulgaris*. Transverse section. Inset shows the approximate location of figure 16.

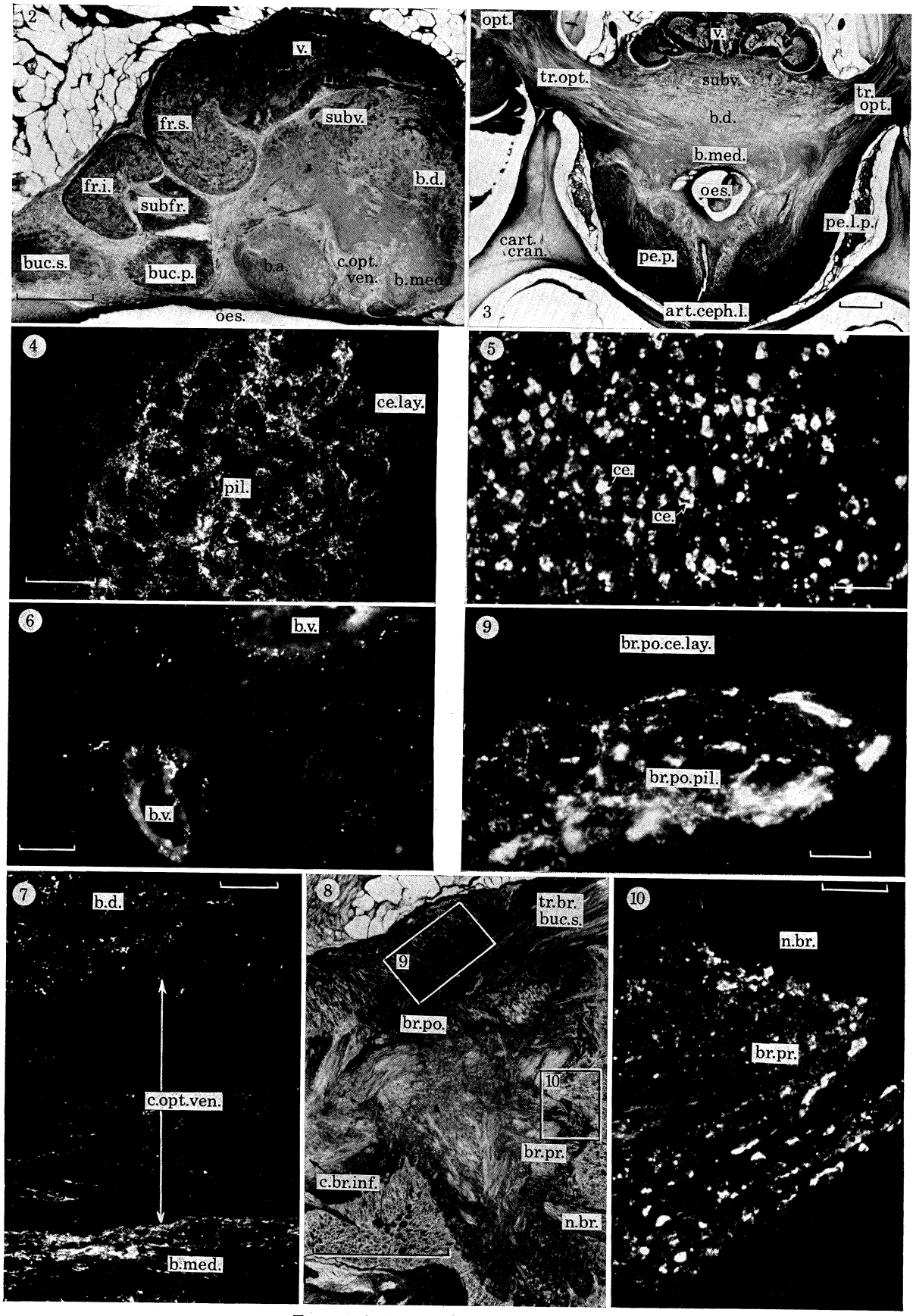
Figure 16. Fluorescence in the neuropil of the anterior pedal lobe of *Octopus vulgaris*. Transverse section.

Figure 17. Cajal preparation of the middle suboesophageal mass of *Octopus vulgaris*. Transverse section. Insets show the approximate locations of figures 18 and 19.

Figure 18. Fluorescence in the ventral part of the anterior pedal lobe of *Eledone cirrosa*. Transverse section.

Figure 19. Fluorescence in the lateral pedal lobe of *Octopus vulgaris*. Transverse section.

Figure 20. Fluorescence in the anterior basal to lateral pedal tract of *Eledone cirrosa*. Transverse section.



FIGURES 2–10. For description see opposite.

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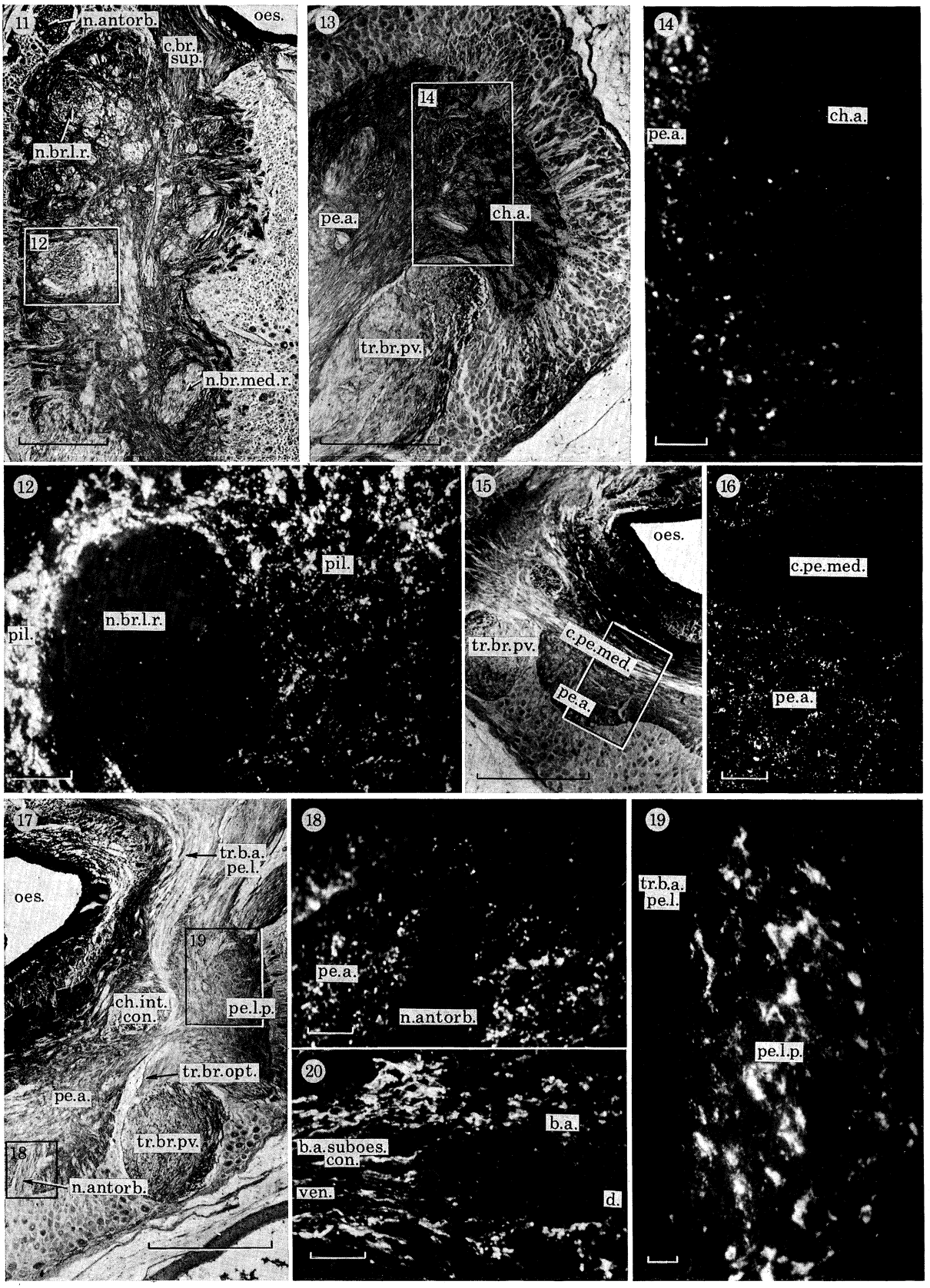
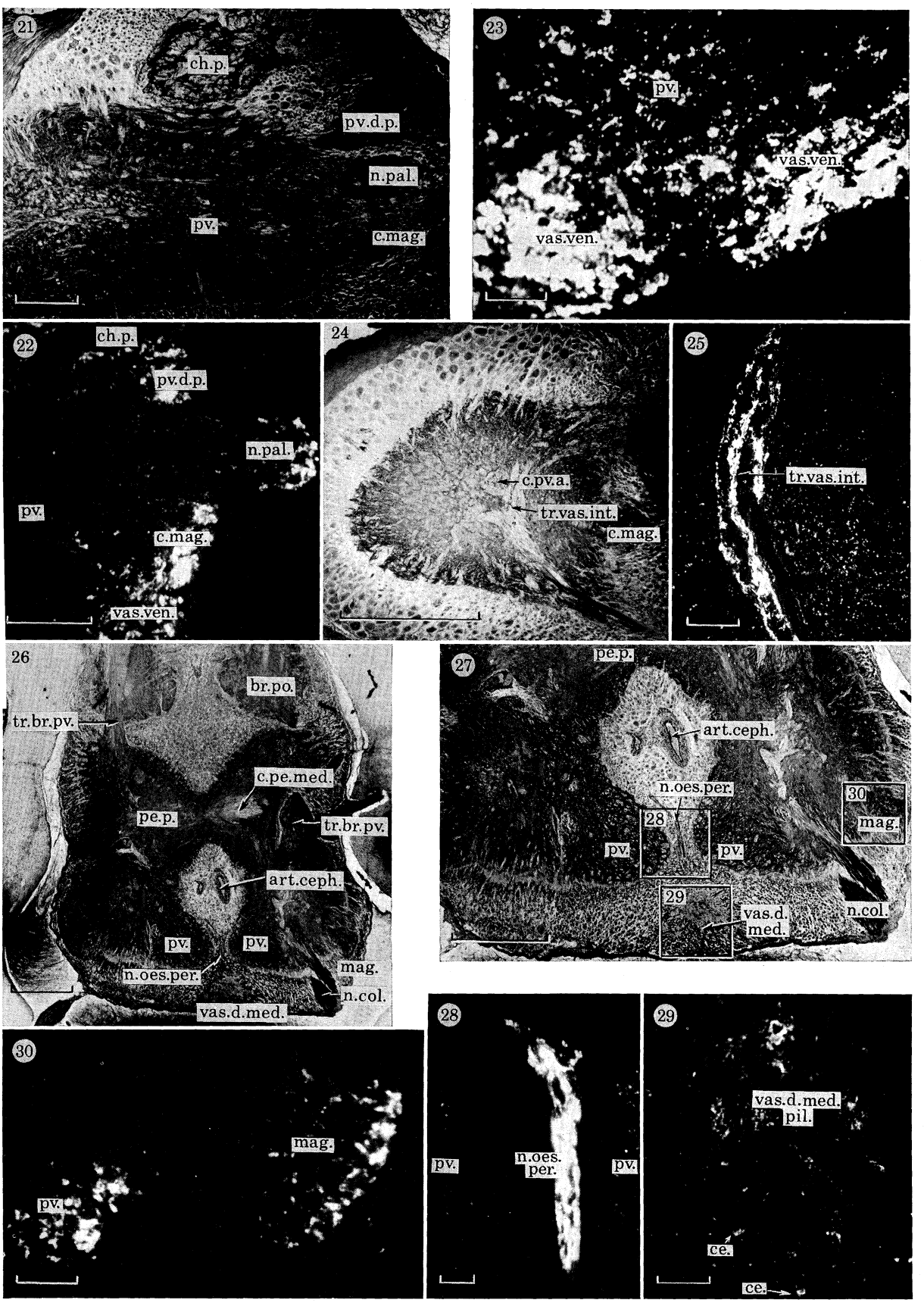
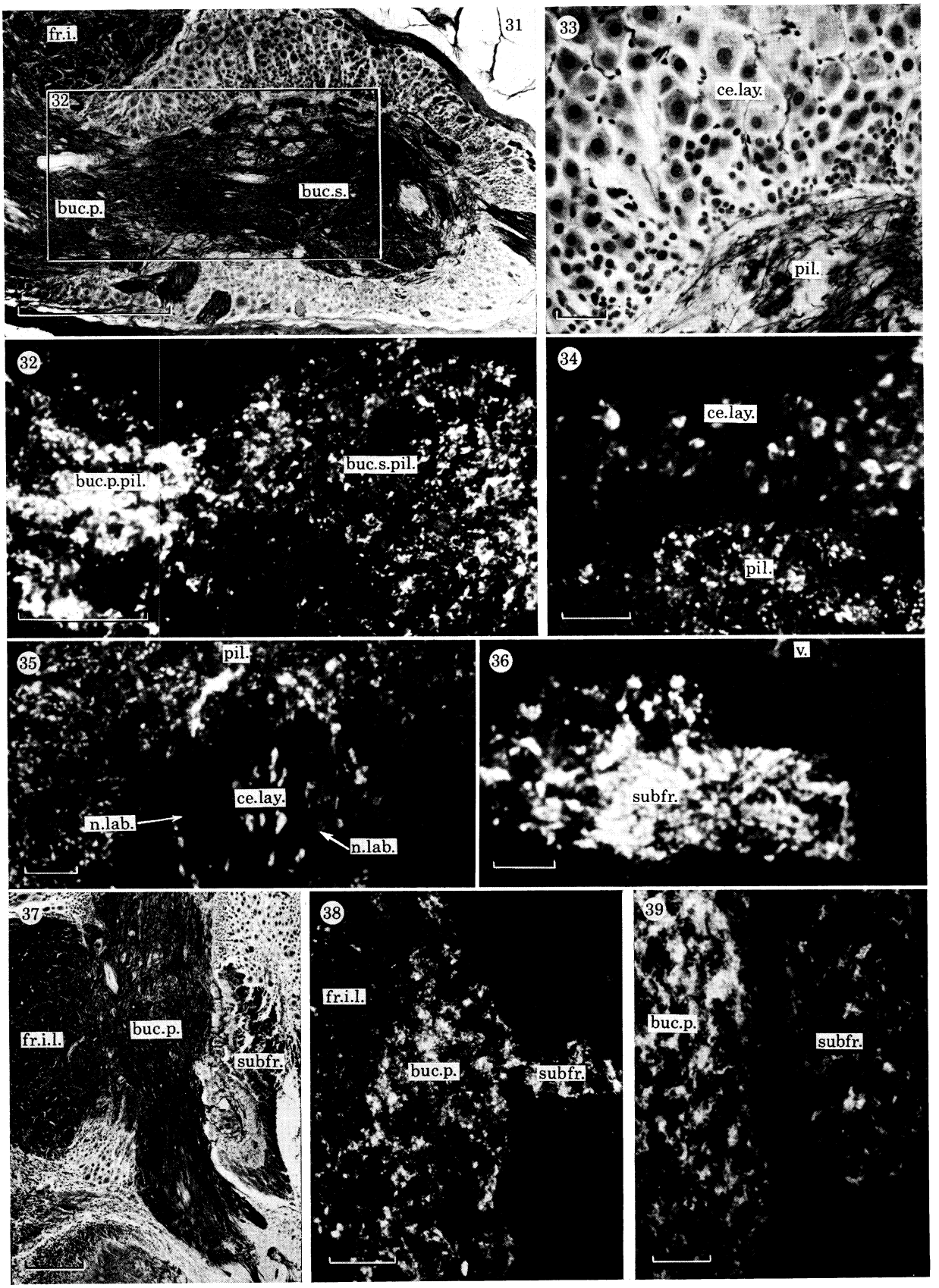


FIGURES 11–20. For description see page 130.



FIGURES 21–30. For description see page 131.



FIGURES 31–39. For description see opposite.

This procedure increased the levels of fluorescence observed.

4. The specificity test of Corrodi *et al.* (1964) was used. After being viewed, the slides were placed in a 1 mg ml^{-1} solution of sodium borohydride in 90% alcohol for 30 min. On being viewed again, all specific fluorescence due to catecholamines and 5HT was found to have disappeared and only non-specific autofluorescence remained. Re-exposing the slides to glyoxylic acid generated weak specific fluorescence.

5. Vapour-steaming the slide for 5 min after exposure to glyoxylic acid eliminated all but the non-specific tissue autofluorescence.

(*e*) *Acetylcholinesterase histochemistry*

For comparison, reference will be made to the localization of acetylcholinesterase in some parts of the brain. For this technique, brains were fixed in a cold sucrose–formalin fixative (Pearson 1967) and were stained by the method of Koelle (1950, 1951) modified by Gomori (1952)

Description of plate 3

Unless otherwise stated scale bars represent 100 μm.

FIGURE 21. Cajal preparation of the posterior suboesophageal mass of *Eledone cirrosa*. Sagittal section. Scale bar, 500 μm.

FIGURE 22. Fluorescence in the posterior suboesophageal mass of *Octopus vulgaris*. Sagittal section.

FIGURE 23. Fluorescence in the neuropil of the palliovisceral and ventral vasomotor lobes of *Eledone cirrosa*. Transverse section.

FIGURE 24. Cajal preparation of the posterior suboesophagal mass of *Octopus vulgaris*. Sagittal section. Inset shows the approximate location of figure 25. Scale bar, 1 mm.

FIGURE 25. Fluorescence in the intervasomotor tract of *Octopus vulgaris*. Sagittal section.

FIGURE 26. Cajal preparation of the suboesophageal mass of *Octopus vulgaris*. Horizontal section. Inset shows the location of figure 27. Scale bar, 1 mm.

FIGURE 27. Cajal preparation of the middle and posterior suboesophageal masses of *Octopus vulgaris*. Horizontal section. Insets show the approximate locations of figures 28–30.

FIGURE 28. Fluorescence in the perioesophageal nerve of *Octopus vulgaris*. Horizontal section.

FIGURE 29. Fluorescence in the neuropil of the ventral vasomotor lobe of *Octopus vulgaris*. Horizontal section.

FIGURE 30. Fluorescence in the lateral magnocellular lobe of *Octopus vulgaris*. Horizontal section.

Description of plate 4

Unless otherwise stated scale bars represent 100 μm.

FIGURE 31. Cajal preparation of the superior and posterior buccal lobes of *Octopus vulgaris*. Sagittal section. Inset shows the approximate location of figure 32.

FIGURE 32. Fluorescence in the superior and posterior buccal lobes of *Octopus vulgaris*. Sagittal section.

FIGURE 33. Cajal preparation of the cell layer of the superior buccal lobe of *Eledone cirrosa*. Transverse section.

FIGURE 34. Fluorescence in the cell layer of the superior buccal lobe of *Octopus vulgaris*. Transverse section.

FIGURE 35. Fluorescence in the superior buccal lobe of *Octopus vulgaris*. Note the 'finely beaded' fluorescence in the labial nerves. Transverse section.

FIGURE 36. Fluorescence in the subfrontal lobe of *Octopus vulgaris*. Sagittal section.

FIGURE 37. Cajal preparation of the front part of the supraoesophageal brain of *Eledone cirrosa*. Horizontal section. Scale bar, 250 μm.

FIGURE 38. Fluorescence in the inferior frontal, posterior buccal and subfrontal lobes of *Octopus vulgaris*, in a horizontal section similar to that shown in figure 37.

FIGURE 39. Fluorescence in the posterior buccal and subfrontal lobes of *Octopus vulgaris*, in a horizontal section more ventral to that of figure 38.

as described by Drury & Wallington (1973), and subjected to the intensification procedure of Henderson (1967). The material stained for acetylcholinesterase was viewed and photographed in the same way as the conventional histological sections.

Results

Introduction

Fluorescence typical of catecholamines and 5HT was demonstrated in the central nervous systems of all the cephalopod species examined. From here on, the term fluorescence will be used to denote specific aminergic fluorescence not present in control sections. The fluoroescence in octopod species was consistently more intense than that in decapods, which agrees with the quantitative data obtained from whole brains by Juorio (1971) and by Juorio & Barlow (1974). For this reason particular attention was paid to the octopod species. In most lobes fluorescence was confined to the neuropil (figure 4), but some cells in the magnocellular, vasomotor, median basal, posterior buccal and superior buccal lobes (figure 5) also displayed fluorescence. Fluorescence was also noted around some blood vessels (figure 6) and along nerve tracts and commissures (figure 7).

In control sections fluorescence was either absent or too weak to photograph. In all tissues, however treated, autofluorescence was present; this appears as small orange granules 1–3 μm in diameter. The specific fluorescence that was obtained was predominantly green, indicating the presence of noradrenalin and dopamine. The yellow fluorescence typical of 5HT was found associated with green fluorescence in the medulla and cortex of the optic lobe, in the anterior basal, subpedunculate, subvertical, precommissural, superior buccal, olfactory, peduncle, pedal and brachial lobes. The only area that appeared to be entirely yellow was the optic gland. The green fluorescence in all lobes faded within 30 min after continuous ultraviolet exposure, but the yellow fluorescence faded after 5 to 10 min.

The results from the brain will be considered in the six following subdivisions:

(*a*) The suboesophageal lobes.

(*b*) The buccal lobe system: the superior and posterior buccal lobes, the inferior frontal and the subfrontal lobes.

(*c*) The vertical lobe system: the vertical, subvertical and the precommissural lobes and the cerebral tract.

(*d*) The basal lobe system: the anterior, median, dorsal, lateral and interbasal lobes and the subpedunculate lobe.

(*e*) The optic lobe.

(*f*) The optic tract lobes: the peduncle and olfactory lobes, the optic gland and the subpedunculate tissue.

(*a*) *Suboesophageal lobes*

The suboesophageal brain of cephalopods comprises three separate regions: the anterior, median and posterior masses. This area has not previously been examined with fluorescence histochemistry. Routinely, the suboesophageal brain was examined after the supraoesophageal brain so that some fading had already occurred and therefore less extensive data is available for this area.

The anterior suboesophageal mass is divided into the pre- and postbrachial lobes (figure 8) and the neuropil of both these regions show widespread fluorescence such as that shown in

figures 9 and 10. The fluorescence in this region was green and yellow, and was particularly intense around the roots of the brachial nerves (figures 11, 12) although no fluorescence was noted in the brachial nerves themselves.

The middle suboesophageal mass is divided into a number of lobes; the neuropil of most of these fluoresce. The fluorescence in this area appears to be less intense than that in either the anterior or posterior suboesophageal masses. No fluorescence was noted in the anterior chromatophore lobe (figures 13, 14) nor in the anterior funnel lobe, nor in the chromatophore connectives nor the pedal commissures. Some sparse fluorescence was noted in the brachiopalliovisceral tract, but the strongest fluorescence in this region was in the neuropil of the anterior, posterior and lateral pedal lobes (figures 15–19). This fluorescence, which was green and yellow, was particularly strong in the lateral lobe (figure 19). Many of the tracts connecting the sub- and supraoesophageal parts of the brain showed fluorescence, as exemplified by the anterior basal to suboesophageal connective (figure 20).

In the posterior suboesophageal mass, the neuropil of the palliovisceral and the dorsoposterior lobe fluoresce but the posterior chromatophore lobe has not been observed to show fluorescence (figures 21, 22). Fluorescence is also absent from all three palliovisceral commissures and from the posterior chromatophore commissure. There is strong fluorescence in the neuropil and in some cell bodies of the vasomotor lobes (figures 23, 29). Of nerve fibres entering or leaving the mass, the visceral nerves show bright fluorescence, as do parts of the collar nerve, pallial nerve and magnocellular commissure, but there has been no fluorescence observed along the lengths of these nerves (figure 22). Particularly strong fluorescence was noted along the intervasomotor tract (figures 24, 25), and along the perioesophageal nerve (figures 26–28). The neuropil and cell layer of the dorsal vasomotor lobe also showed fluorescence (figures 27, 29), as did the neuropil of the magnocellular lobe (figures 27, 30).

(*b*) *Buccal lobe system*

In the octopod species examined, fluorescence has been observed in the neuropil of the superior and posterior buccal lobes and in the subfrontal and median and lateral inferior frontal lobes. The superior and posterior buccal lobes showed the most intense fluorescence, and both inferior frontal lobes the least intense fluorescence of this group of lobes. The fluorescence of the decapod posterior buccal lobe was less intense than that in any of the octopod lobes. In two animals of each species the neuropil of the inferior buccal lobe of *Eledone cirrosa* and of the inferior and superior buccal lobes of *Sepia officinalis* were examined and also showed fluorescence.

The fluorescence in the superior and posterior buccal lobes was particularly strong in the neuropil (figures 31, 32). Fluorescence was also located in the cell layers of both buccal lobes, there being a higher proportion of fluorescent cells in the superior lobe (figure 5). Figure 34 shows the distinct gap between the fluorescent cells and the fluorescent neuropil of the superior buccal lobe. This area corresponds to the layer of small cells that surrounds the neuropil. The fluorescence is confined to the larger, more peripheral cells that are clearly shown in the Cajal preparation (figure 33). Some of the cells and part of the neuropil of the buccal lobes exhibited yellow fluorescence characteristic of 5HT, but this was not restricted to any particular area. No cell layer fluorescence was seen in any of the buccal lobes of any decapod species examined.

The superior buccal lobe receives nerves from the posterior salivary glands and as these salivary glands enter the lobe posteriorly they are seen to fluoresce quite strongly. Similarly,

the bundles of labial nerves that enter the superior buccal lobe anteriorly show the pattern of fluorescence typical of nerve tracts (figure 35).

The subfrontal lobe showed very strong fluorescence in the neuropil (figures 36–39) but no fluorescence was observed in the cell layer. In contrast, the fluorescence in the neuropil of both the median and the lateral inferior frontal lobes was less dense and, when compared with the Cajal preparation, rather irregular in distribution (figures 40, 41).

The only representative of these lobes that is present in the decapod brain, the posterior buccal lobe, fluoresced less intensely than the buccal lobes of octopods. The fluorescence in this lobe was confined entirely to the neuropil (figures 42, 43).

(*c*) *Vertical lobe system*

In the vertical lobe of all species examined, bright fluorescence was located in both the inner and outer neuropil but none was noted in the cell layer (figures 44–46). This distribution of fluorescence is shown in horizontal section through a median lateral lobule of the vertical lobe of *Octopus vulgaris* (figure 46), while the transverse section (figures 44, 45) clearly shows the regions of neuropil, the outer neuropil fluorescing in a band beneath the cell layer, where the fibres from the superior frontal lobe terminate, and the inner neuropil where the subvertical

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Description of plate 5

Unless otherwise stated scale bars represent 100 μm.

Figure 40. Cajal preparation of the median inferior frontal lobe of *Octopus vulgaris*. Sagittal section.

Figure 41. Fluorescence in the median inferior frontal lobe of *Octopus vulgaris*. Sagittal section.

Figure 42. Cajal preparation of the supraoesophageal lobes of *Sepia officinalis*. Inset shows the approximate location of figure 43. Sagittal section. Scale bar, 500 μm.

Figure 43. Fluorescence in part of the neuropil of the posterior buccal lobe of *Sepia officinalis*. Sagittal section.

Figure 44. Cajal preparation of the supraoesophageal lobes of *Octopus vulgaris*. Transverse section. Insets show the approximate locations of figures 45 and 49. Scale bar, 500 μm.

Figure 45. Fluorescence in one gyrus of the vertical lobe of *Eledone cirrosa*. Transverse section.

Figure 46. Fluorescence in a median–lateral gyrus of the vertical lobe of *Octopus vulgaris*. Horizontal section.

Figure 47. Cajal preparation of the vertical and subvertical lobes of *Octopus vulgaris*. Sagittal section.

Figure 48. Aminergic fluorescence in the vertical and subvertical lobes of *Eledone cirrosa*. Note the sparse fluorescence in the vertical–subvertical tracts. Sagittal section.

Figure 49. Fluorescence in the subvertical and precommissural lobes of *Octopus vulgaris*. Transverse section.

Description of plate 6

Unless otherwise stated scale bars represent 100 μm.

Figure 50. Fluorescence in the subvertical lobe of *Eledone cirrosa*. Some fluorescence in the vertical–subvertical tracts. Horizontal section.

Figure 51. Cajal preparation of the superior frontal lobe of *Octopus vulgaris*. Transverse section. Insets show the approximate locations of figures 52 and 53.

Figure 52. Fluorescence in the lateral superior frontal lobe of *Eledone cirrosa*. Transverse section.

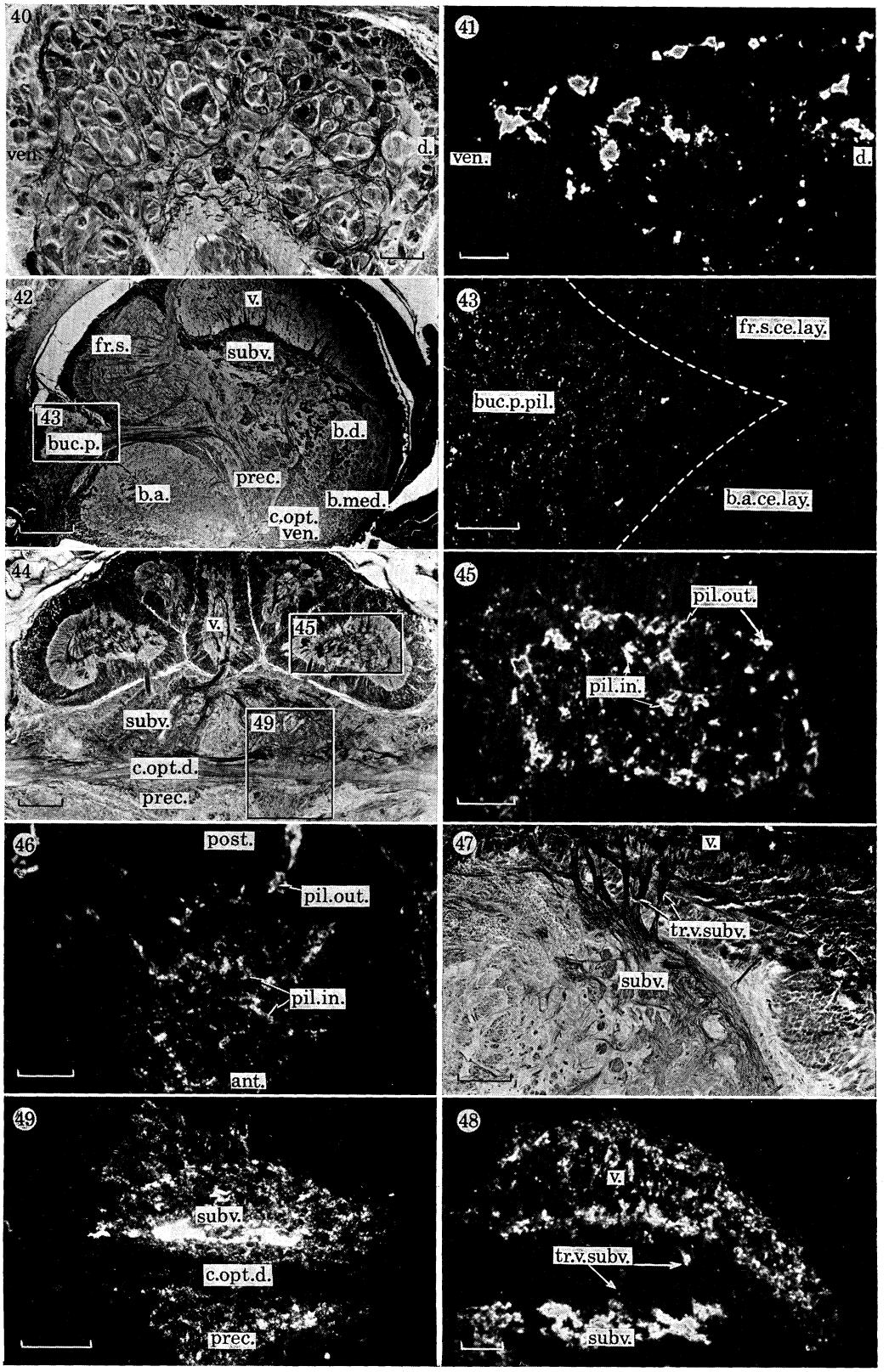
Figure 53. Fluorescence in the median superior frontal lobe of *Eledone cirrosa*. Transverse section.

Figure 54. Cajal preparation of the superior frontal lobe of *Eledone cirrosa*. Sagittal section. Scale bar, 250 μm.

Figure 55. Fluorescence in the superior frontal lobe of *Octopus vulgaris*. Sagittal section.

Figure 56. Cajal preparation of part of the supraoesophageal brain of *Eledone cirrosa*. Sagittal section. Inset shows the approximate location of figure 57.

Figure 57. Fluorescence in the cerebral tract of *Eledone cirrosa*. Sagittal section.



FIGURES 40–49. For description see opposite.

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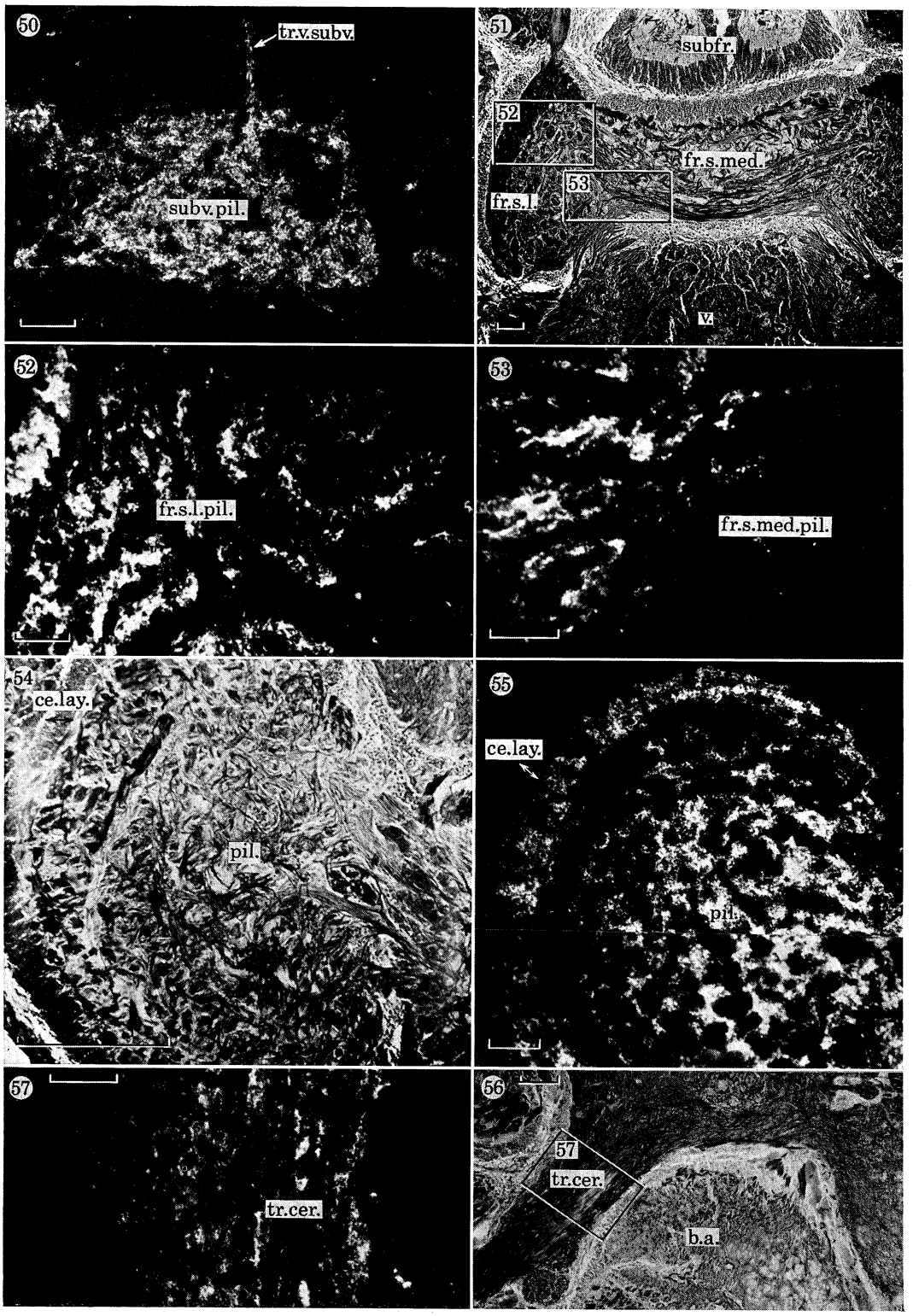
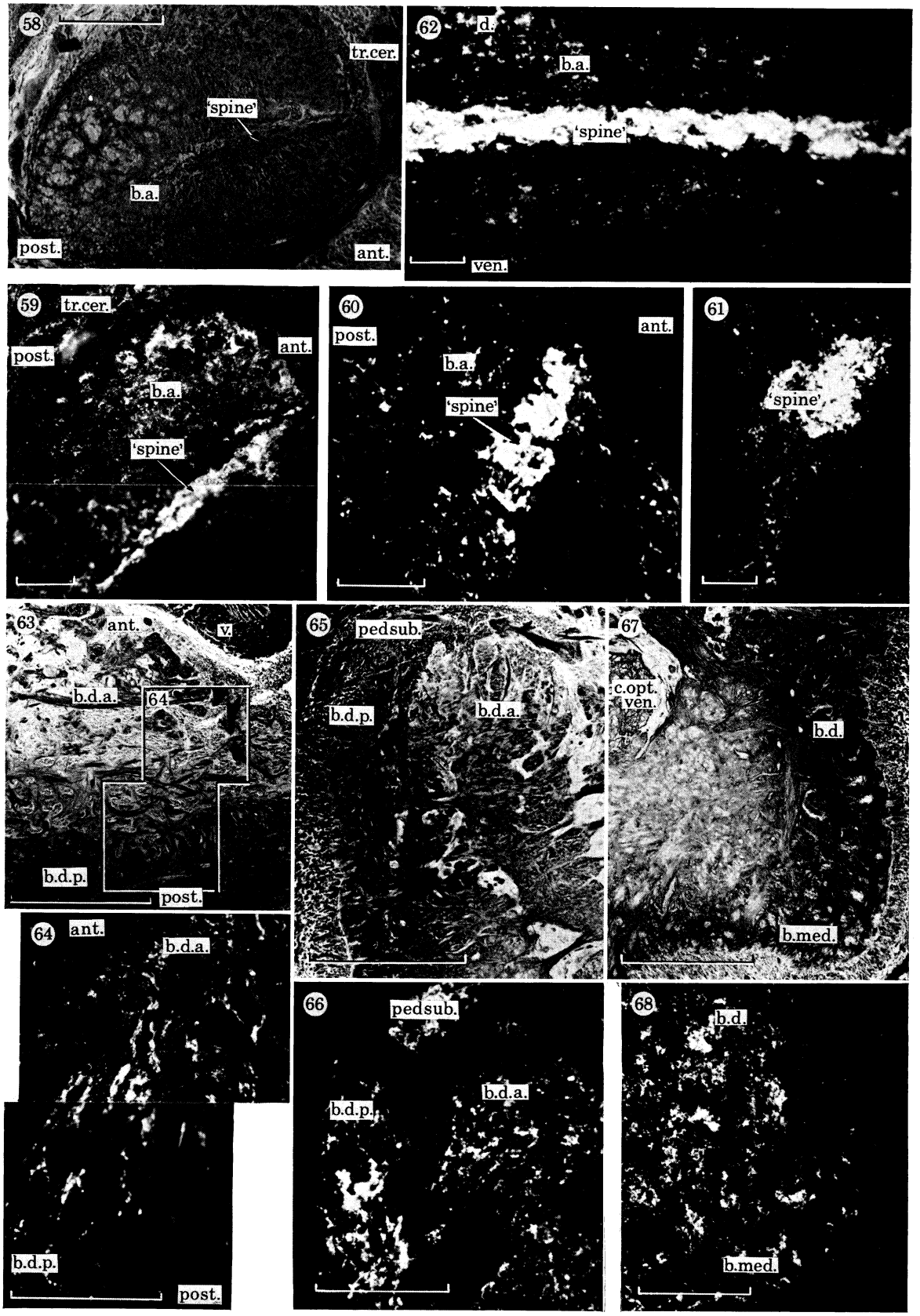
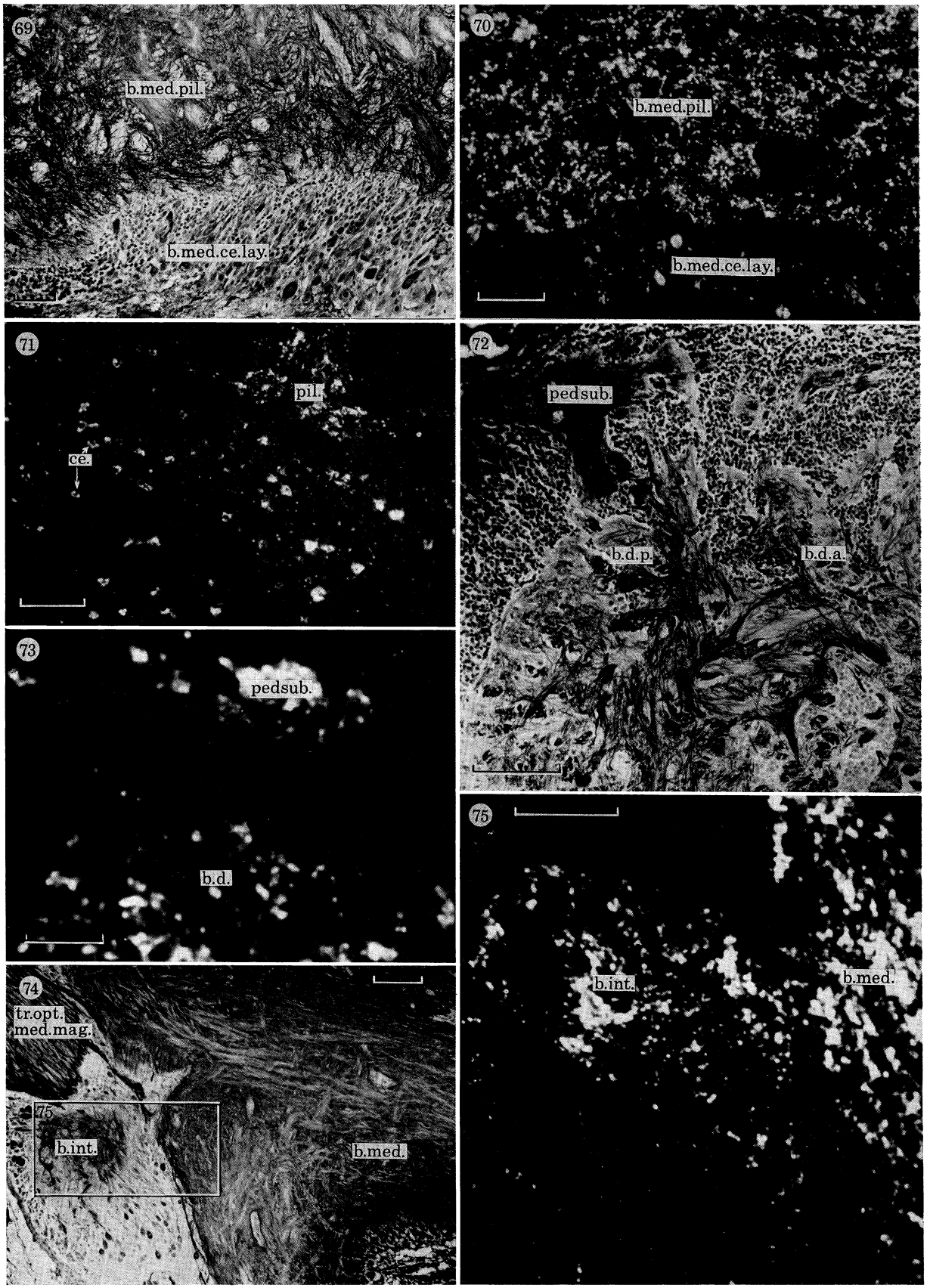


FIGURES 50–57. For description see page 134.



FIGURES 58–68. For description see page 135.



FIGURES 69–75. For description see opposite.

fibres terminate, as also shown in figures 47 and 48. This latter figure also shows some sparse fluorescence in the subvertical–vertical tracts, and the intense fluorescence in the subvertical lobe itself.

The subvertical lobe is one of the most intensely fluorescent of all the supraoesophageal lobes (figures 48–50). The fluorescence in the neuropil is extremely bright and there is no reaction in the cell layer. Figure 49 shows the fluorescence in both the subvertical and precommissural lobes. Much of the fluorescence in both lobes is yellow.

In the lateral and median superior frontal lobes of all species examined, no fluorescence was noted in the cell layer, although both neuropil fluoresced brightly (figures 51–55). The neuropil of the lateral part of the lobe is consistently brighter than that of the median part (figures 52, 53).

The cerebral tract showed aminergic fluorescence along its length (figures 56, 57). A separate, well defined subpedunculate nerve was never observed in fluorescent sections.

(*d*) *Basal lobe system*

The anterior basal lobe is considered to be divided into two parts one anterior and one posterior. One part extends transversely, while the other extends dorso-ventrally, and both

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Description of plate 7

Unless otherwise stated scale bars represent 100 μm.

Figure 58. Cajal preparation of the anterior basal lobe of *Eledone cirrosa*. Sagittal section. Scale bar, 250 μm.

Figure 59. Fluorescence in the anterior basal lobe of *Octopus vulgaris*; showing particularly prominent fluorescence in the 'spine' region of the anterior basal lobe. Sagittal section.

Figure 60. Fluorescence in the anterior anterior basal lobe of *Sepia officinalis*. Sagittal section.

Figure 61. Fluorescence in the 'spine' of the anterior anterior basal lobe of *Sepia officinalis*. Sagittal section.

Figure 62. Fluorescence in the anterior basal lobe of *Eledone cirrosa*; showing strong fluorescence in the spine of the posterior anterior basal lobe. Transverse section.

Figure 63. Cajal preparation of the dorsal basal lobe of *Eledone cirrosa*. Horizontal section. Inset shows the approximate location of figure 64. Scale bar, 250 μm.

Figure 64. Fluorescence in the dorsal basal lobe of *Octopus vulgaris*. Horizontal section. Scale bar, 250 μm.

Figure 65. Cajal preparation of the dorsal basal lobe of *Eledone cirrosa*, showing the 'spine' area of the neuropil. Sagittal section.

Figure 66. Fluorescence in the dorsal basal lobe of *Eledone cirrosa*, showing the arrangement of fibres in the 'spine' area. Sagittal section. Scale bar, 250 μm.

Figure 67. Cajal preparation showing the confluent neuropil of the median and dorsal basal lobes of *Octopus vulgaris*. Sagittal section. Scale bar, 250 μm.

Figure 68. Fluorescence in the neuropil of the median basal lobe of *Octopus vulgaris*. Sagittal section.

Description of plate 8

Unless otherwise stated scale bars represent 100 μm.

Figure 69. Cajal preparation of the ventral part of the median basal lobe of *Eledone cirrosa*. Sagittal section.

Figure 70. Fluorescence in the ventral part of the median basal lobe of *Octopus vulgaris*. Sagittal section.

Figure 71. Fluorescence through the cell layer of the median basal lobe of *Eledone cirrosa*. Sagittal section.

Figure 72. Cajal preparation of the median basal and interbasal lobes of *Octopus vulgaris*. Transverse section. Scale bar, 250 μm.

Figure 73. Fluorescence in the neuropil of the median basal and interbasal lobes of *Eledone cirrosa*. Transverse section.

Figure 74. Cajal preparation of the subpedunculate lobe of *Eledone cirrosa*. Sagittal section.

Figure 75. Fluorescence in the neuropil of the dorsal basal lobe and subpedunculate lobe of *Octopus vulgaris*. Sagittal section.

regions contain areas of very fine parallel fibres, similar in morphology to the spine region of the peduncle lobe (Young 1977). Both these areas will be referred to as 'spines'.

The neuropil of the anterior basal lobes of all species examined displayed strong green fluorescence, the fluorescence being more intense in the octopod species. No fluorescence was observed in any cell bodies. Of particular interest is the very intense fluorescence in the 'spine' regions of the neuropil, the fluorescence in these regions being green and yellow (figures 58–62). The 'spines' of both octopod and decapod species displayed this strong, very characteristic fluorescence.

The dorsal basal lobe showed fluorescence in the neuropil but none in the cell layer (figures 63–66), the fluorescent neuropil being contiguous with that of the median basal lobe. Indeed, in some fluorescent sections it is very difficult to distinguish the two (figures 67, 68). However, in horizontal sections through the dorsal basal lobe, the strands of neuropil of the anterior and posterior parts of the lobe can be clearly seen fluorescing while the intermittent groups of cells do not fluoresce (figures 63, 64). The dorsal basal lobe also contains an array of parallel fibres similar to those of the peduncle and anterior basal lobes. This 'spine' region contains strong fluorescence (figures 64, 65).

In the median basal lobe, fluorescence was located in the neuropil (figures 67, 68) and in some of the cell bodies of the ventral cell layer (figures 69–71). The fluorescence was predominantly in the large cells of at least 10 μm diameter. It is difficult to distinguish the neuropil of the lateral basal lobes as it is continuous with the neuropil of the median basal lobe. However, the interbasal lobe did show fluorescence (figures 74, 75). The two optic commissures, dorsal and ventral, displayed slightly beaded fluorescence (figures 7, 49).

The subpedunculate lobe yields intense green and yellow fluorescence, clearly distinct from the fluorescence of both the dorsal basal and subvertical lobes (figures 72, 73).

(*e*) *Optic lobe*

The optic lobe of all species examined displayed strong fluorescence in the cortex and in the medulla. These divisions of the optic lobe are shown in figure 76. The cortex consists of an inner and an outer granular cell layer, with a plexiform layer between them. The plexiform layer is a region of synaptic contact and is organized into eight layers, four in the radial plane (r1–r4) and four in the tangential plane (t1–t4) (Young 1971). Decapods have an additional layer, the inner plexiform layer (see figure 78).

In the cortex neither the inner nor the outer cell layers fluoresced. The plexiform layer fluoresced very brightly, but it was not possible to distinguish a consistently precise pattern in the radial and tangential layers of every animal examined. It was especially difficult to distinguish the layers in the octopod species where the fluorescence was the most intense (figure 77). The less intensely fluorescent decapod plexiform layers were easier to distinguish, as shown in *Sepia officinalis* (figures 78, 79). There is no fluorescence in r1, but t1 is very bright; it would also appear that r2, r3 and r4 are all weakly fluorescent, while t2 and t3 are non-fluorescent and t4 is very brightly fluorescent. In *Sepiola atlantica* there is also a clearly non-fluorescent band in the region of r3/t3 (figure 80). In the decapod species the inner plexiform also showed fluorescence (figures 79 and 80). In all species examined the fluorescence was green and only in *Octopus vulgaris* was strong yellow fluorescence observed. This was in the t1/t2 region of the plexiform layer. In the optic lobes of octopods there were distinctly fluorescent fibres leaving the plexiform layer and passing through the inner granular layer into the medulla (figure 81). There were no such fluorescent fibres in the decapod brains examined.

In the medulla of the optic lobe the neuropil fluoresced brightly in all genera examined (figure 82).

(*f*) *Optic tract lobes*

The peduncle lobe, which is divided into two clearly distinct regions, the spine and the basal zone (Messenger 1967*a*), showed fluorescence in the neuropil of both areas; there was no fluorescence in the cell layer.

In the peduncle lobe spine the fluorescence was green and yellow. One particularly interesting finding was that the two banks of the spine fluoresced differentially (figures 83–86). Although this difference was not quantified, it was obvious to the eye and on film that the more lateral bank was consistently brighter than the median bank. A previously unreported fact is that the lateral bank is itself subdivided into two separate elements, the more lateral of which fluoresces the more brightly (figures 84, 86). This intensity difference was very pronounced in both *Eledone* and *Octopus* but was slight in the decapod species examined, probably in keeping with the generally lower level of fluorescence observed in the decapods.

The basal zone neuropil fluoresced in all species examined, and some of the optico-peduncular tracts also displayed fluorescence (figures 87, 88).

In *Eledone cirossa* the olfactory lobe is divided into two lobules, and in *Octopus vulgaris* it is divided into three lobules (Messenger 1965). In *Eledone cirrosa* the neuropil of both lobules fluoresced brightly (see figure 4), but there was noticeably brighter fluorescence from the anterior lobule (figure 89). In *Octopus vulgaris* all three lobules fluoresced but the neuropil of the median lobule was the most intense (figure 90). One consistent feature of this region was that the olfactory nerve displayed bright green fluorescence along its length as it entered the olfactory lobe (figures 89, 91).

Other components of this region of the cephalopod brain were also examined. Dots of fluorescence were noted across the optic tract that were attributed to the optico-peduncular tracts (figures 92, 93). The optic gland, an endocrine organ (Wells & Wells 1959), displayed yellow fluorescence typical of 5HT (figure 94). A particularly interesting finding was the presence, between the optic tract and the optic lobe, of deep yellow fluorescing material, which represents strands of the subpedunculate tissue (figures 95–97). This tissue arises in the optic lobes as strands of cells around blood vessels at the hilum of the lobe and ends in a ring of tissue in the orbit (Boycott & Young 1956). The deep yellow fluorescence observed in this lobe, which was absent from control sections, is unlike the bright yellow fluorescence observed elsewhere. The possibility that it was caused by strands of white body was dismissed by examining preparations of white body tissue, which does not show fluorescence.

Discussion

(*a*) *General*

Specific aminergic fluorescence characteristic of dopamine, noradrenalin and 5HT has been observed in most regions of the cephalopod c.n.s. The present study has been in greater detail and included more species than the previous work of Matus (1973). Also, the technique employed here used glyoxylic acid, a chemical more sensitive for catecholamines and 5HT than formaldehyde (Lindvall *et al.* 1975), and one that has already been shown to be effective in other invertebrate nervous systems (Bolstad *et al.* 1979). Juorio & Killick (1972) noted that in the brain of *Octopus vulgaris* these amines were stored in reserpine-sensitive granules and

postulated that they may act as neurotransmitters. Other evidence, such as the specific neuronal uptake mechanisms for some of these amines (see, for example, Pollard *et al.* (1973)), increases the possibility that they act as neurotransmitters (see review by Tansey (1979)).

In the neuropil, the fluorescence observed was predominantly green, indicating the presence of dopamine and noradrenalin. The closely related amine adrenalin, which is also known to cause fluorescence, was below the level of sensitivity of the technique employed by Juorio (1971) and Juorio & Killick (1972) in an examination of *Octopus* brain. Yellow fluorescence typical of 5HT was located in the medulla and cortex of the optic lobe, in the superior buccal, anterior basal, precommissural, subvertical, subpedunculate, olfactory, peduncle, brachial and pedal lobes. One particular problem with fluorescence studies is to ascertain that the yellow fluorescence is indeed due to 5HT and not to a high concentration effect of catecholamines, for it has been demonstrated with the formaldehyde technique that cells containing large amounts of catecholamines will appear to be yellowish in the microscope (Bjorklund *et al.* 1972). This has been ascribed either to a misrepresentation of the colour by the eye (the Bezold–Bruicke effect (Ritzen 1967)) or to a concentration-dependent redshift in the emission maximum from around 470 nm to longer wavelengths (Caspersson *et al.* 1966; Jonsson 1971). However, the yellow fluorescence seen in the cephalopod brain was qualitatively similar to the fluorescence seen in the posterior salivary glands of *Octopus vulgaris* and *Eledone cirrosa*, which contain large amounts of 5HT (455 $\mu g\ g^{-1}$ and 238 $\mu g\ g^{-1}$ respectively (Juorio & Killick 1973)). The distribution of yellow fluorescence seen in the present work agrees closely with the quantitative analysis of 5HT by Juorio (1971). The formaldehyde-induced fluorescence study by Matus (1973) noted yellow fluorescence only in the peduncle lobe, which is probably because the fluorescence yields

Description of plate 9

Unless otherwise stated scale bars represent 100 μm.

Figure 76. Normal Cajal preparation of the optic lobe and optic tract lobes of *Octopus vulgaris*. Transverse section. Scale bar, 1 mm.

Figure 77. Fluorescence in the cortex of the optic lobe of *Octopus vulgaris*. Transverse section. Scale bar, 200 μm.

Figure 78. Cajal preparation of the plexiform layer of the optic lobe of *Sepia officinalis*. Horizontal section.

Figure 79. Fluorescence in the plexiform layer of the optic lobe of *Sepia officinalis*. Horizontal section.

Figure 80. Fluorescence in the cortex of the optic lobe of *Sepiola atlantica*. Horizontal section.

Figure 81. Fluorescence in the cortex of the optic lobe of *Eledone cirrosa*. The arrows indicate the strongly fluorescent fibres noted in the optic lobes of octopods. Horizontal section.

Figure 82. Fluorescence in the medulla of the optic lobe of *Eledone cirrosa*. Horizontal section.

Description of plate 10

Unless otherwise stated scale bars represent 100 μm.

Figure 83. Cajal preparation of the optic tract lobes of *Octopus vulgaris*. Horizontal section. Scale bar, 250 μm.

Figure 84. Fluorescence in the spine of the peduncle lobe of *Octopus vulgaris*. Horizontal section.

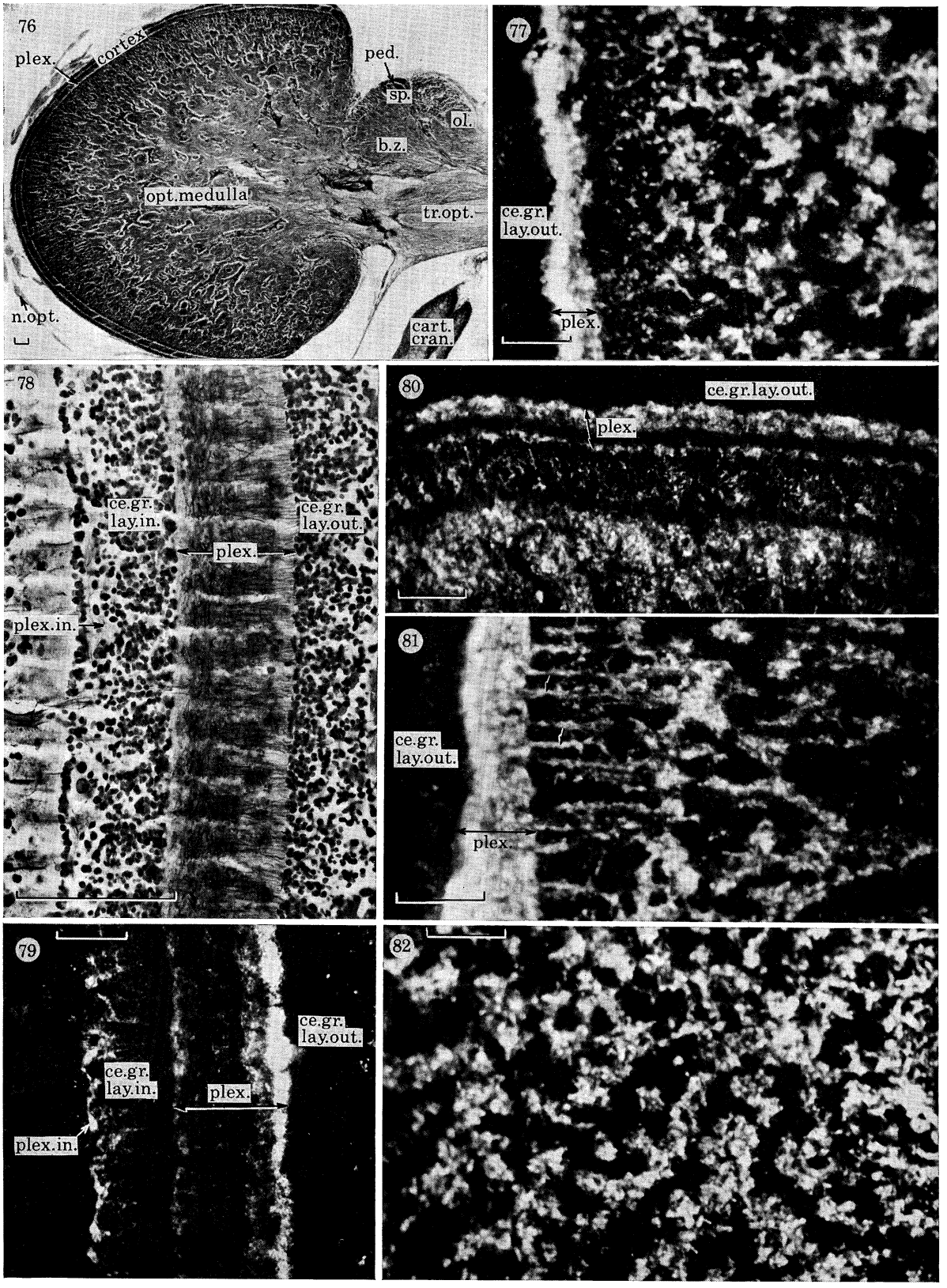
Figure 85. Cajal preparation of the optic tract lobes of *Eledone cirrosa*. Transverse section. Scale bar, 250 μm.

Figure 86. Fluorescence in the peduncle and olfactory lobes of *Octopus vulgaris*. Transverse section.

Figure 87. Cajal preparation of the peduncle basal zone and optico-peduncular tracts of *Eledone cirrosa*. Horizontal section.

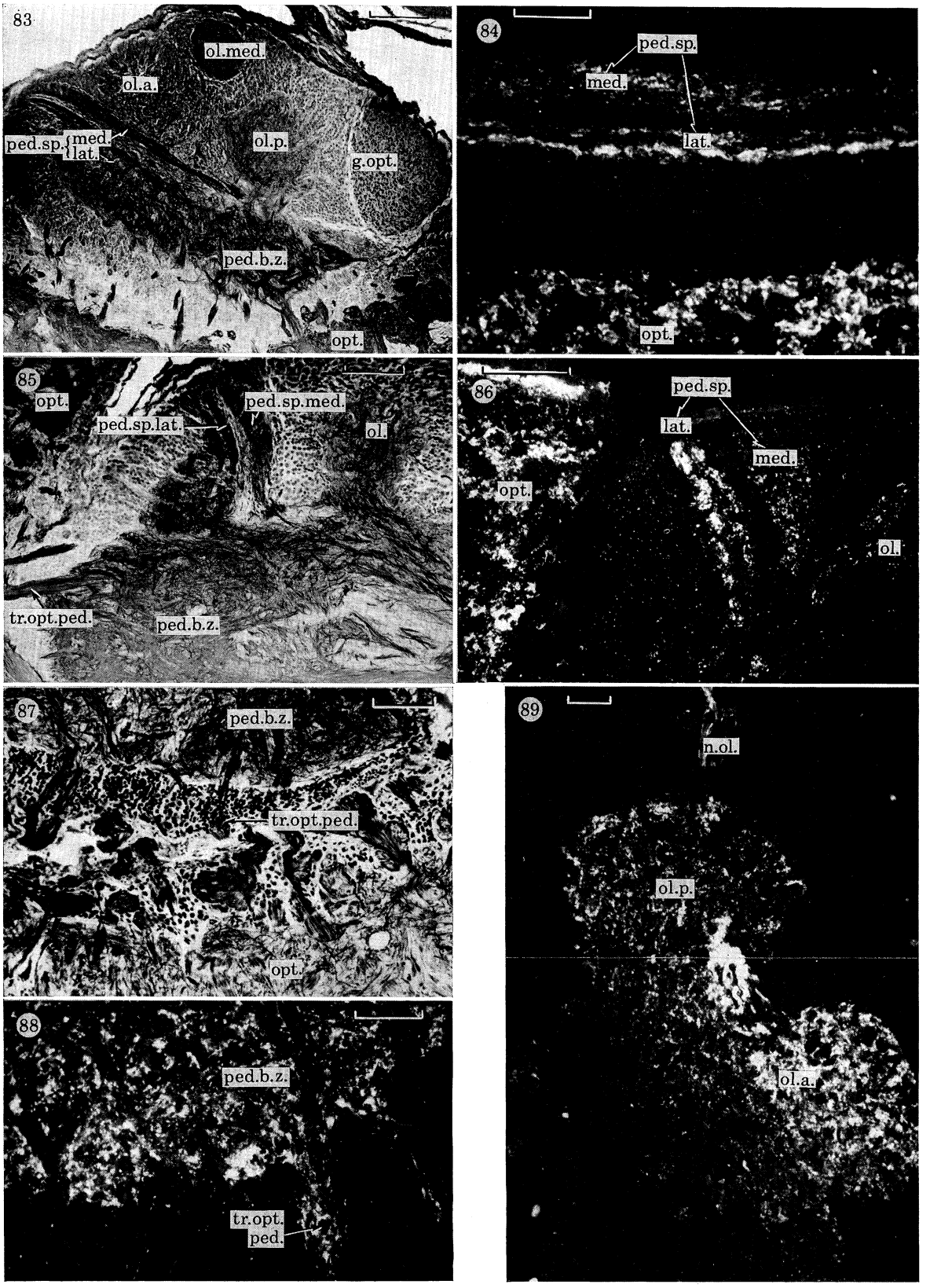
Figure 88. Fluorescence in the basal zone of the peduncle lobe of *Eledone cirrosa*. Note the fluorescence in some of the optico-peduncular tracts. Horizontal section.

Figure 89. Fluorescence in the lobules of the olfactory lobe and olfactory nerve of *Eledone cirrosa*. Horizontal section. Scale bar, 250 μm.

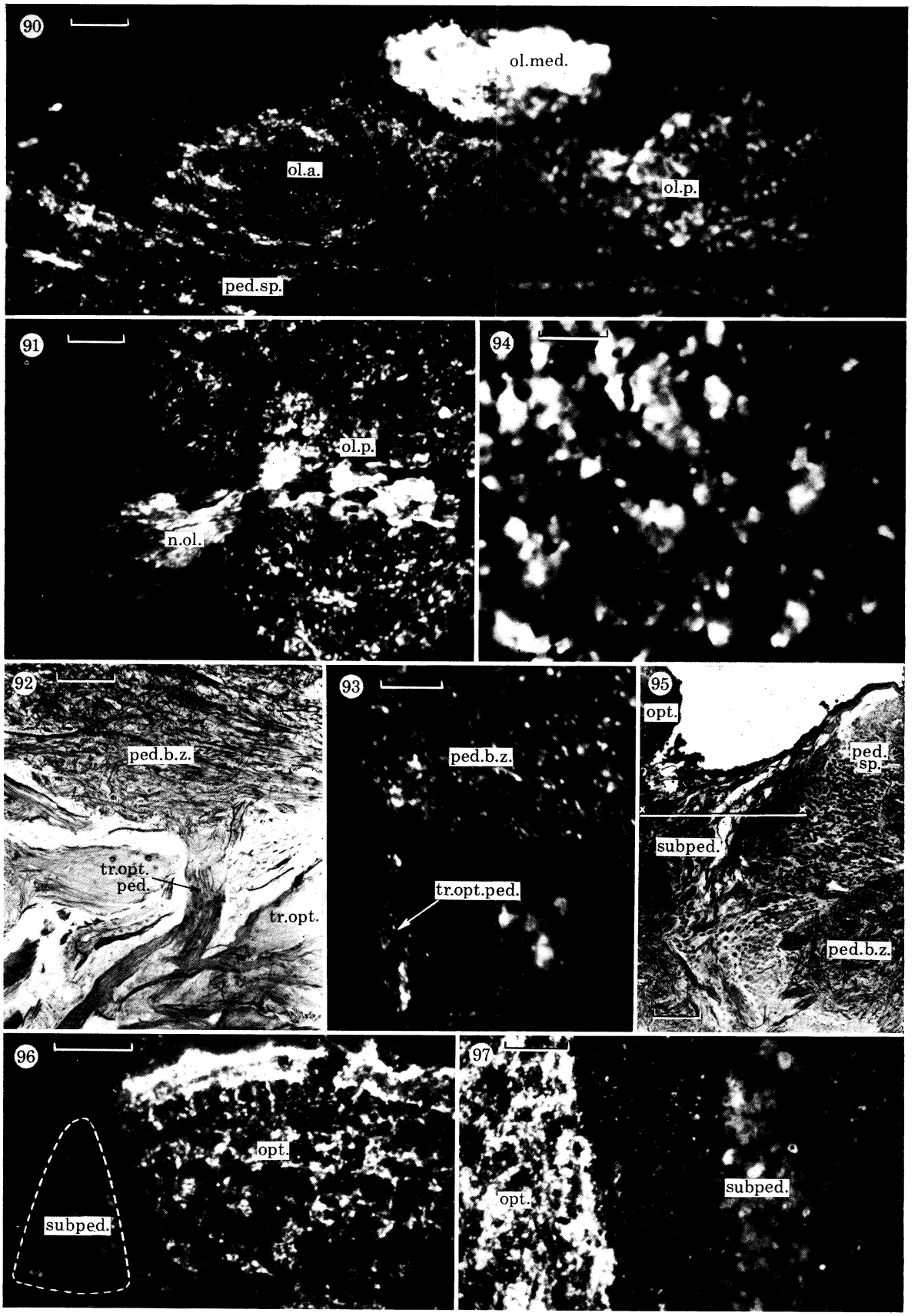


FIGURES 76–82. For description see opposite.

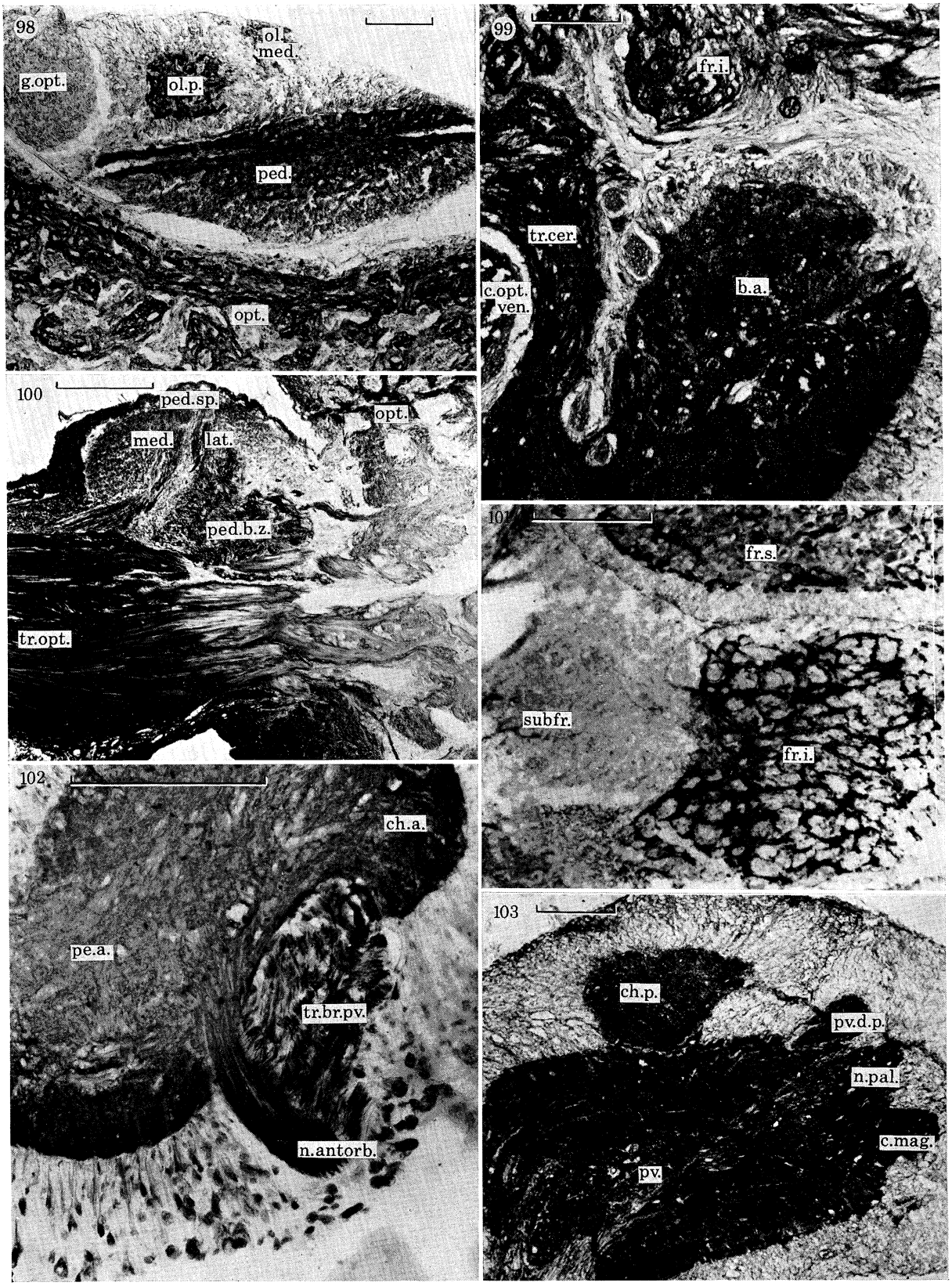
(*Facing p.* 138)



FIGURES 83–89. For description see page 138.



FIGURES 90–97. For description see page 139.



FIGURES 98–103. For description see opposite.

for 5HT are lower in the formaldehyde reaction than in the glyoxylic technique used in this study (Lindvall *et al.* 1975).

Aminergic fluorescence has also been noted in the cell layers of several lobes: the superior and posterior buccal, the median basal, magnocellular and vasomotor lobes, in comparison with Matus's (1973) observation of fluorescing cells only in the buccal lobes. Nerve tracts, on the whole, did not show strong fluorescence along their length, but showed streaky patches of fluorescence. This type of fluorescence, which has been described as 'finely beaded' by Brimijoin (1977) in his study of fluorescence in the rabbit sciatic nerve, may be accounted for by the axonal transport of biogenic amines along nerve tracts. This type of fluorescence was not seen in some nerves, e.g. optic nerves. The olfactory nerve was unusual in that it showed strong dense fluorescence.

As noted previously the fluorescence was less intense in decapod species than in octopod species, which is in agreement with the quantitative data obtained by Juorio & Barlow (1974). Also, specific areas, such as the lateral and median inferior frontal lobes, seemed to exhibit less intense fluorescence than other areas. There has not been a quantitative study of the glyoxylic acid-induced fluorescence, although there have been a number of such studies on the formaldehyde-induced fluorescence technique (Jonsson 1971; Dowson 1973; Kopin *et al.* 1974; Stanford 1976). These investigations have shown that there is a connection between fluorescence

Description of plate 11

Unless otherwise stated scale bars represent 100 μm.

Figure 90. Fluorescence in the olfactory lobe and peduncle lobe of *Octopus vulgaris*. Note the particularly bright reaction in the median lobule of the olfactory lobe. See figure 83 for approximate orientation. Horizontal section.

Figure 91. Fluorescence in the posterior lobule of the olfactory lobe of *Eledone cirrosa*. Horizontal section.

Figure 92. Cajal preparation of the optic tract and peduncle basal zone of *Eledone cirrosa*. Transverse section.

Figure 93. Fluorescence in the basal zone of the peduncle lobe and across the optic tract of *Eledone cirrosa*. Transverse section.

Figure 94. Fluorescence in the optic gland of *Eledone cirrosa*. Horizontal section. Scale bar, 250 μm.

Figure 95. Cajal preparation showing the strands of subpedunculate tissue of *Eledone cirrosa*. Transverse section. X–X indicates the position of the horizontal section in figure 97.

Figure 96. Fluorescence in the optic lobe and subpedunculate tissue of *Eledone cirrosa*. Transverse section.

Figure 97. Fluorescence in the optic lobe and subpedunculate tissue of *Eledone cirrosa*. Horizontal section.

Description of plate 12

Unless otherwise stated scale bars represent 250 μm.

Figure 98. Acetylcholinesterase distribution in the optic lobe and optic tract lobes of *Octopus vulgaris*. Horizontal section. Compare the enzyme distribution with the fluorescence shown in figures 84, 89 and 90.

Figure 99. Acetylcholinesterase distribution in some of the supraoesophageal lobes of *Octopus vulgaris*. Sagittal section. Compare the enzyme distribution in the anterior basal lobe with the fluorescence shown in figure 59.

Figure 100. Cajal preparation of the optic tract lobes of *Eledone cirrosa*, showing tracts entering the lateral and median banks of the peduncle spine. Transverse section.

Figure 101. Acetylcholinesterase distribution in the inferior frontal and subfrontal lobes of *Octopus vulgaris*. Sagittal section. Compare with the Cajal preparation and fluorescence distribution shown in figures 40 and 41.

Figure 102. Acetylcholinesterase distribution in the middle suboesophageal mass of *Eledone cirrosa*. Transverse section. Scale bar, 1 mm. Compare with the Cajal preparation and fluorescence distribution shown in figures 13 and 14.

Figure 103. Acetylcholinesterase distribution in the posterior suboesophageal mass of *Octopus vulgaris*. Sagittal section. Compare with the Cajal preparation and fluorescence distribution shown in figures 21 and 22.

intensity and tissue catecholamine concentration and it is therefore probable that, in the present research, an increase in the fluorescence intensity in sections of the same thickness indicates an increase in catecholamine concentration.

(*b*) *Peduncle and basal lobes*

The optic tract lobes of *Octopus vulgaris* develop as lateral extensions of the basal lobes (Messenger 1965, 1967*a*) and in many other genera the peduncle complex is also an extension of the dorsal basal lobe (Messenger 1965*a*, 1979). Evidence from *Octopus vulgaris* has indicated structural similarities between the peduncle and anterior basal lobes (Young 1971; Woodhams 1977), and more recent and complete evidence from studies on *Loligo* (Young 1977; Messenger 1979) has confirmed this close morphological and functional relationship between the peduncle and basal lobes, and so it is convenient to discuss these two areas together.

Messenger (1965, 1967*a*, *b*) noted that the anterior basal lobe was closely associated with the peduncle lobe in visuomotor control of *Octopus vulgaris*. In *Octopus* the anterior basal lobe receives the largest efferent tract from the peduncle lobe (see also Woodhams (1975)). This tract is derived largely from the anterior and middle regions of the peduncle spine and runs downwards and forwards into the ipsilateral anterior basal lobe. Its fibres end in the lateral and dorsal regions, which contain large numbers of small cells and have many parallel fibres organized like the peduncle spine. There has been some evidence that the arrays of parallel fibres in the peduncle and anterior basal lobes are associated with a similar array in the dorsal basal lobe to form a functional analogue of the vertebrate cerebellum (Hobbs & Young 1973; Young 1977; Woodhams 1977).

The histochemical evidence in this paper points to an additional similarity between the peduncle and basal lobes, 'elongate' patterns of fluorescence being noted in the spine regions of all these lobes. Particularly striking, however, are the 'spines' of the peduncle and anterior basal lobes (one in the peduncle lobe, two in the anterior basal lobe) in that both contained yellow fluorescence in addition to the green fluorescence in the remaining neuropil of both lobes (Messenger & Tansey 1979). However, in sections stained for acetylcholinesterase (figures 98, 99) this similarity between the two 'spine' areas is not so apparent.

It is of particular interest that there is an appreciable difference in the intensity of fluorescence between the median and lateral banks of the peduncle spine of octopods. It is perhaps worth stressing that Woodhams (1975) has presented ultrastructural evidence for chemical synapses in the peduncle spine. Woodhams (1975) has shown that of the afferents into the peduncle spine those from the contralateral peduncle lobe have been shown to project principally to the median bank (see, for example, figure 100), but, as yet, no principal input to the lateral bank has been defined. The precise pattern of aminergic fluorescence in the peduncle lobe offers a new technique for further elaborating the connectivity of this region.

(*c*) *Other supraoesophageal lobes*

Previous histochemical investigations of the supraoesophageal lobes have been concerned with monoamines in *Octopus vulgaris* (Barlow 1971; Matus 1973), acetylcholinesterase in *O. vulgaris* (Barlow 1971, 1977) and cholinesterase in *Sepia officinalis* (Chichery & Chichery 1974).

In the vertical lobe of *Octopus vulgaris* and in that of *Eledone cirrosa*, fluorescence is located in both inner and outer components of the neuropil. Barlow (1971) located fluorescence beneath the amacrine cell layer and postulated that this was in the region of the termination of the

fibres from the median superior frontal lobe, which are also known to contain many dense-cored vesicles that may be aminergic (Gray 1970). The more sensitive glyoxylic acid method detected a strong band of fluorescence in this region, thus reinforcing the previous view (Barlow 1971) that the superior frontal afferents to the vertical lobe include adrenergic fibres. Similarly, the present study detected fluoresecnce in the inner band of neuropil, where the afferents from the subvertical lobe terminate, again suggesting that some of these afferents are adrenergic. However, some of the fluorescence in the neuropil may be attributable to the fibres of the 'intrinsic' cells, the small cells whose axons do not leave the lobe. Without examining the patterns of fluorescence following selective lesions of the two afferent inputs it is not possible to distinguish precisely the origins of the bands of fluorescence in the vertical lobe neuropil.

In the superior frontal lobe itself, both the lateral and median parts displayed fluorescence. Matus (1973) notes that the deeper fibres of this lobe did not show fluorescence, although they did fluoresce in the present study. The difference is again probably attributable to the increased sensitivity of the technique used in the present study. Indeed, the superior frontal lobes, particularly of the octopod species, showed a very distinctive layer pattern that had not previously been noticed.

The fluorescence in the subvertical lobe is very intense, as is the fluorescence in the closely associated precommissural lobe. Again, this intensity effect was not observed by Matus (1973), and it may well be connected with the fact that these two lobes, which are broadly continuous, are in the centre of the cephalopod brain. They receive inputs from many systems, and therefore also lie at the functional centre of the brain. The intense fluorescence in these two lobes may therefore be a reflection of their important integrative role.

In the buccal lobes it is of particular interest that strong fluorescence has been found in the cell layer of the superior, and to a lesser extent, in the posterior buccal lobes. It has previously been shown with formaldehyde fluorescence histochemistry that some of the cells in both these buccal lobes fluoresce (Martin & Barlow 1972; Matus 1973), although the present study has shown this fluorescence to be more intense and widespread than previously thought. There is also evidence for neurosecretory cells in the superior buccal lobe of *Octopus vulgaris* (Bonichon 1968). So far these cells have not been compared with the fluorescing cells, although the glyoxylic acid technique does offer the possibility of doing this. It has also been shown that some of the superior buccal cells incorporate tritiated 5HT (Martin & Barlow 1972), although it is not certain that the incorporation is into cells that show fluorescence.

The two sets of nerve fibres that run from the superior buccal lobes, the labial nerves that pass to the lips and the salivary nerves that arise from the superior buccal and the subradular ganglia and innervate the posterior salivary glands, were observed to have the 'finely beaded' type of fluorescence. The glands themselves contain a large number of biologically active amines (Hartman *et al.* 1960; Juorio & Killick 1973). Ligation experiments have shown that these nerves transport noradrenalin from the brain to the gland (Barlow *et al.* 1974), which accounts for the patterns of fluorescence in these nerves.

The bright fluorescence in the subfrontal lobe is particularly interesting in comparison with results from acetylcholinesterase histochemistry, (figure 101) as there is no positive reaction in this lobe for acetylcholinesterase (Barlow 1977; Tansey, unpublished results). Although the presence of acetylcholinesterase is not a reliable indicator of cholinergic pathways, its absence, particularly from an area with strong fluorescence, is good evidence that most, if not all, the afferents to this region are adrenergic.

(*d*) *Optic lobe and other optic tract lobes*

In the medulla of the optic lobe the neuropil fluoresced brightly in all genera examined although once again the fluorescence was more intense in the octopods. Matus (1973) described the medulla of *Octopus vulgaris* as having a central area of bright fluorescence surrounded by a region of little or no fluorescence. Both these regions fluoresced brightly in this study, the only difference being that the yellow fluorescence was more prominent in the central area.

The neuropil of the olfactory lobes of all cephalopods examined displayed bright, regular fluorescence. In *Octopus vulgaris* the middle lobule was consistently brighter than the other two lobules, which is in agreement with the anatomical findings of Messenger (1965) that the larger of the two branches of the olfactory nerve terminates in this lobule. Although the olfactory nerve fluoresced very brightly it was not possible to trace it for a great distance, nor to see it divide.

The optic glands consistently showed a 'hazy' yellow, fast-fading fluorescence, typical of 5HT and in agreement with the quantitative analysis by Juorio (1971). An attempt to show aminergic fluorescence in the optic gland nerve of *Octopus bimaculatus* (Nishioka *et al.* 1970) showed very sparse fluorescence of a type not encountered in the present work.

(*e*) *Suboesophageal brain*

This region of the cephalopod brain has not previously been examined histochemically. There was widespread fluorescence in the neuropil of most lobes, although those in the middle suboesophageal mass appeared to fluoresce less, which is in agreement with the quantitative data of Juorio (1971).

In the anterior suboesophageal mass the very prominent fluorescence around the roots of the brachial nerve may well indicate the terminations of the afferent fibres within the brachial lobes. Although fluorescence was not observed along the brachial nerves as they entered the brachial lobe, transverse sections across the arms did show some fluorescence in the nerves.

In the middle suboesophageal mass, as mentioned, the fluorescence was less intense than in the other two suboesophageal regions. However, aminergic fluorescence was located in a number of lobes, but, more significantly perhaps, not in the anterior chromatophore lobe. Neither was fluorescence located in the posterior chromatophore lobe, nor in the chromatophore interconnectives. This again is interesting when contrasted with results from acetylcholinesterase histochemistry, as both the chromatophore lobes give a positive reaction for the enzyme (figures 102, 103). Extensive work has shown that the innervation of the chromatophores is probably cholinergic (Florey 1966; Florey & Kriebel 1969), and the results presented in this paper are further evidence that the chromatophore system is cholinergic.

To summarize, the present study, by a rapid and sensitive technique not previously used in cephalopods, has shown a widespread distribution of catecholamines and 5HT in the brains of a number of genera of cephalopods. Moreover, the evidence suggests that dopamine, noradrenalin and 5HT are widely but not uniformly distributed in the cephalopod brain. These amines are presumably related to function in some way, but at present it is only possible to speculate about this. Perhaps these aminergic systems could be inhibitory modulators of excitatory cholinergic mechanisms?

I am immensely grateful to Dr J. B. Messenger and Professor J. Z. Young, F.R.S., for their encouragement during the course of this work and for their critical comments on earlier drafts

of the manuscript. This work was performed during the tenure of a S.R.C. Research Studentship, and thanks are due to the Research Fund of the University of Sheffield and to the Marshall and Orr Bequest of the Royal Society, for funding two visits to Naples. I am also grateful to the Directors and their staffs of the Stazione Zoologica, Naples, and the Marine Laboratory, Plymouth, for providing their facilities and assistance. I would also like to thank Professor I. Chester Jones for providing the facilities of his Department, and Mr D. I. Hollingworth for his expert photographic assistance.

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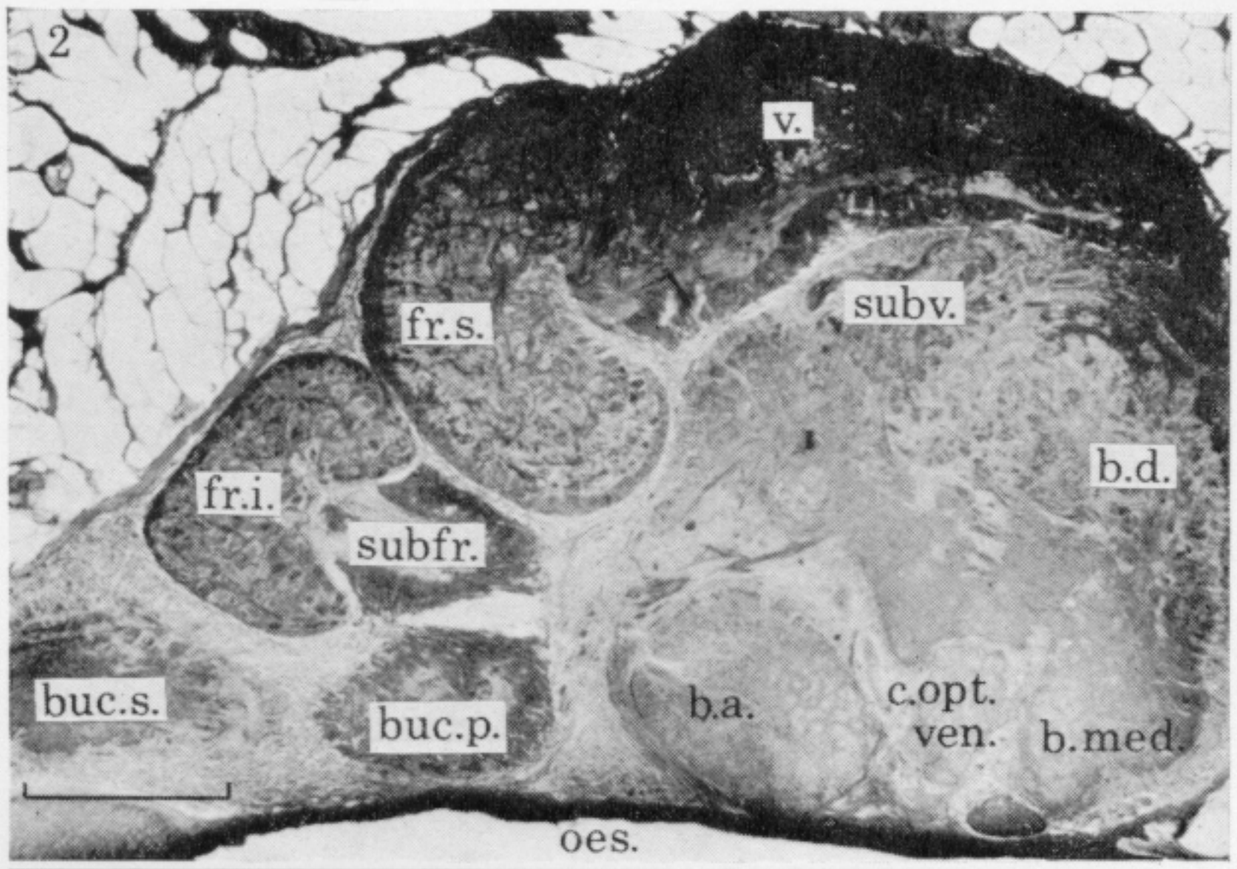
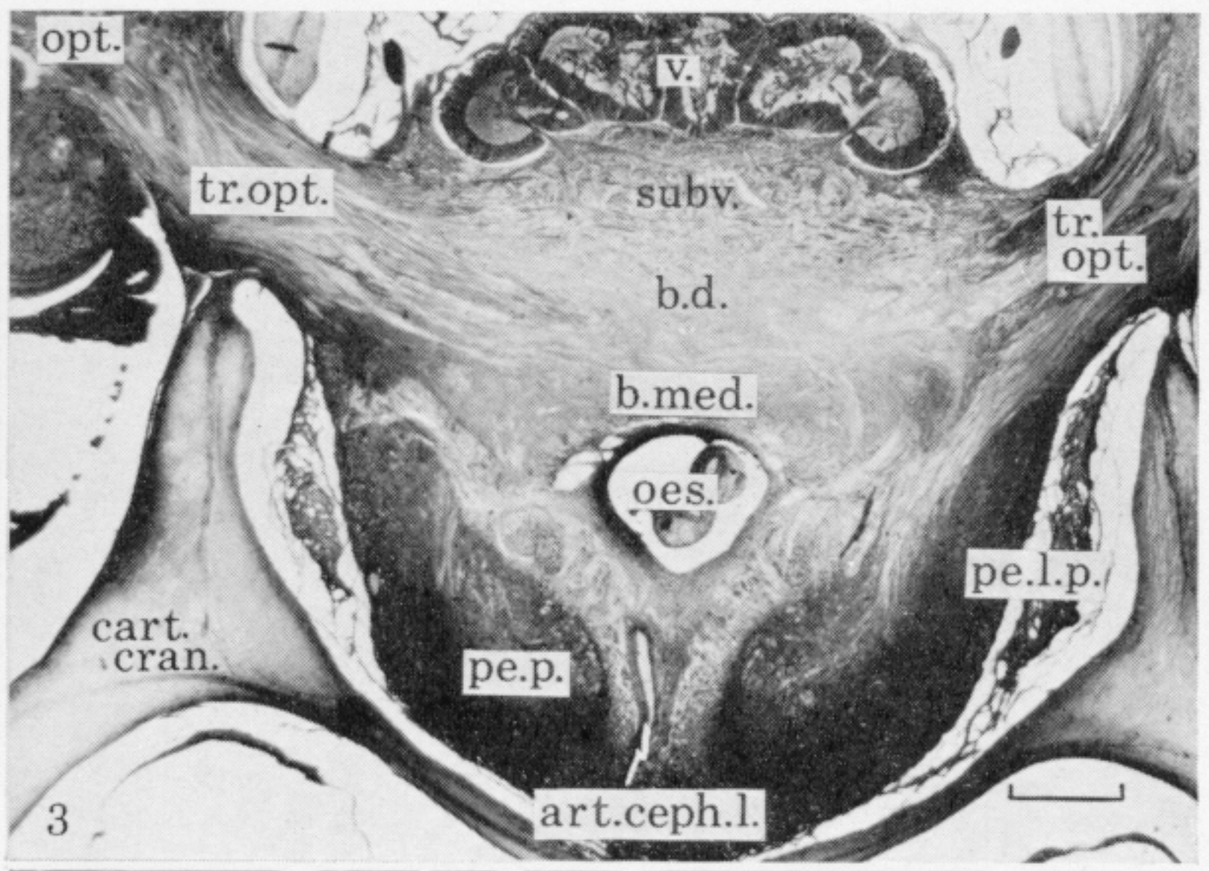
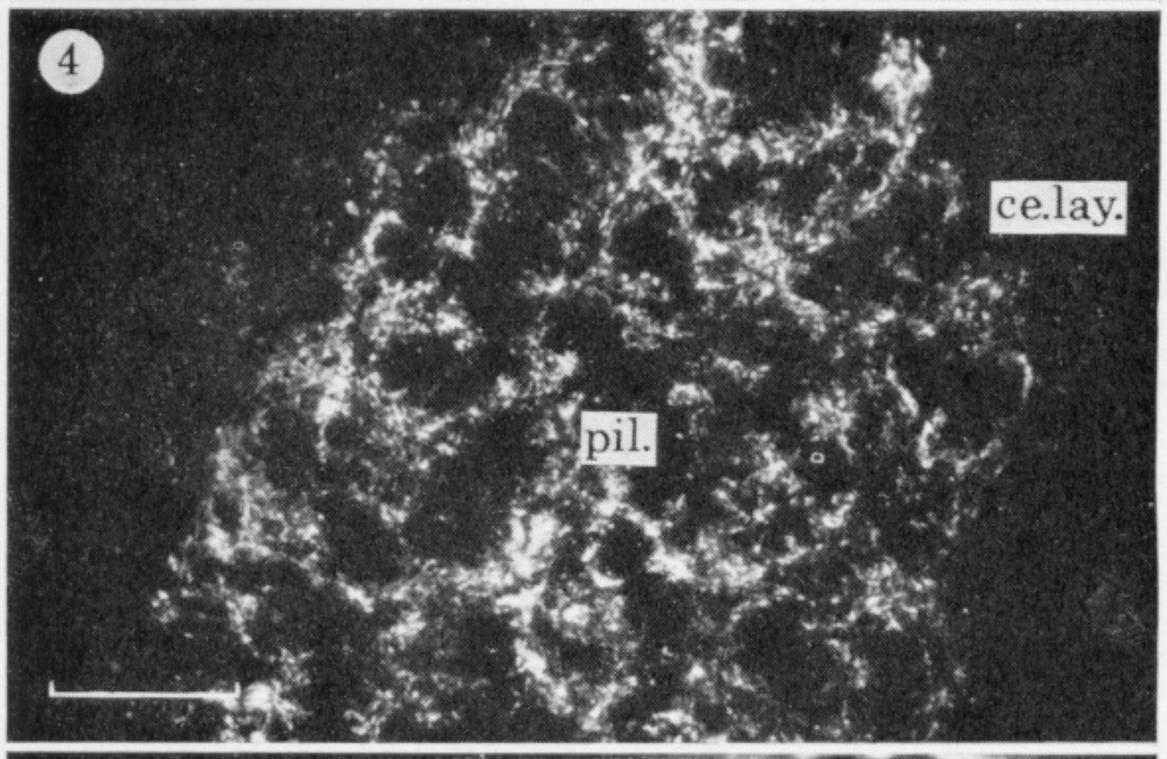
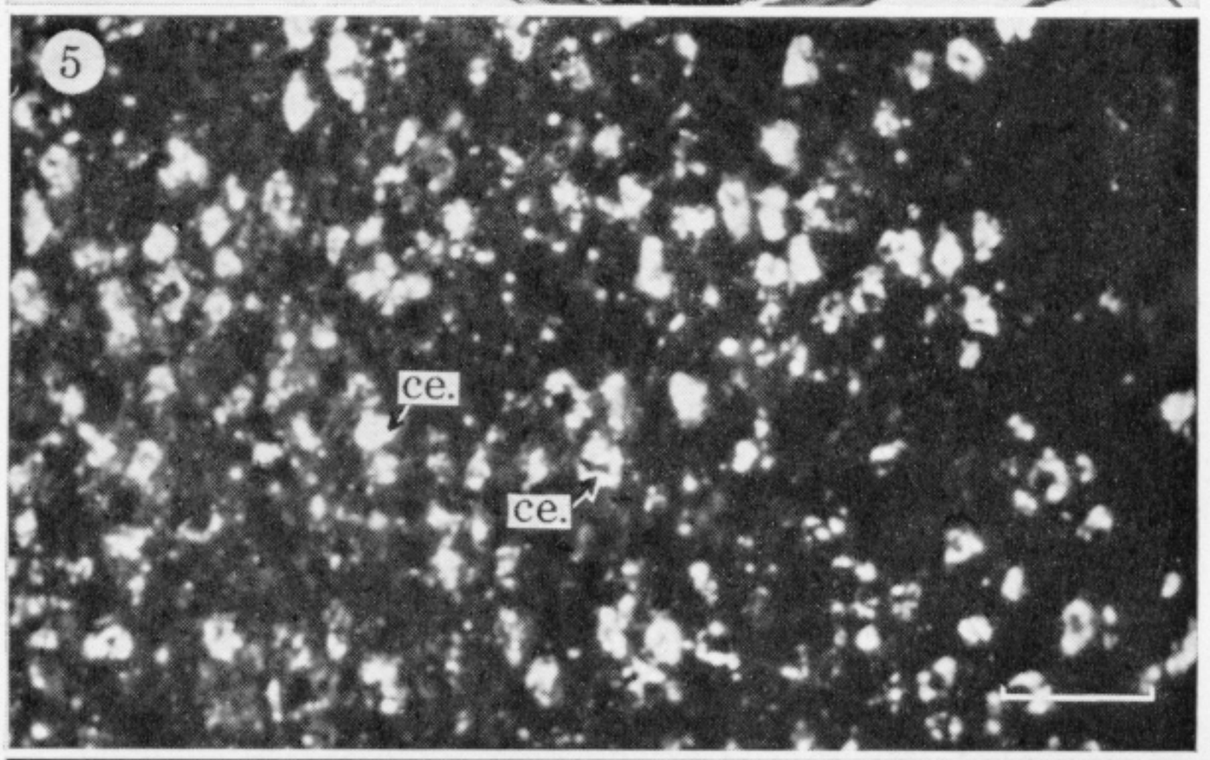
Explanation of abbreviations used on figures

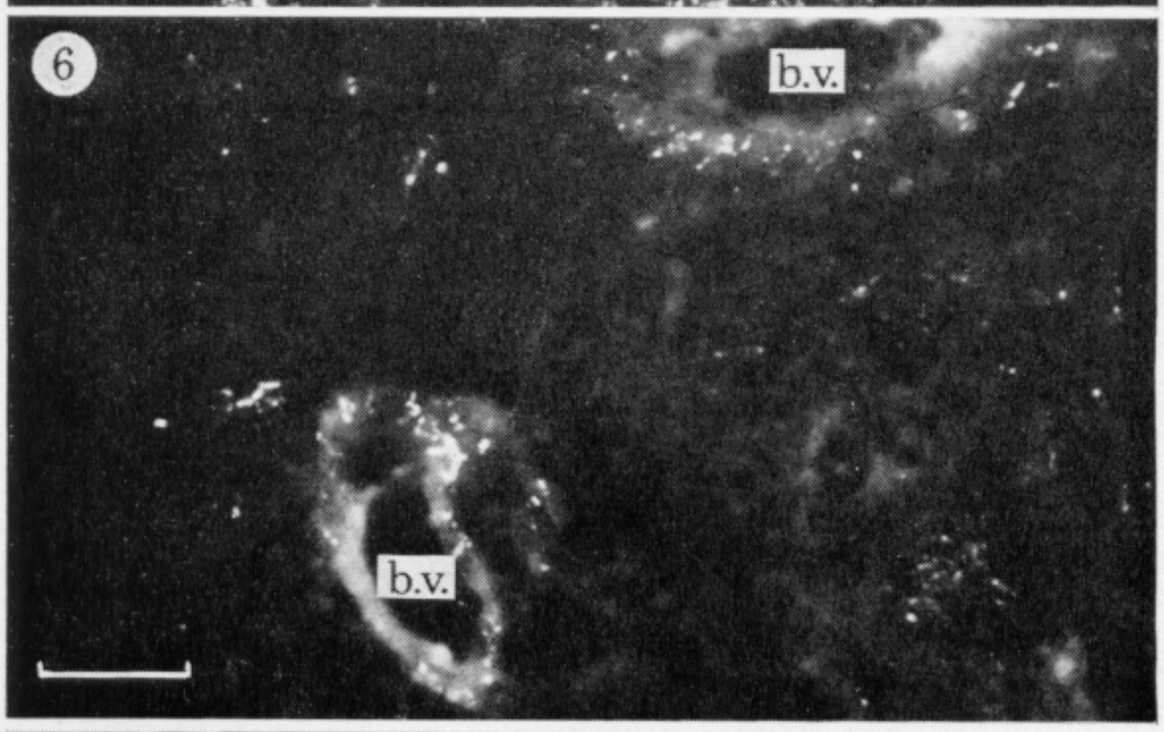
a., ant.	anterior
art. ceph.	cephalic artery
art. ceph. l.	lateral cephalic artery
b.a.	anterior basal lobe
b.a. suboes. con.	anterior basal–suboesophageal connective
b.z.	basal zone
br.	brachial lobe
br. po.	postbrachial lobe
br. pr.	prebrachial lobe
buc. p.	posterior buccal lobe
buc. s.	superior buccal lobe
b.d.	dorsal basal lobe
b.d.a.	anterior dorsal basal lobe
b.d.p.	posterior dorsal basal lobe
b. int.	interbasal lobe
b. med.	median basal lobe
b.v.	blood vessel
c. br. inf.	infrabrachial commissure
c. br. sup.	suprabrachial commissure
c. mag.	magnocellular commissure
c. opt. d.	dorsal optic commissure
c. opt. ven.	ventral optic commissure
c. pe. med.	medial pedal commissure
c. pv. a.	anterior palliovisceral commissure
cart. cran.	cranial cartilage
ce.	cell
ce. gr. lay. in.	inner granule cell layer
ce. gr. lay. out.	outer granule cell layer
ce. lay.	cell layer
ch. a.	anterior chromatophore lobe
ch. int. con.	interchromatophore connective
ch. p.	posterior chromatophore lobe
d.	dorsal
fr. i.	inferior frontal lobe
fr. i. l.	lateral inferior frontal lobe
fr. s.	superior frontal lobe
fr. s. l.	lateral superior frontal lobe
fr. s. med.	median superior frontal lobe
g. opt.	optic gland
lat.	lateral
mag.	magnocellular lobe
med.	median
n.	nerve
n. aorta	aortic nerve
n. antorb.	antorbital nerve
n. br.	brachial nerve
n. br. l. r.	lateral root of the brachial nerve
n. br. med. r.	median root of the brachial nerve
n. col.	collar nerve
n. lab.	labial nerve
n. oes. per.	perioesophageal nerve
n. ol.	olfactory nerve
n. pal.	pallial nerve
oes.	oesophagus
ol.	olfactory lobe
ol. med.	median olfactory lobe
ol. p.	posterior olfactory lobe
ol. a.	anterior olfactory lobe
opt.	optic lobe
p., post.	posterior
pe. a.	anterior pedal lobe
pe. l.	lateral pedal lobe
pe. p.	posterior pedal lobe
ped.	peduncle lobe
ped. b. z.	peduncle basal zone
ped. sp.	peduncle spine
ped. sp. lat.	lateral bank of the peduncle spine
ped. sp. med.	median bank of the peduncle spine
ped. sub.	subpedunculate lobe
pil.	neuropil
pil. in.	inner neuropil
pil. out.	outer neuropil
plex.	plexiform layer
plex. in.	inner plexiform layer
prec.	precommissural lobe
pv.	palliovisceral lobe
pv. d. p.	posterior-dorsal palliovisceral lobe
sp.	spine
subfr.	subfrontal
subped.	subpedunculate tissue
subv.	subvertical lobe
tr. b.a. pe. l.	anterior basal-lateral pedal lobe tract
tr. br. opt.	brachial-optic lobe tract
tr. br. pv.	brachial-palliovisceral lobe tract
tr. cer.	cerebral tract
tr. opt.	optic tract
tr. opt. ped.	optic-peduncle lobe tract
tr. opt. med. mag.	median optic-magnocellular lobe tract
tr. v. subv.	vertical–subvertical tract
tr. vas. int.	intervasomotor tract
v.	vertical lobe
vas.	vasomotor lobe
vas. d. med.	median dorsal vasomotor lobe
vas. ven.	ventral vasomotor lobe
ven.	ventral

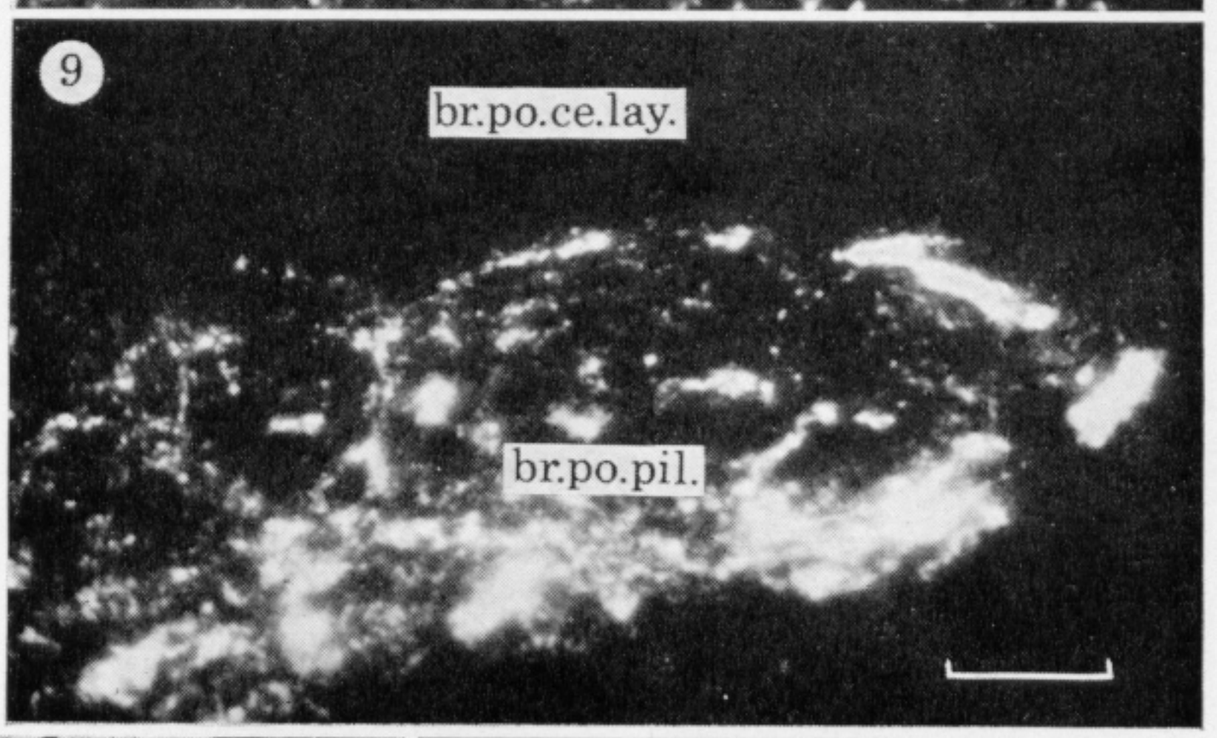


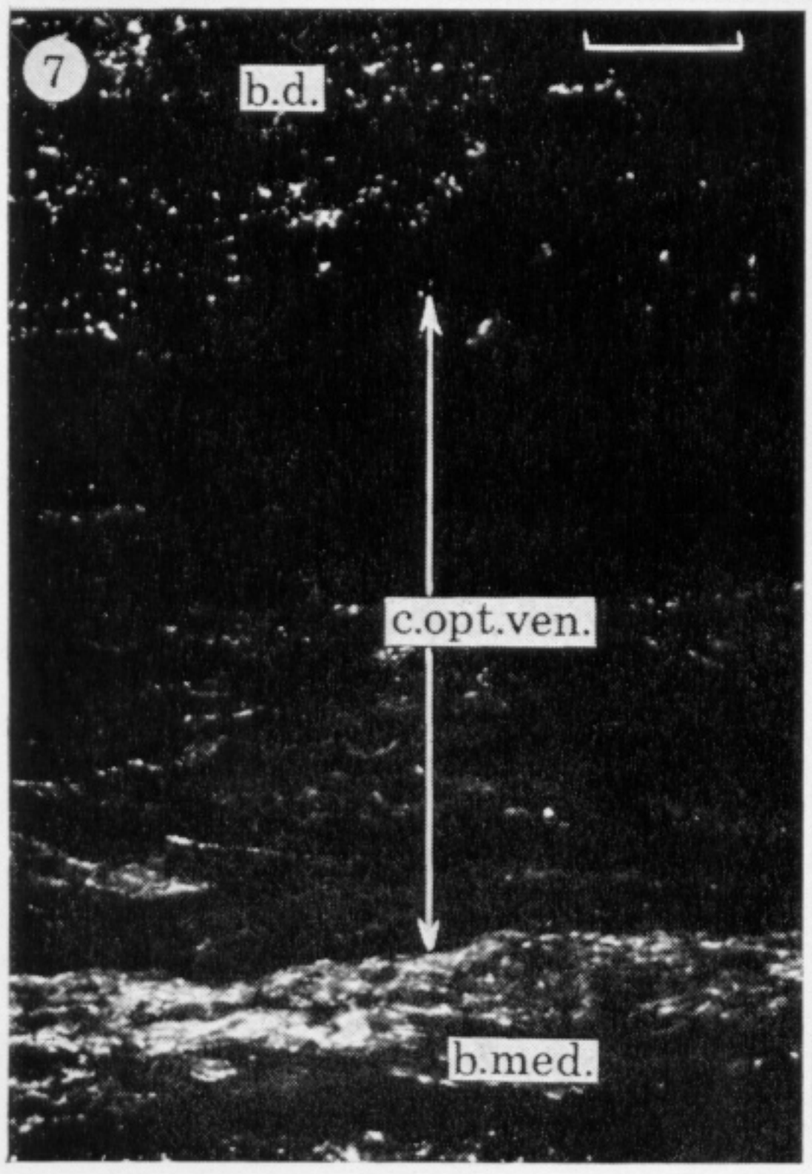


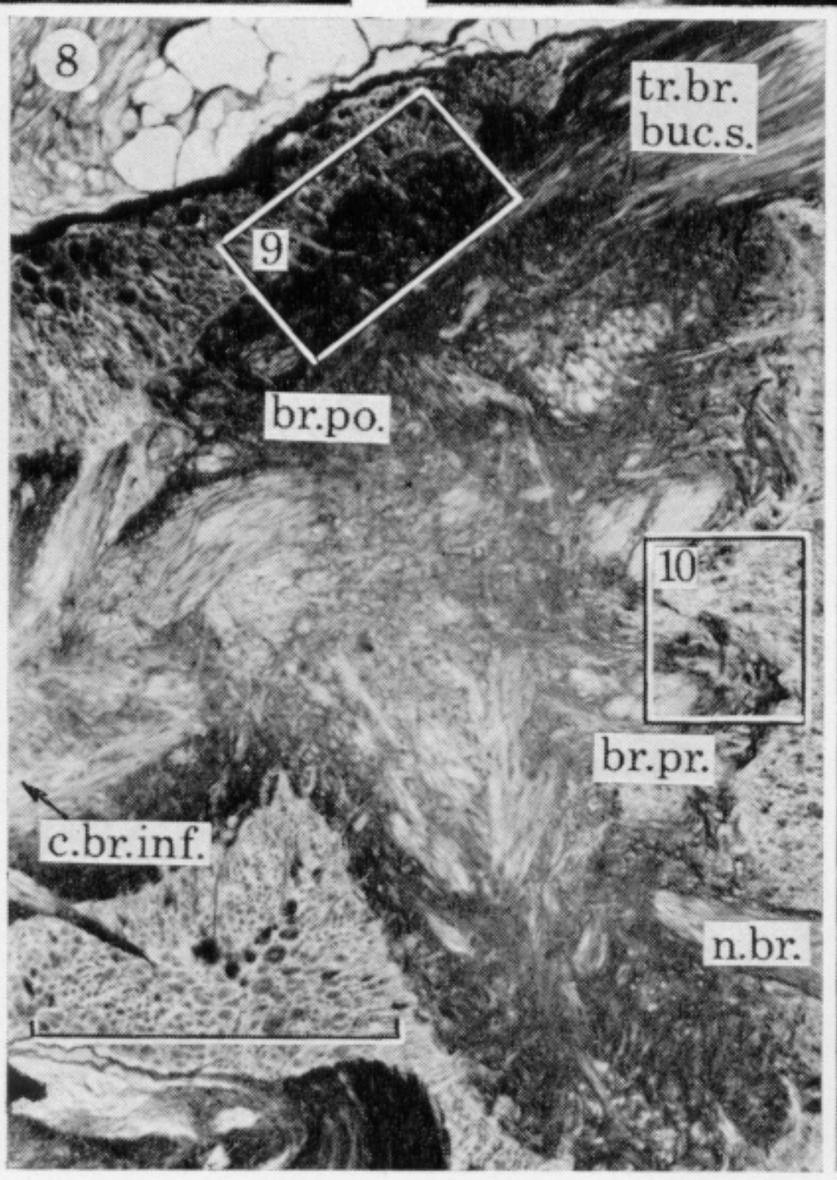


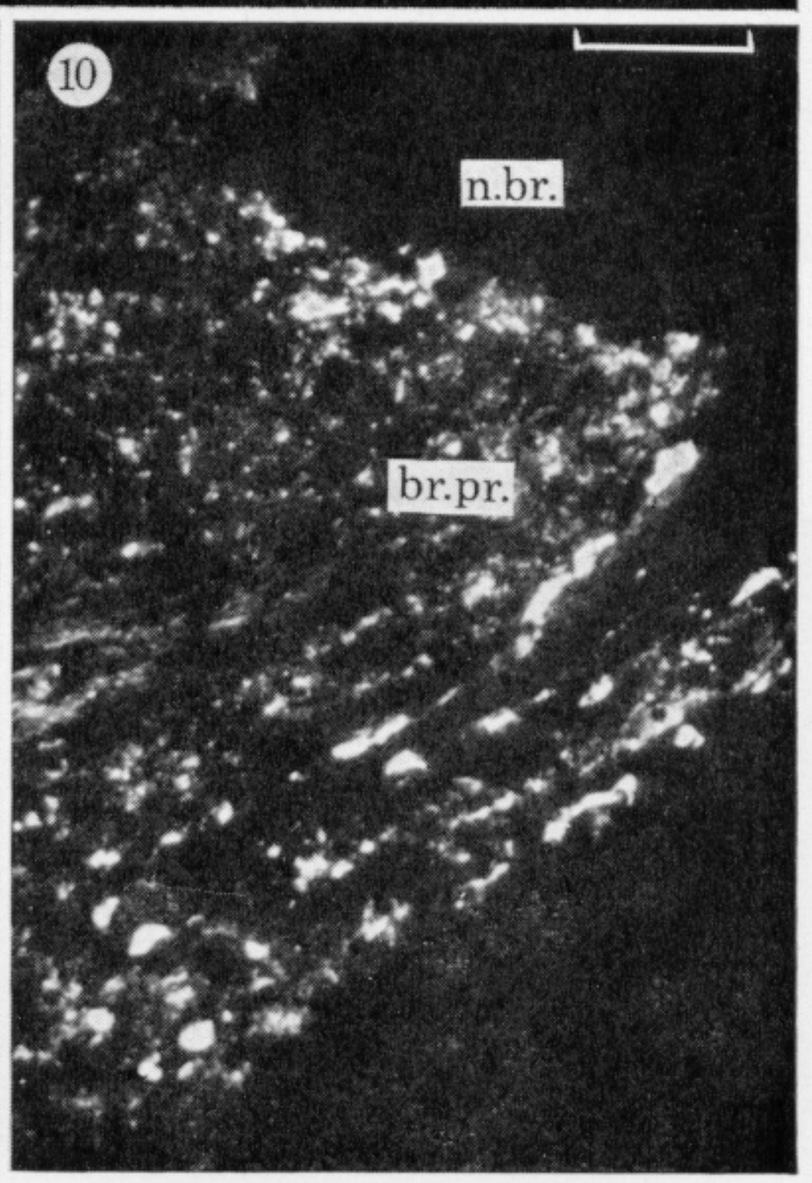




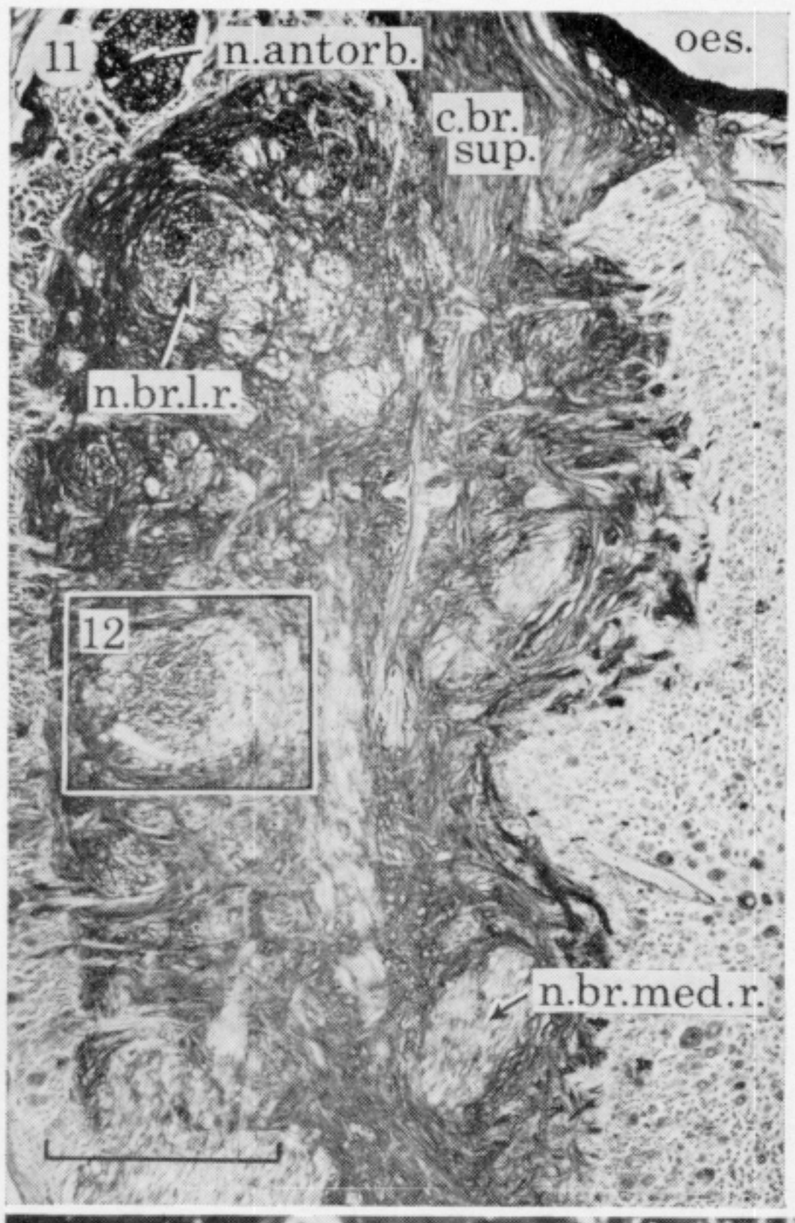


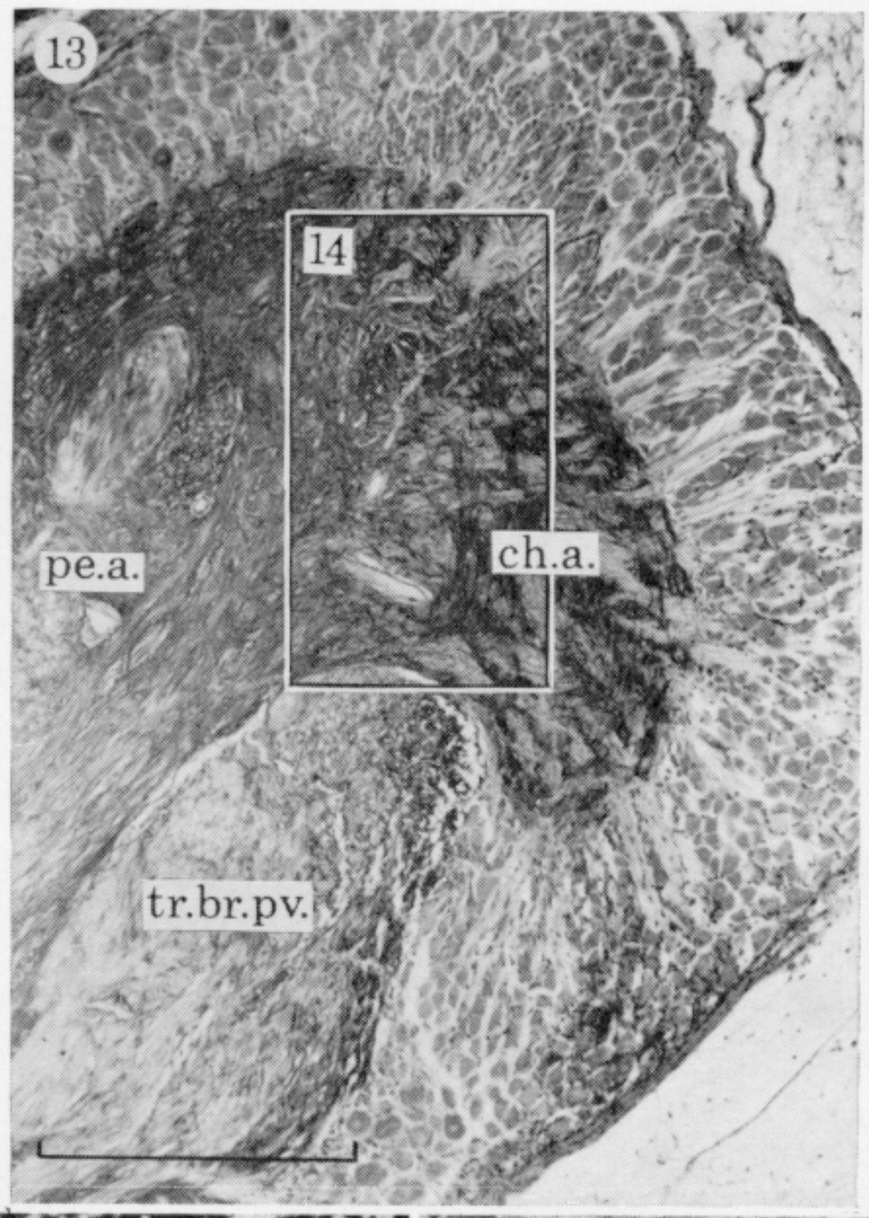


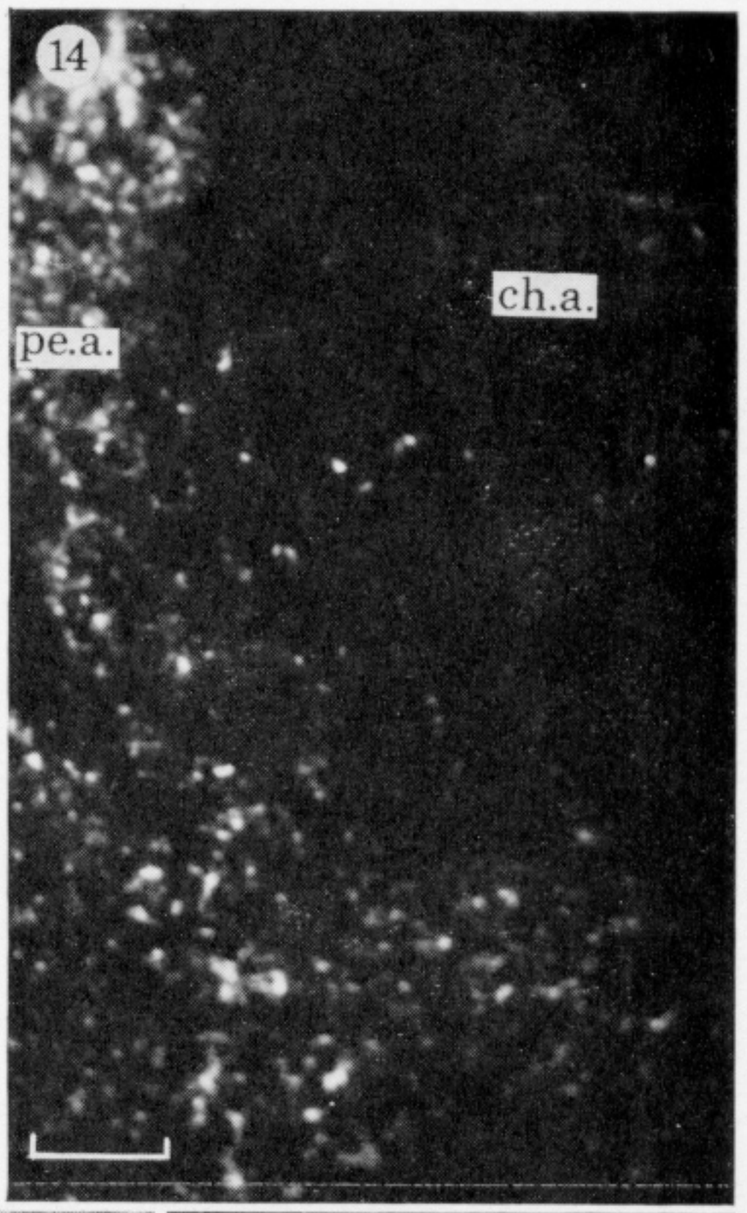


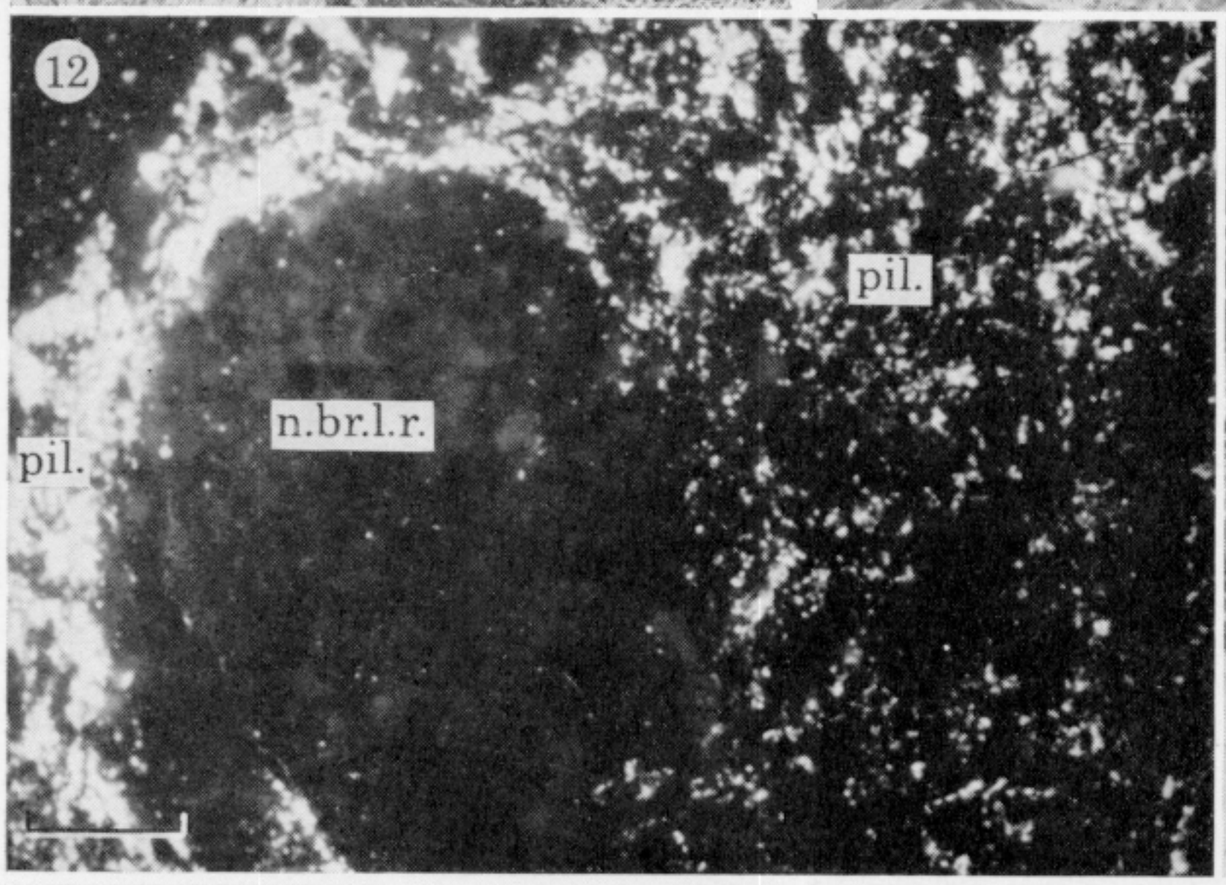


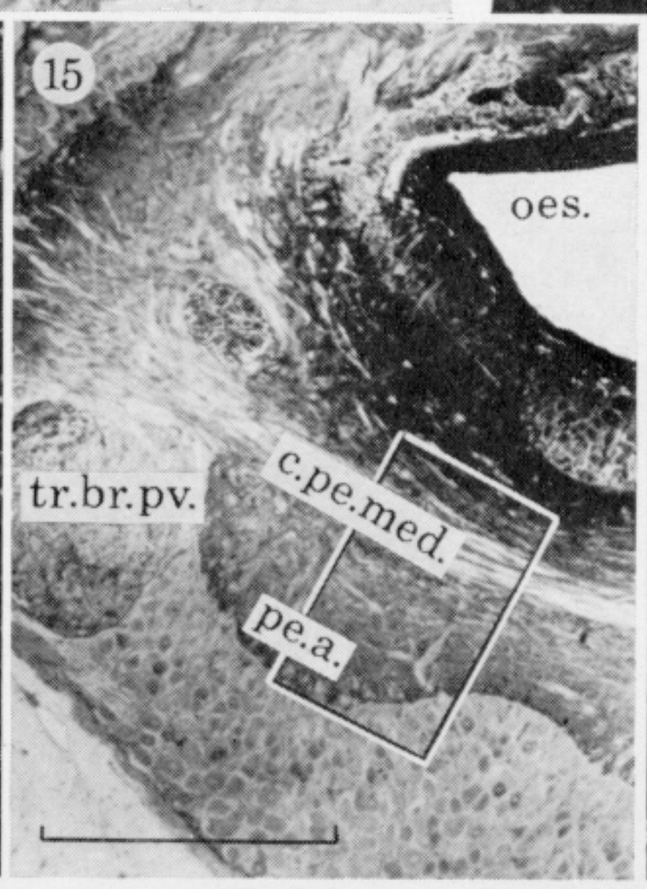
FIGURES 2–10. For description see opposite.

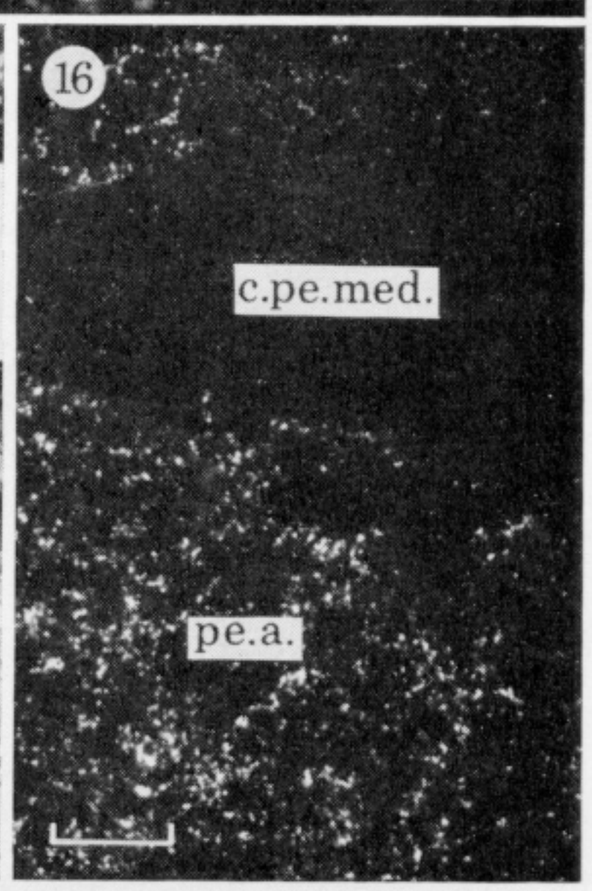


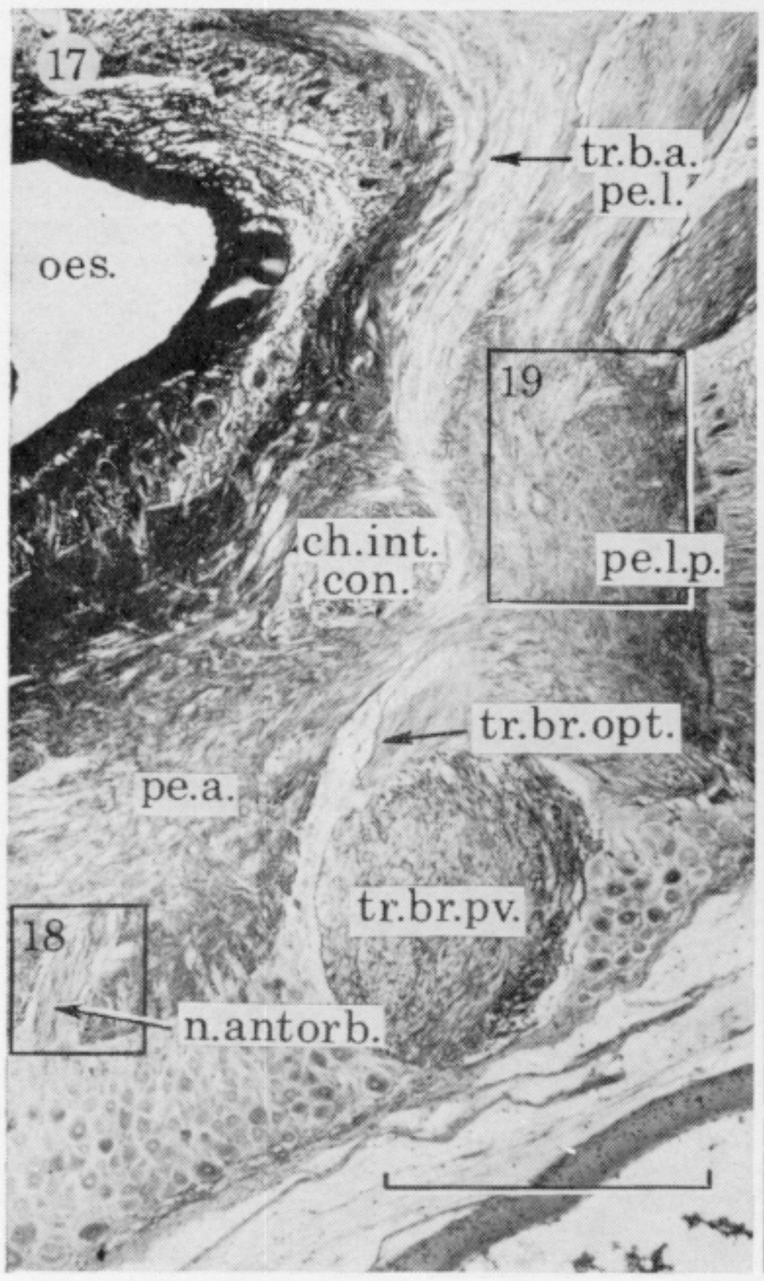


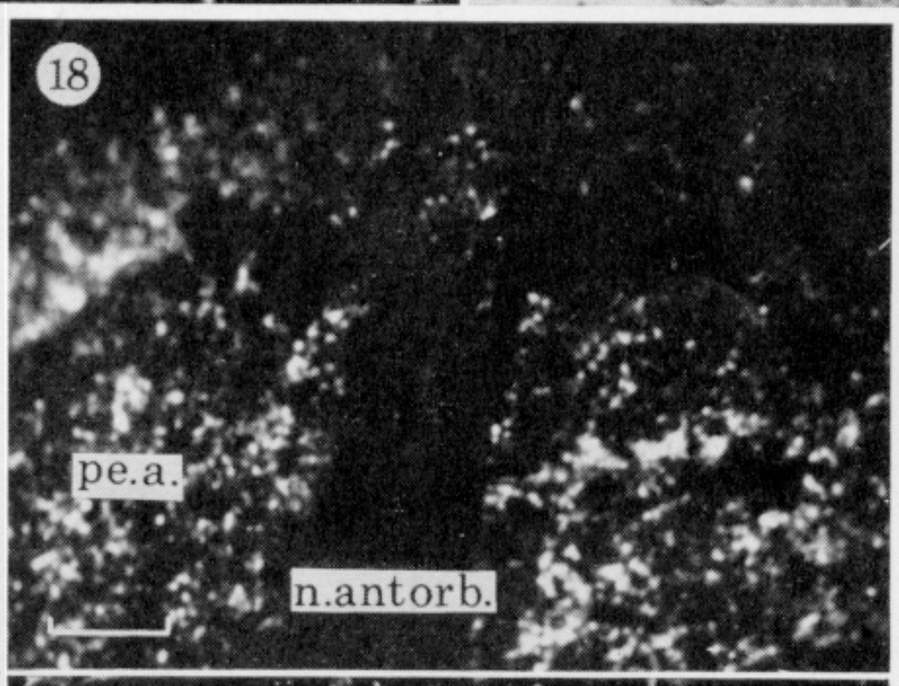


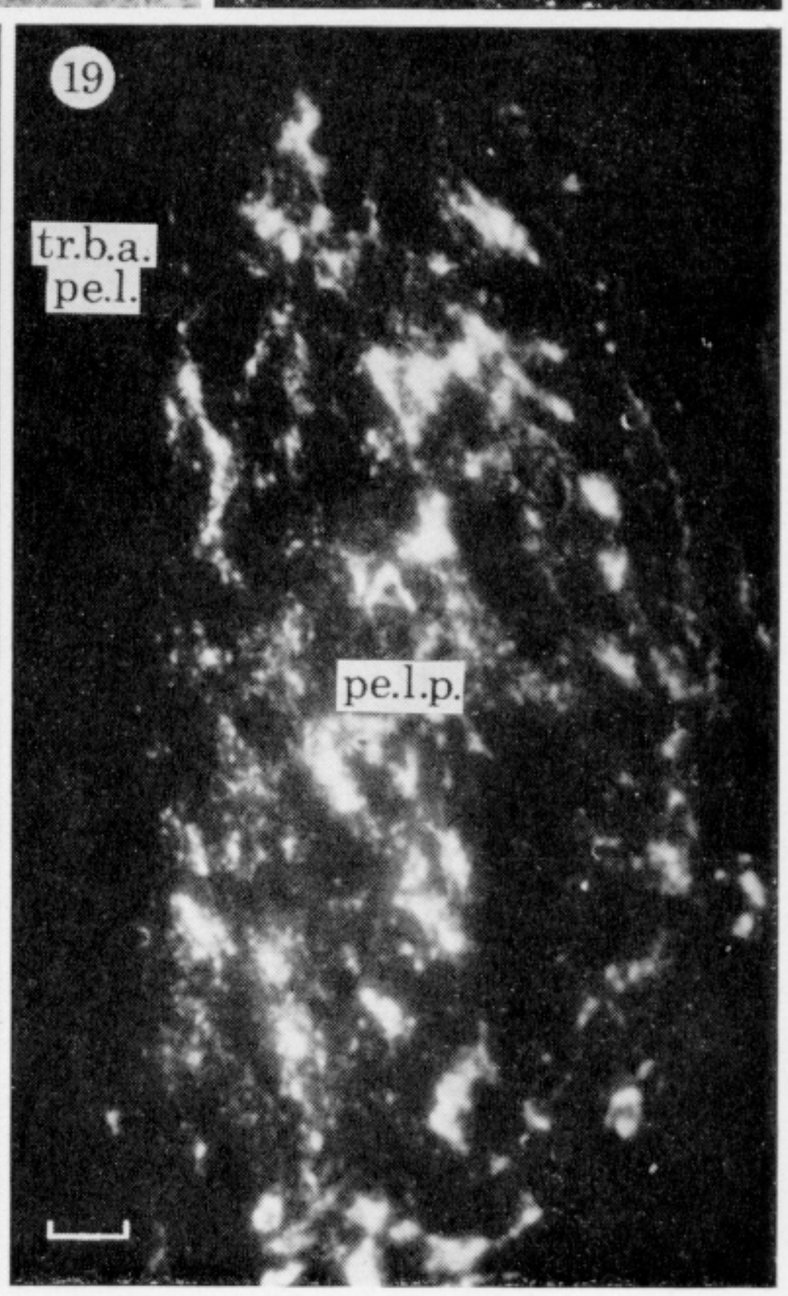




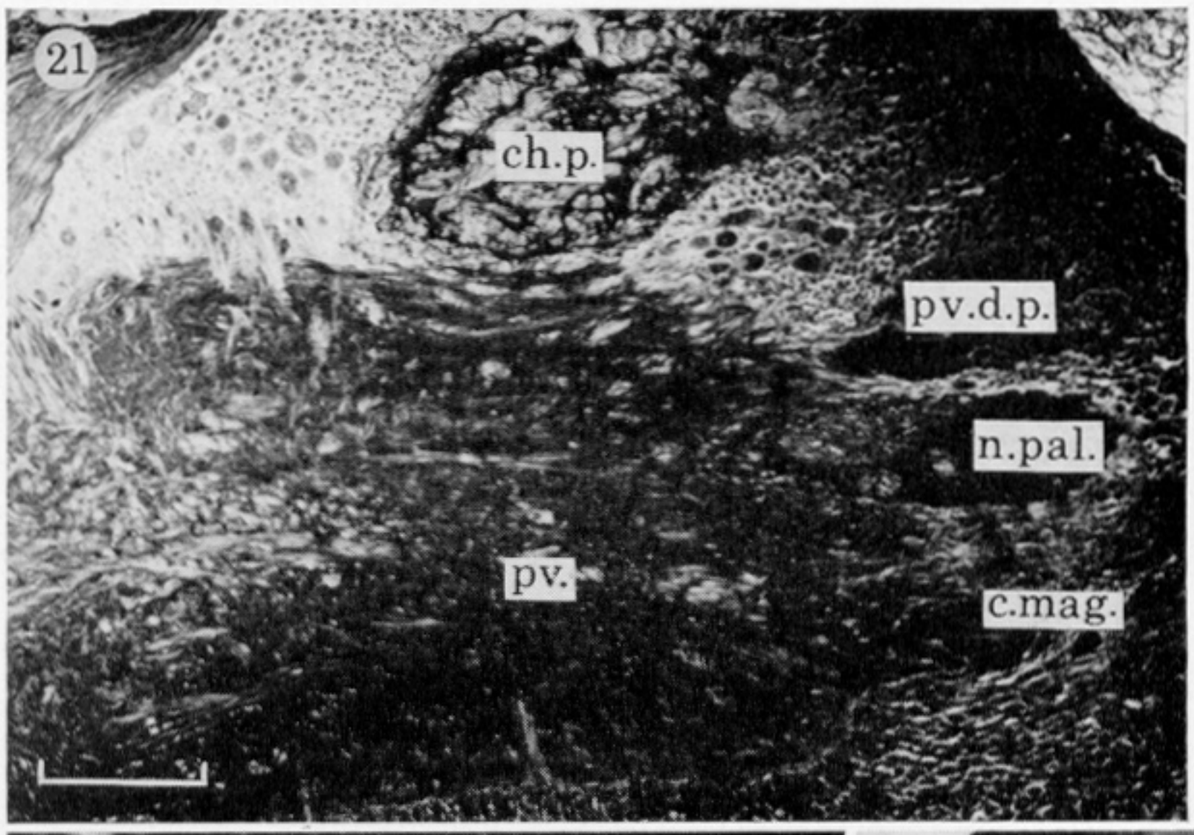


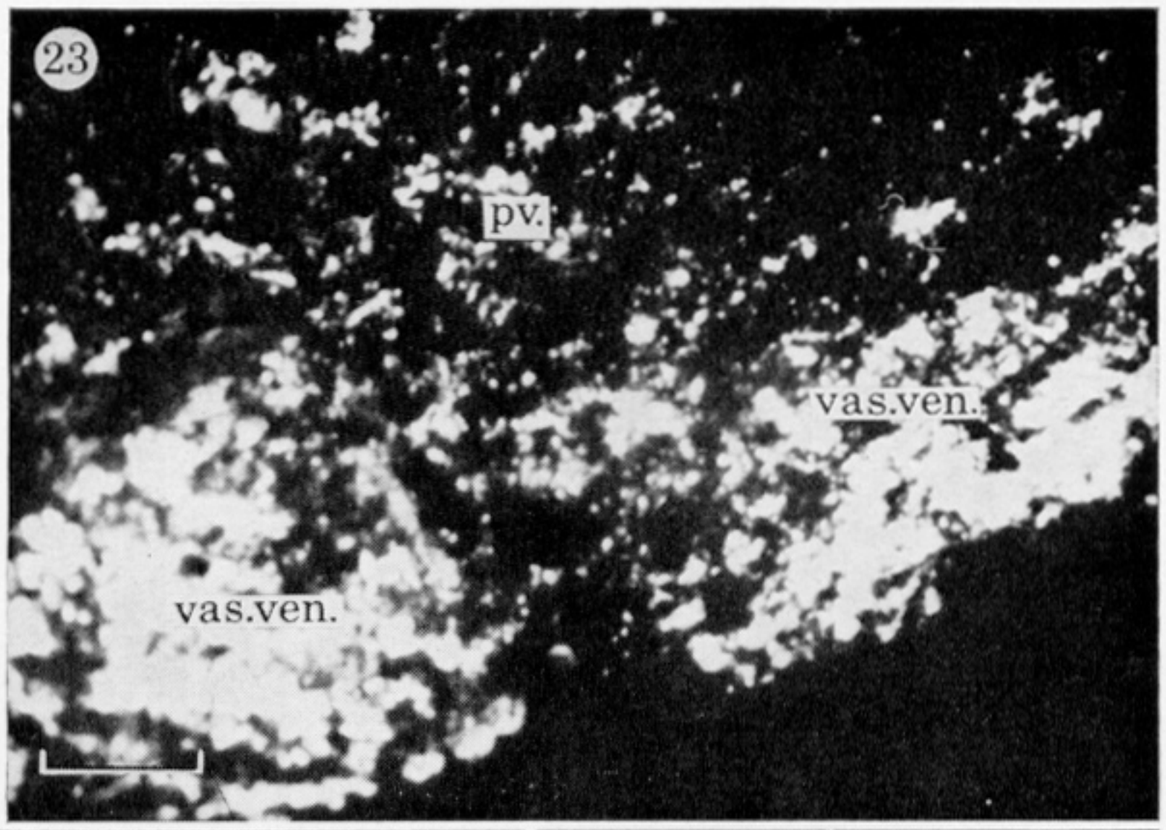


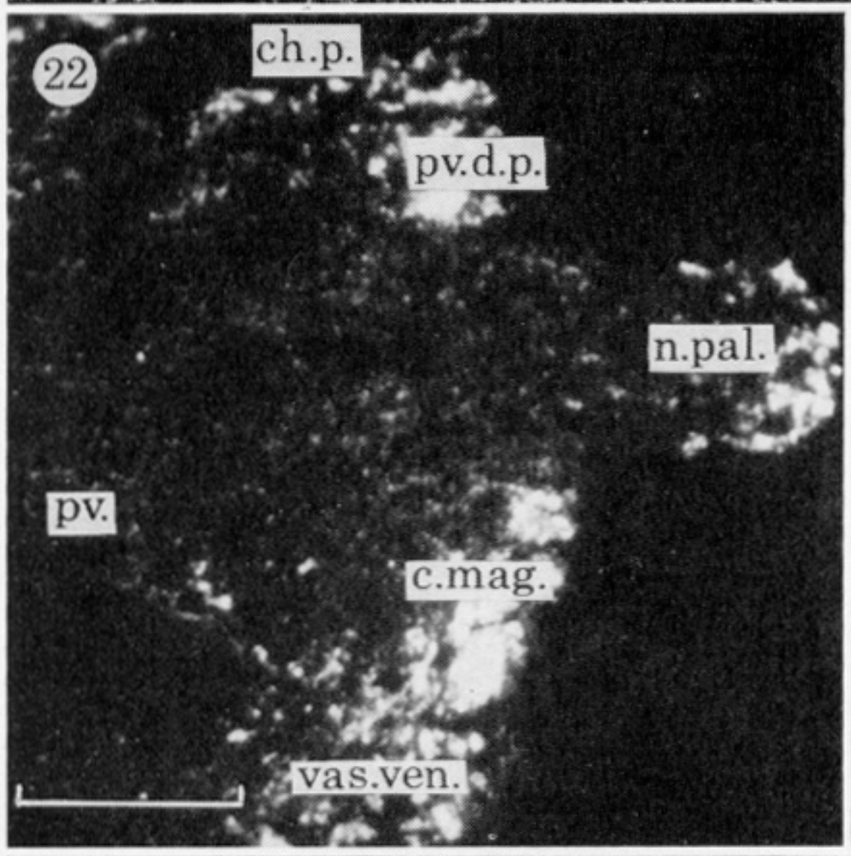


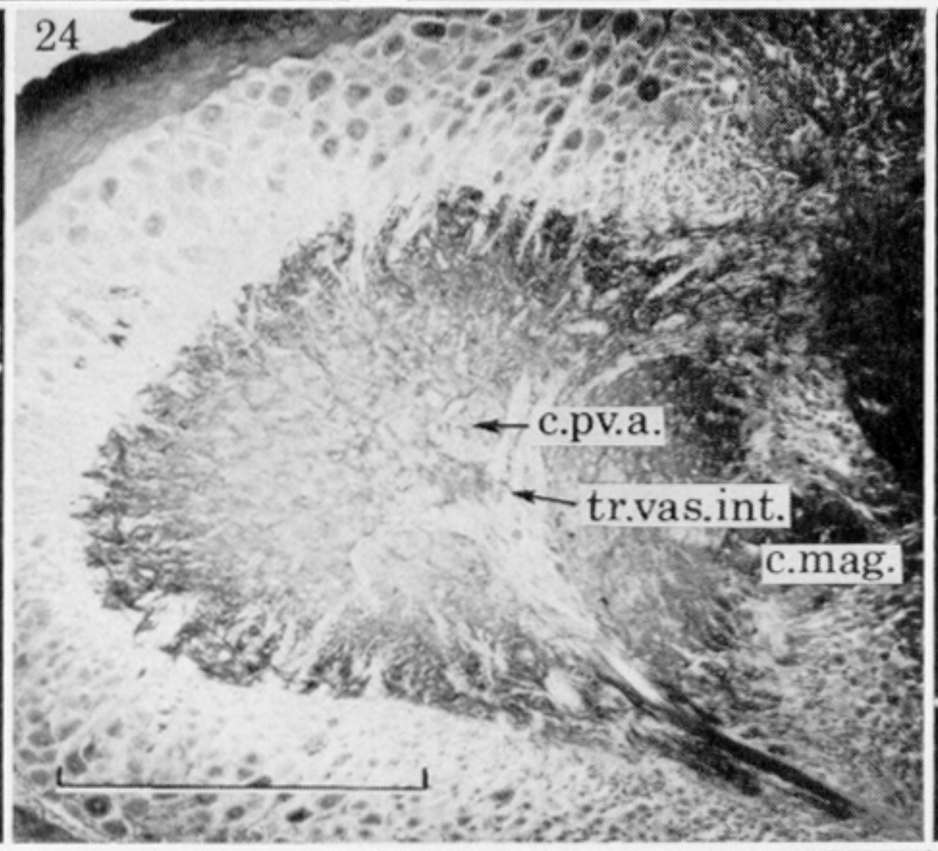


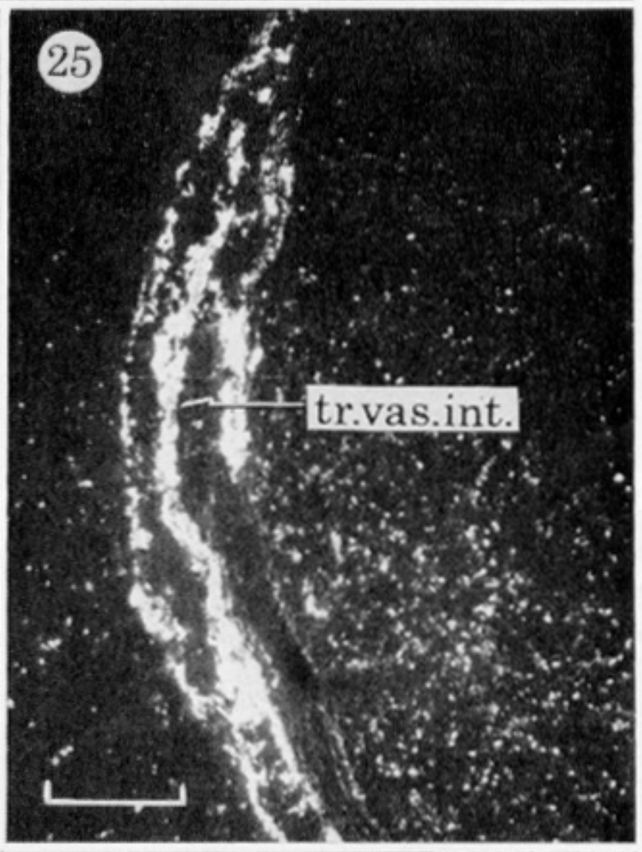
FIGURES 11–20. For description see page 130.

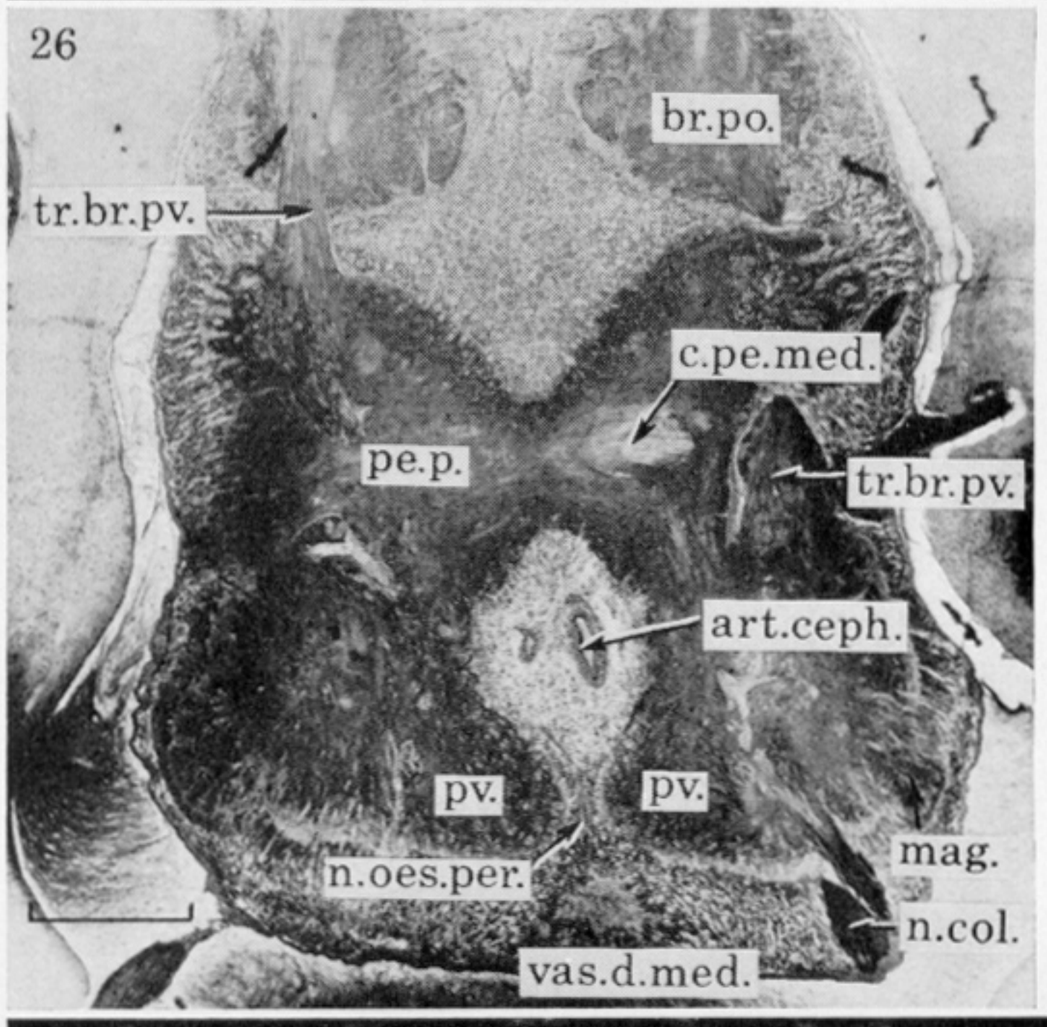


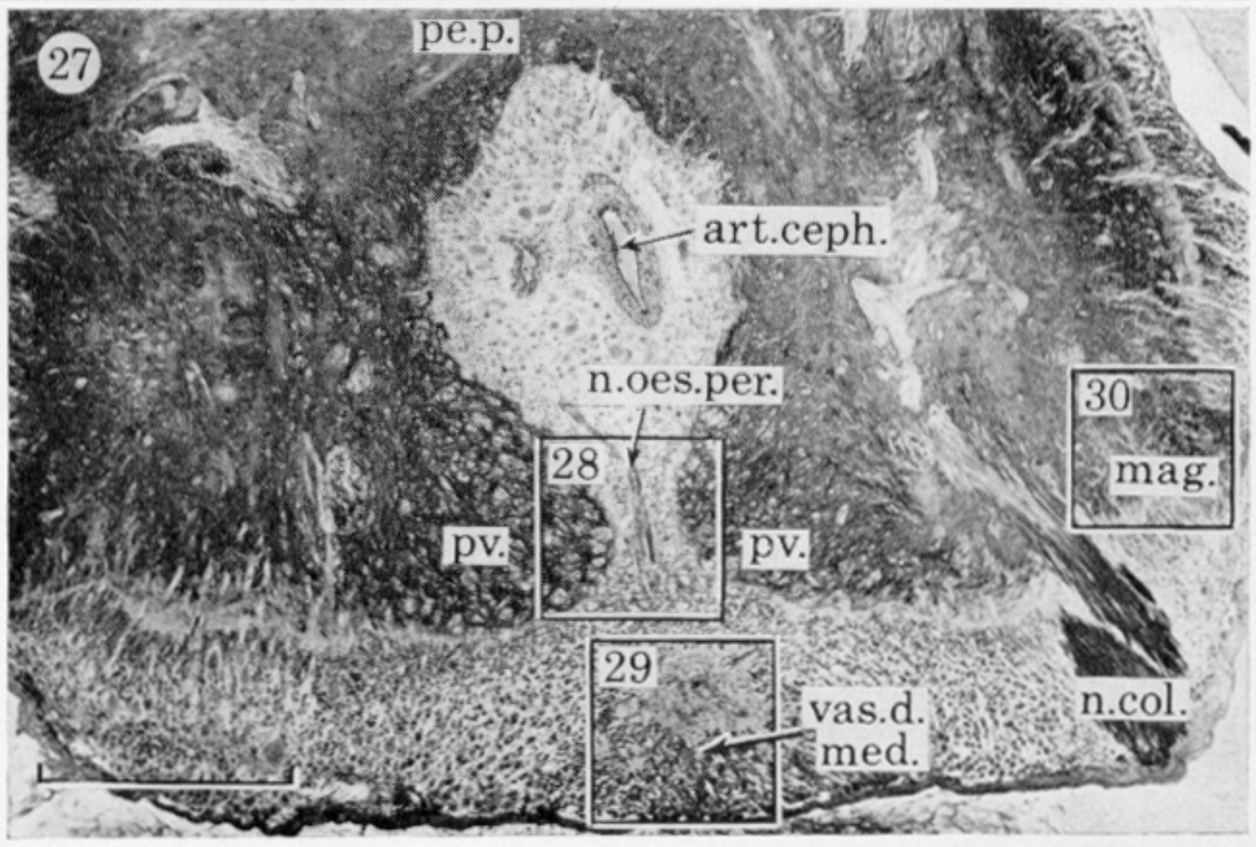


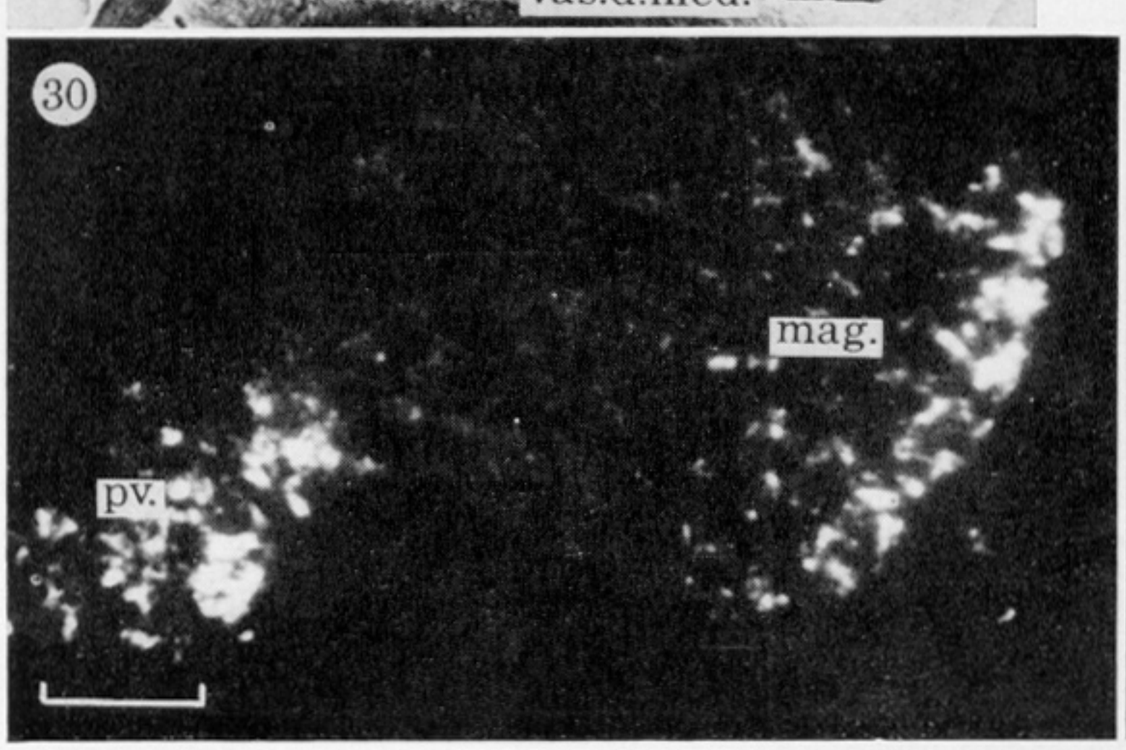


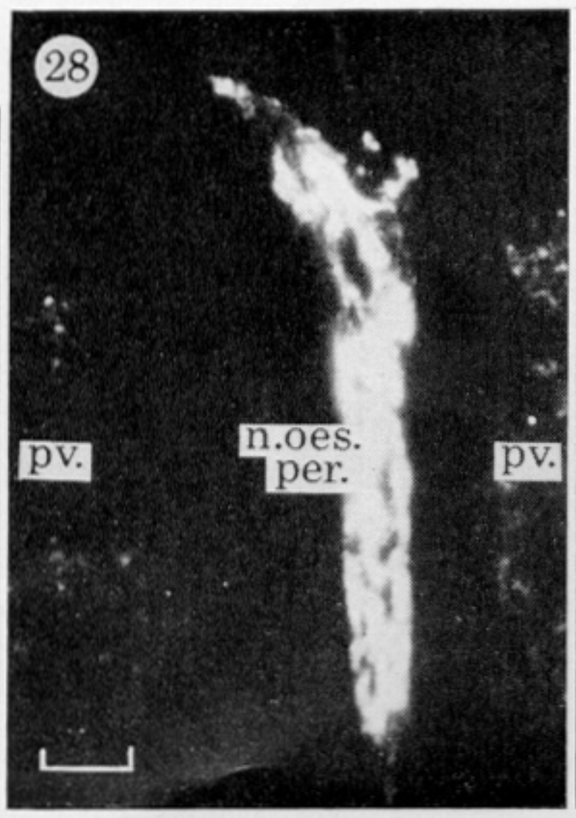


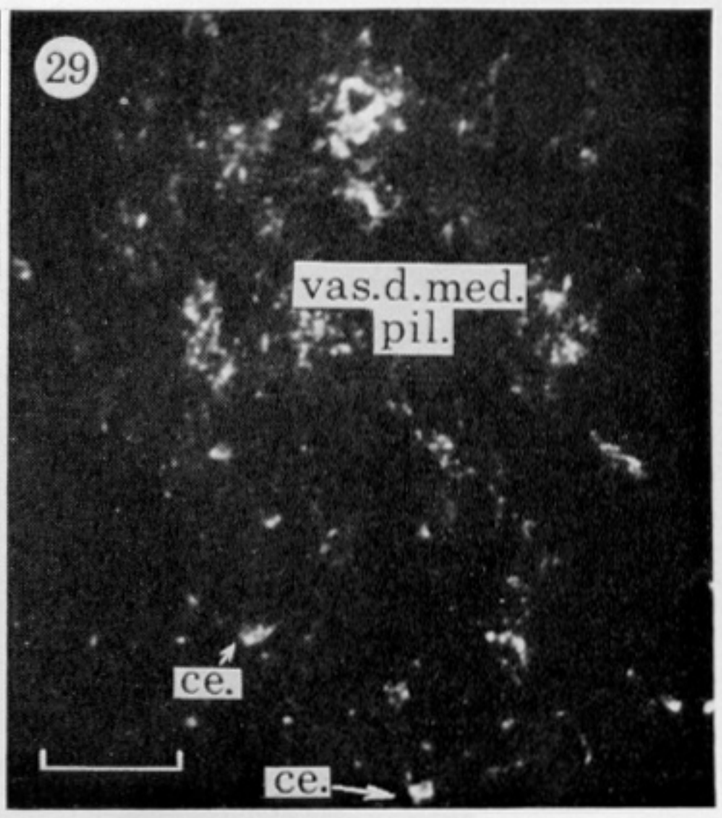




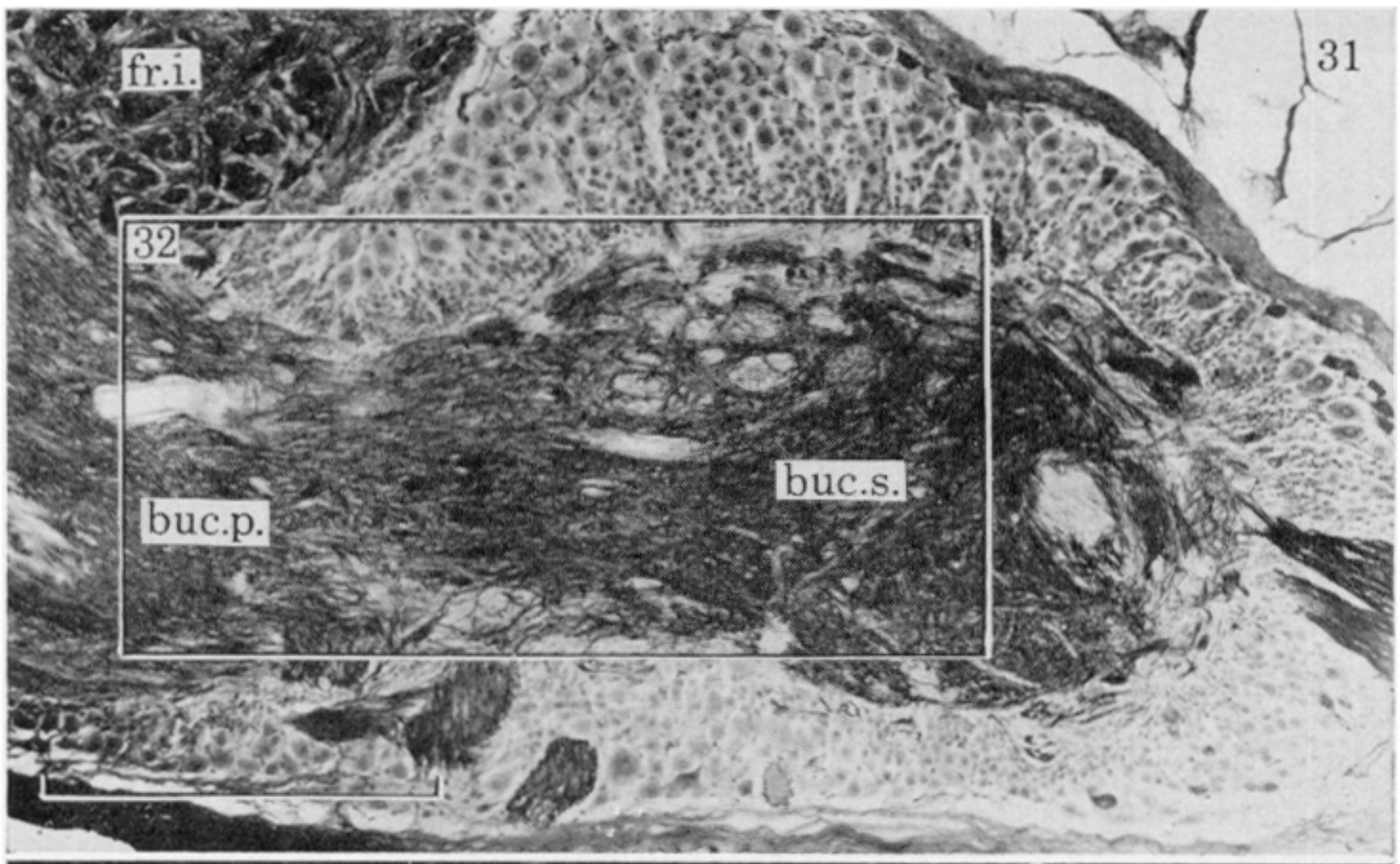


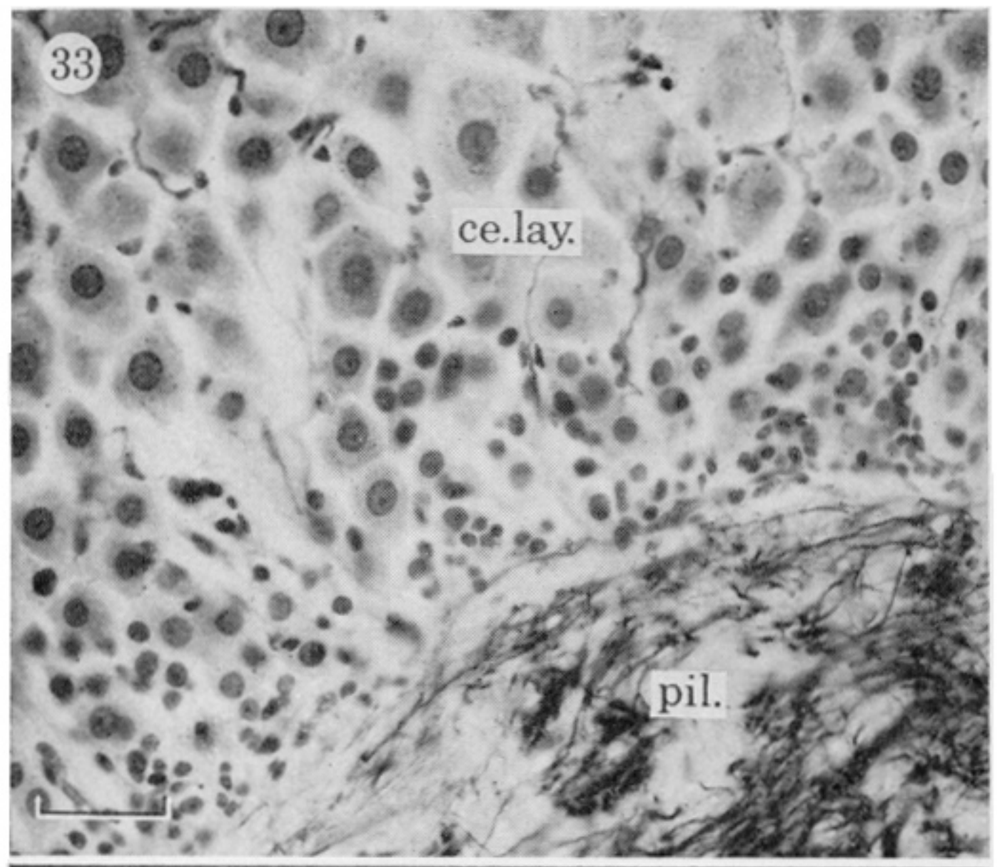


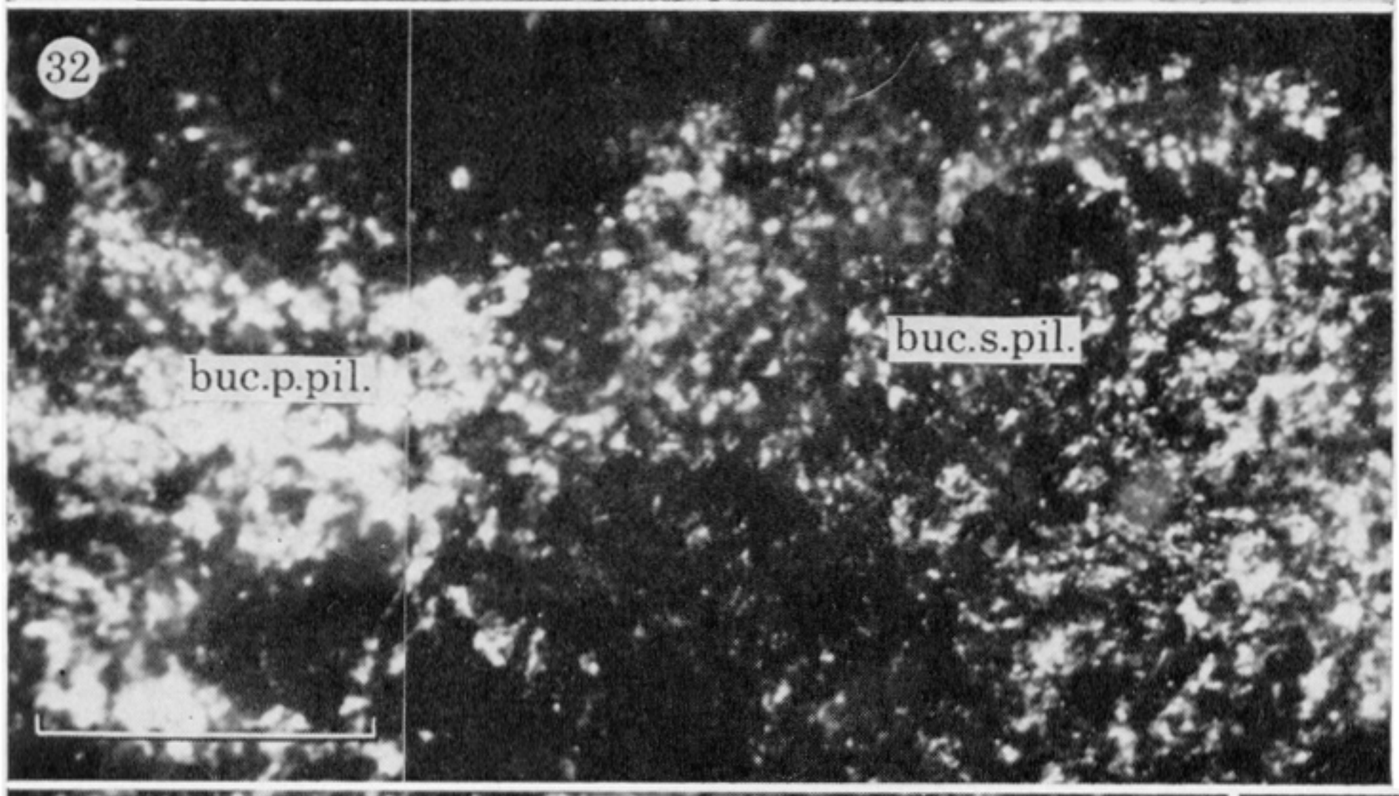


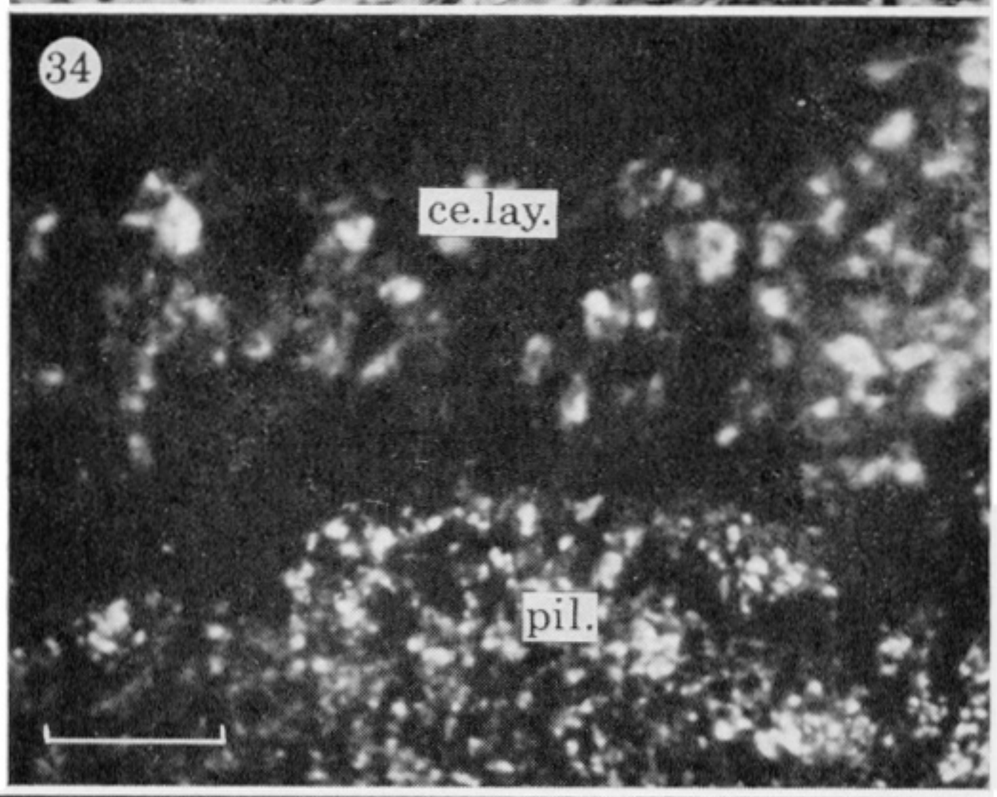


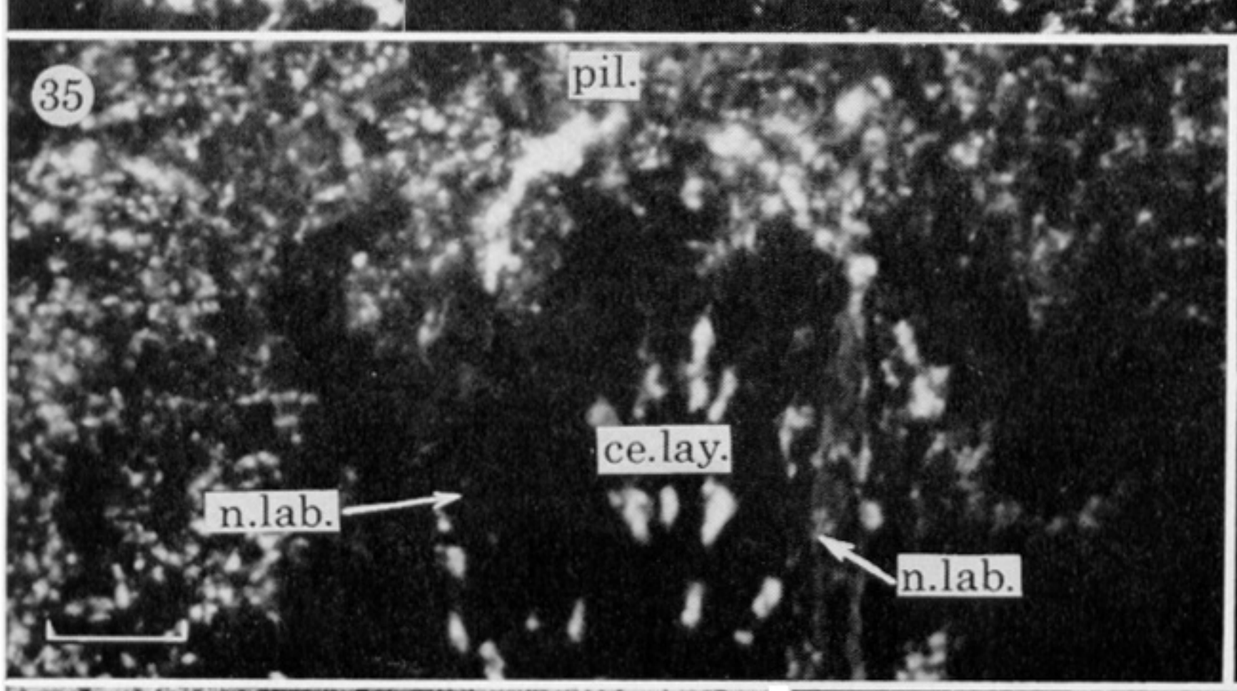
FIGURES 21–30. For description see page 131.

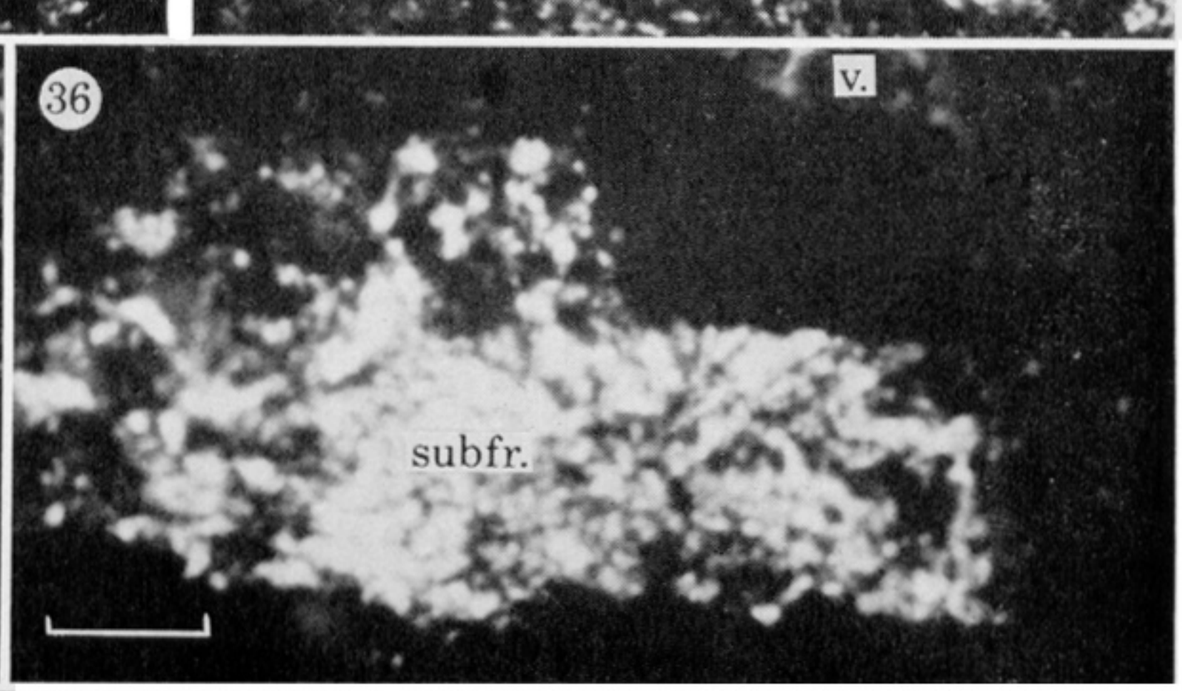


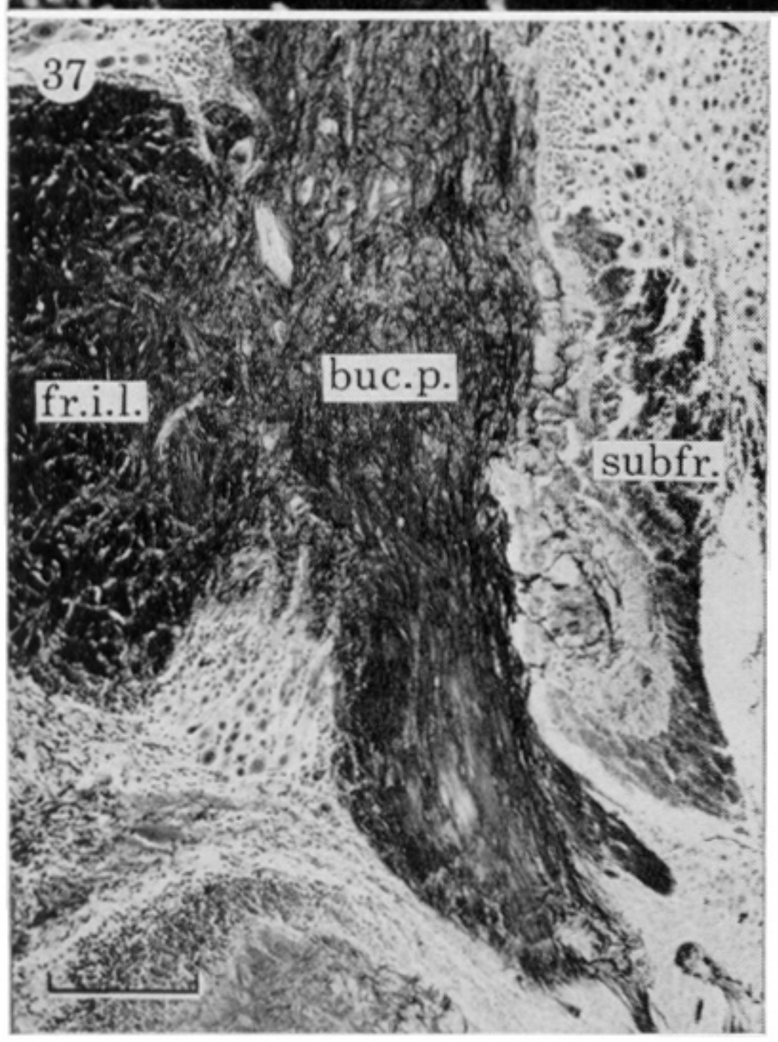


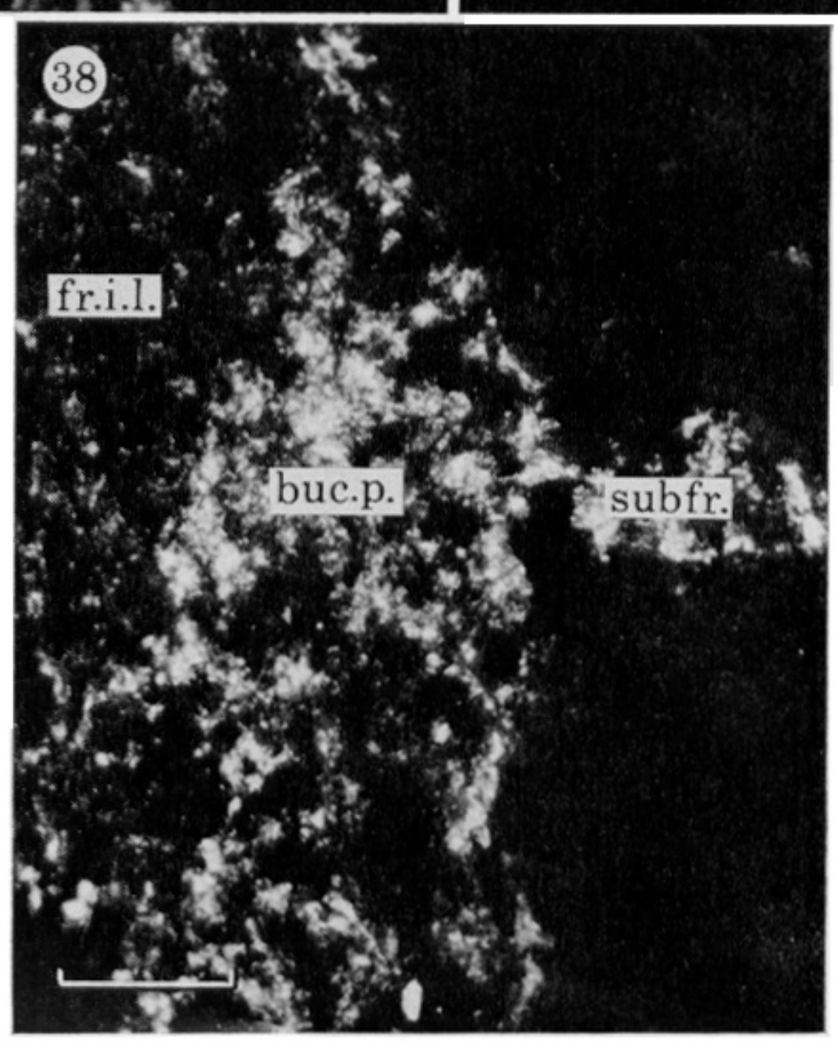


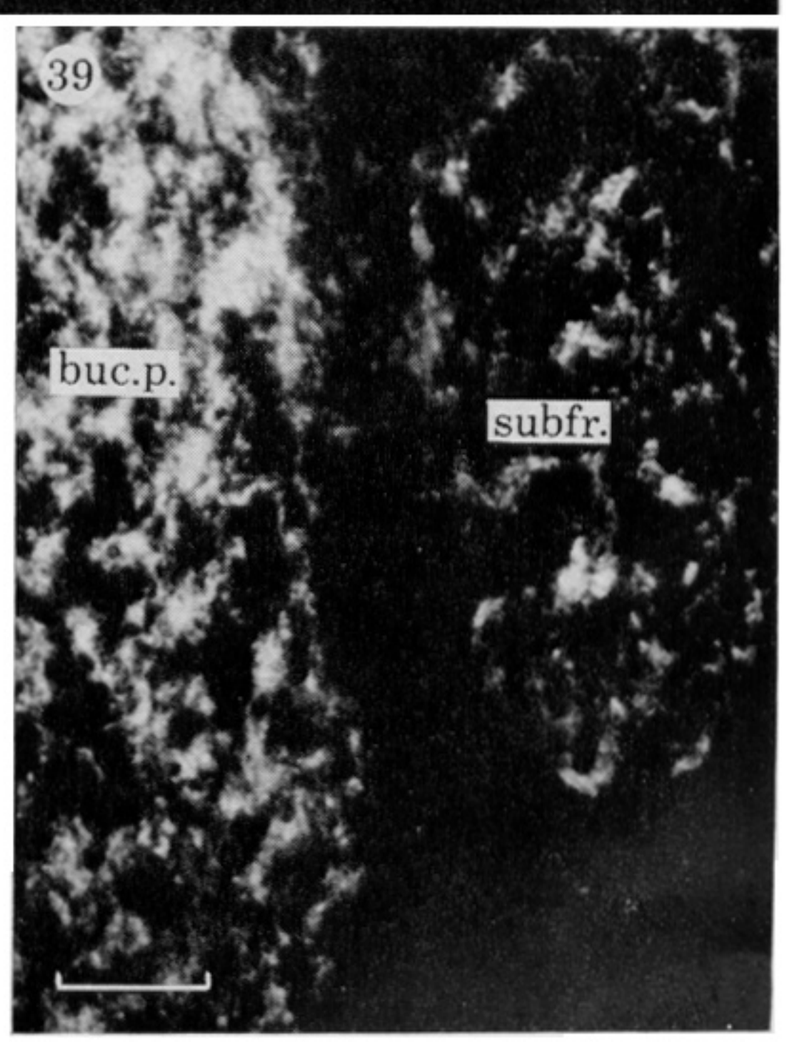




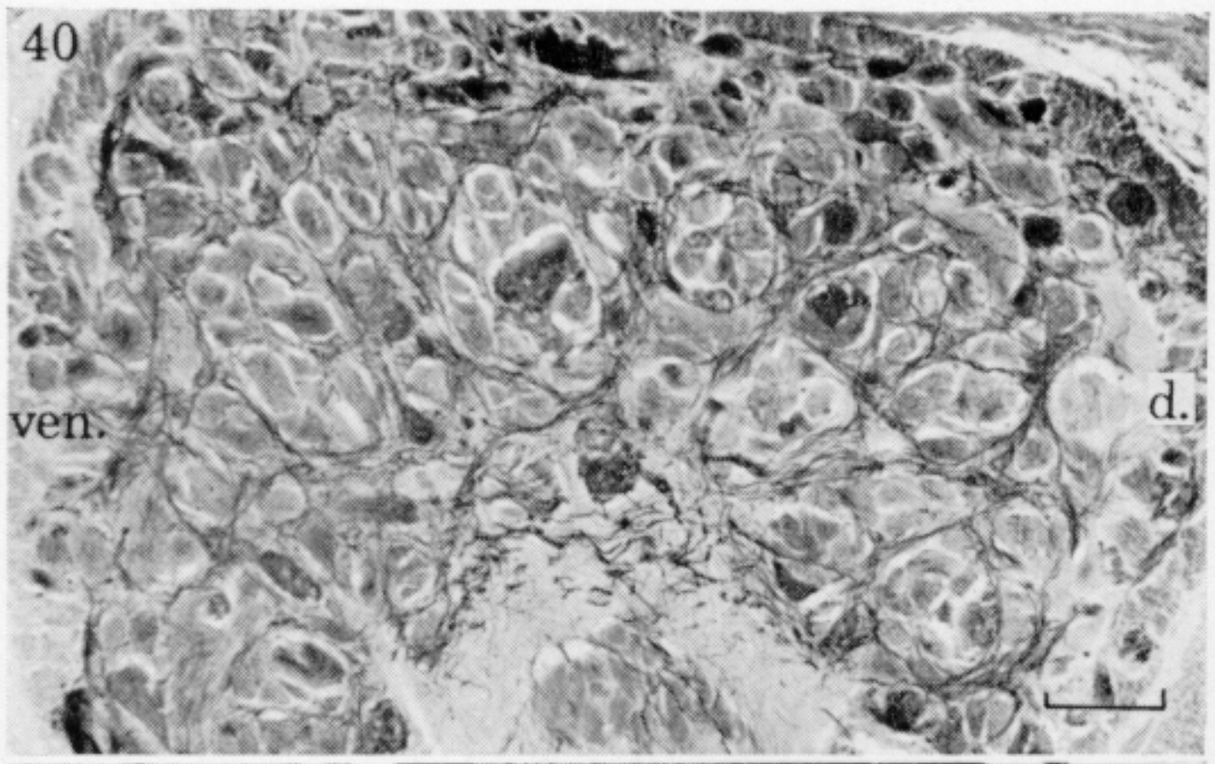


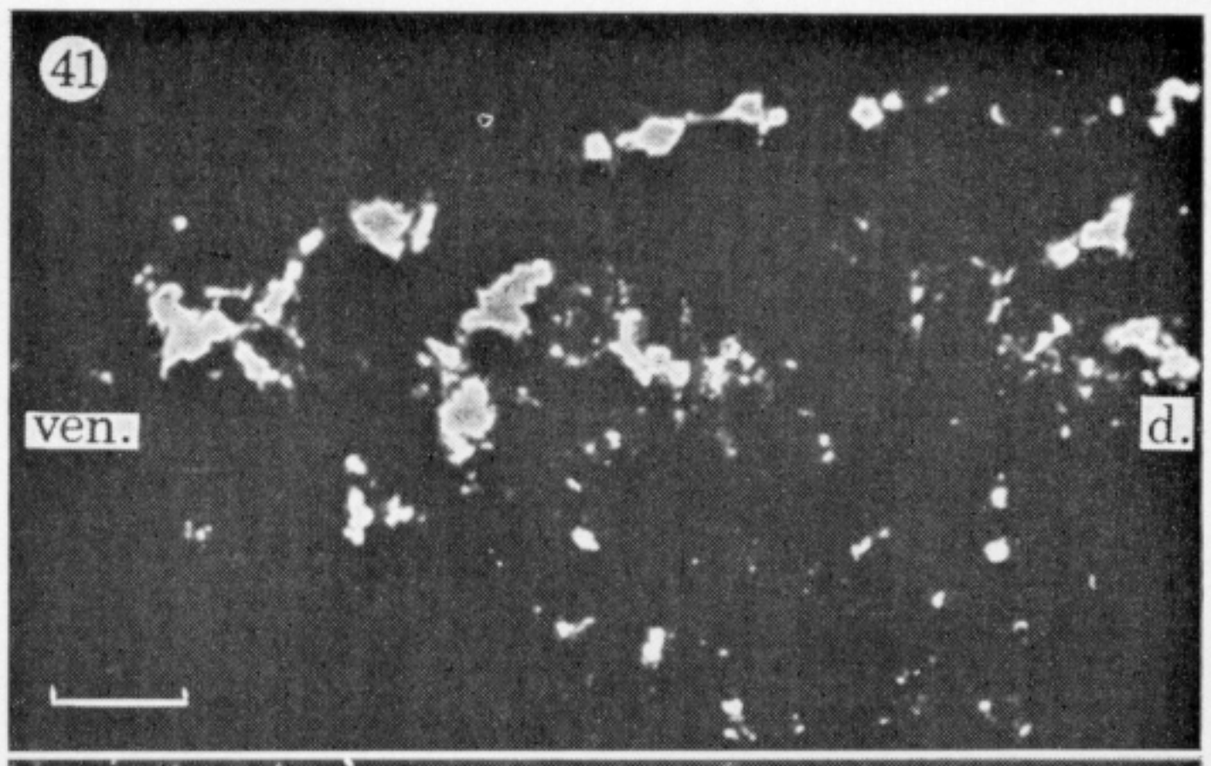


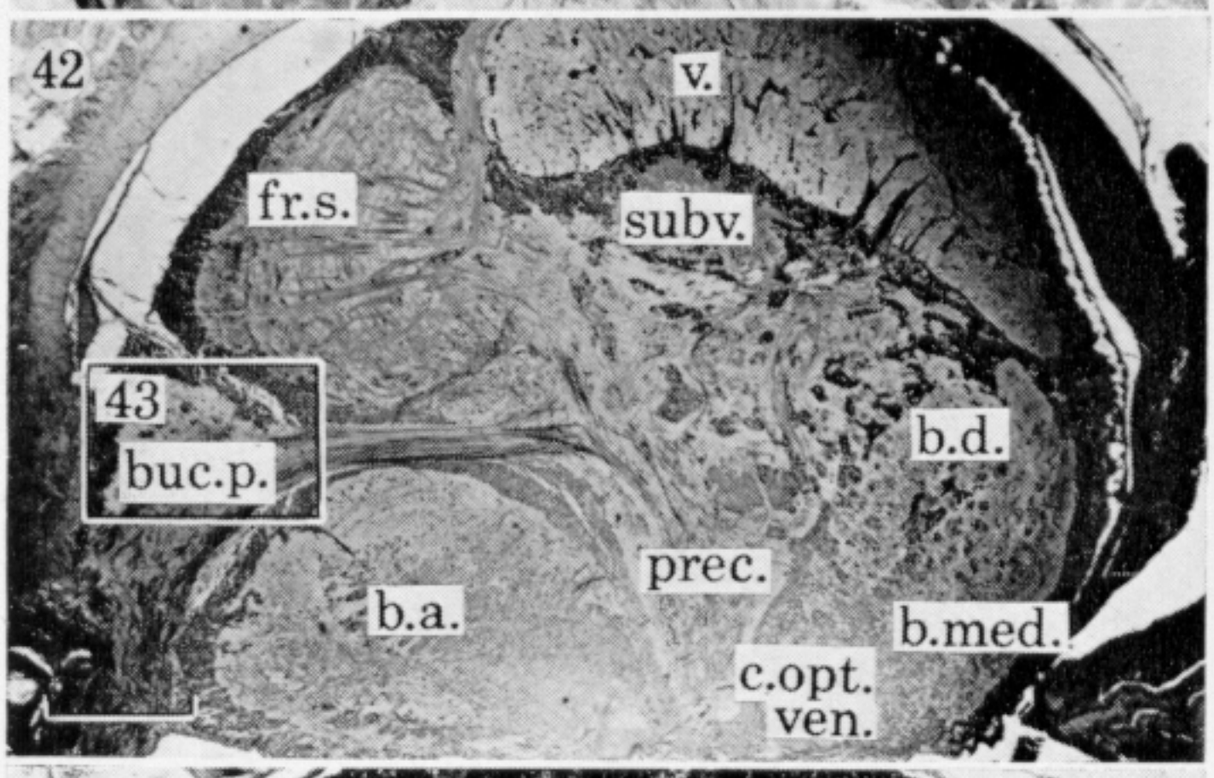


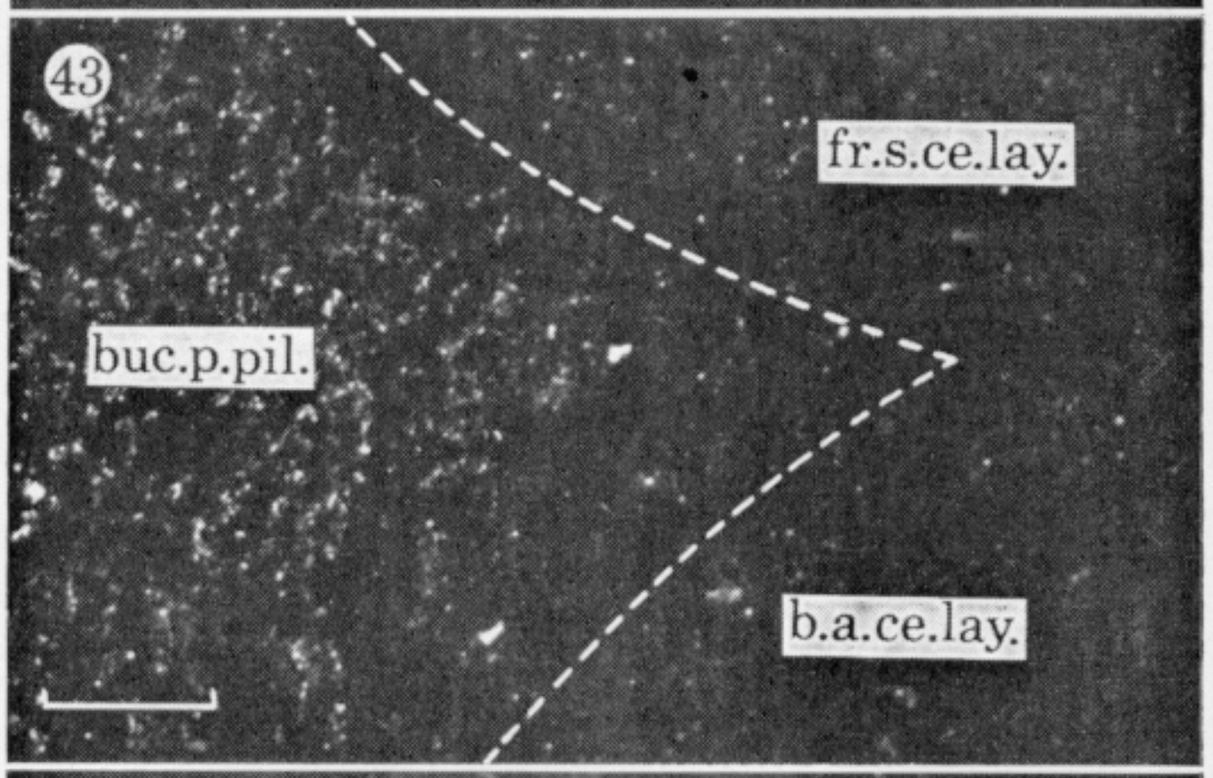


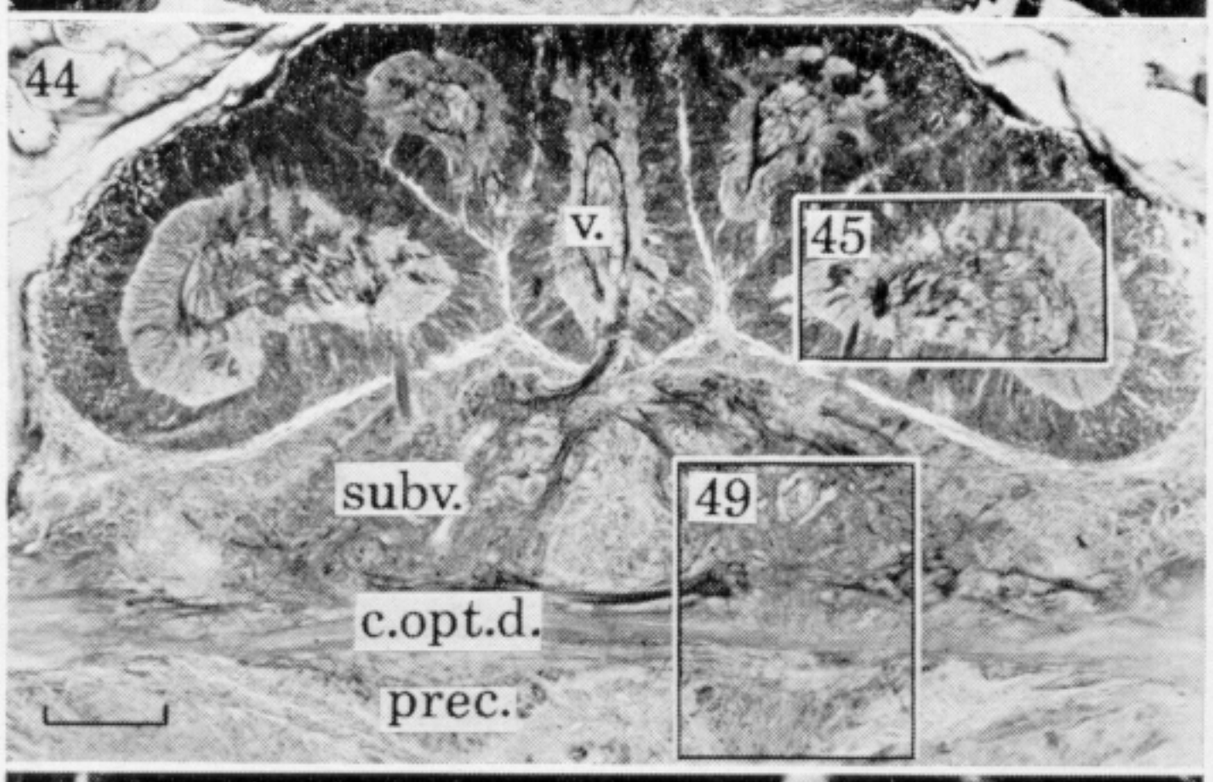
FIGURES 31–39. For description see opposite.

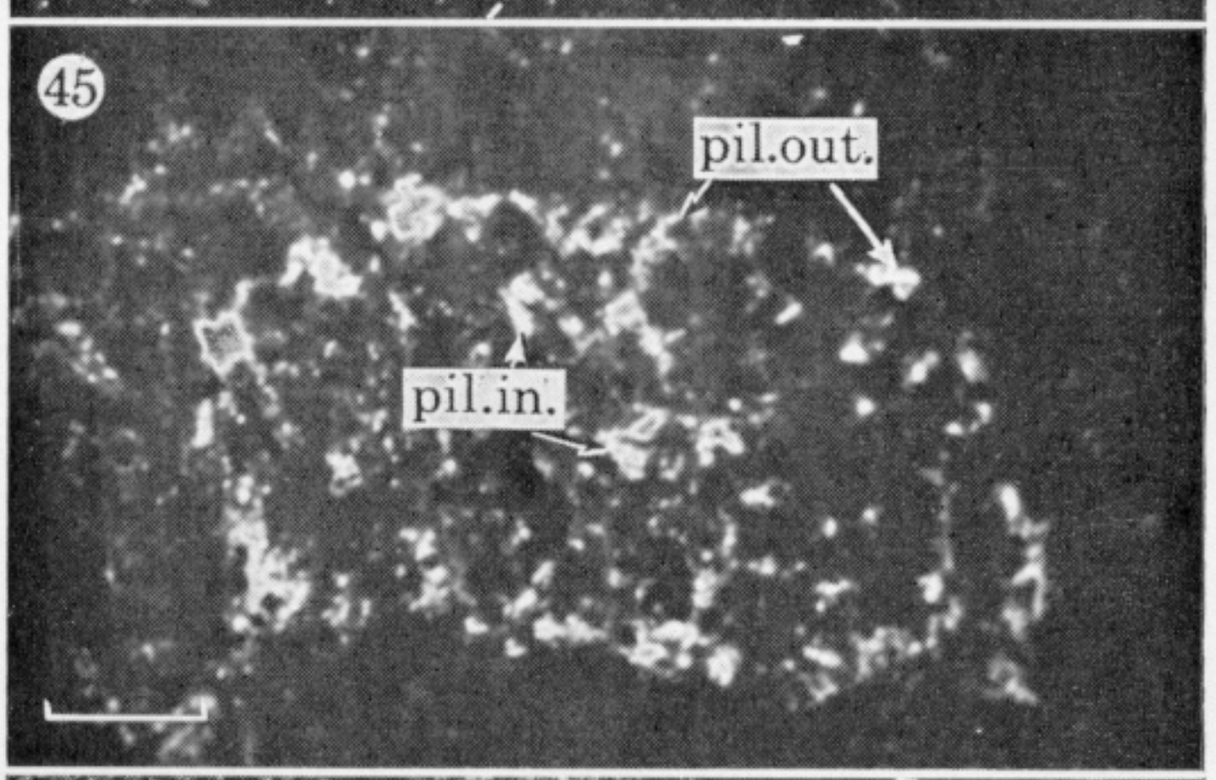


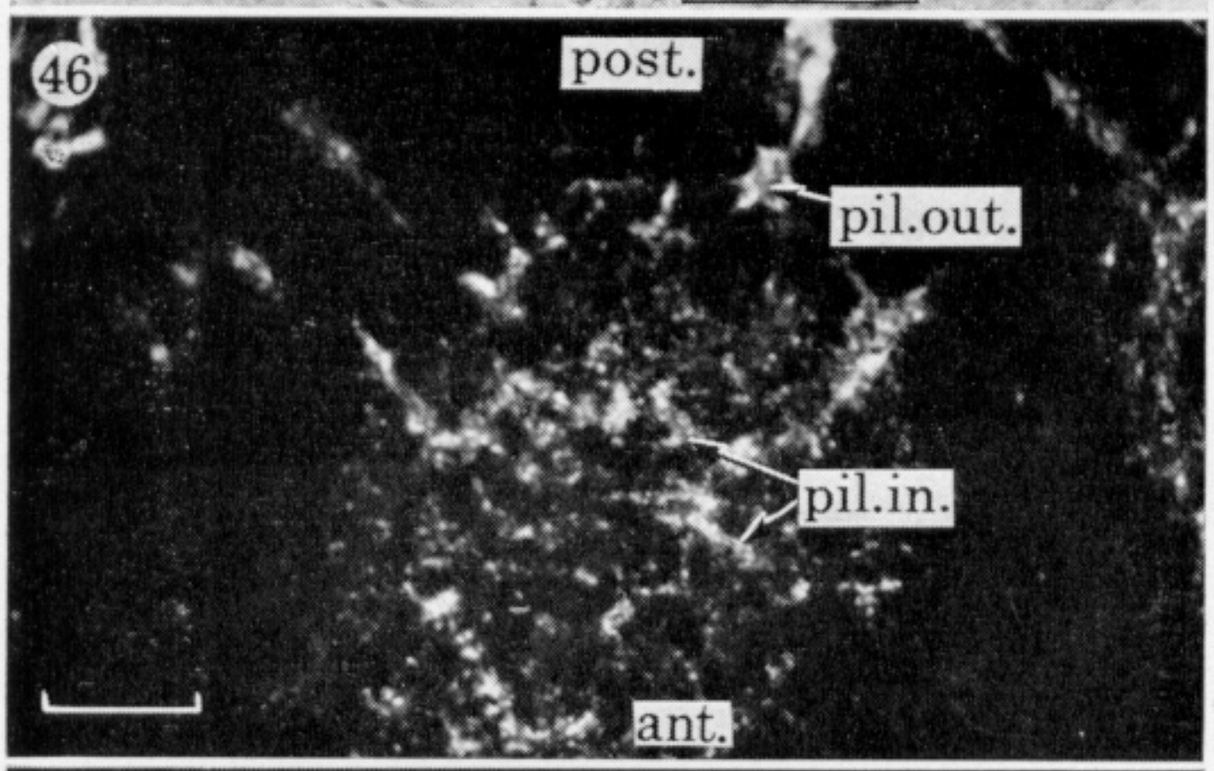


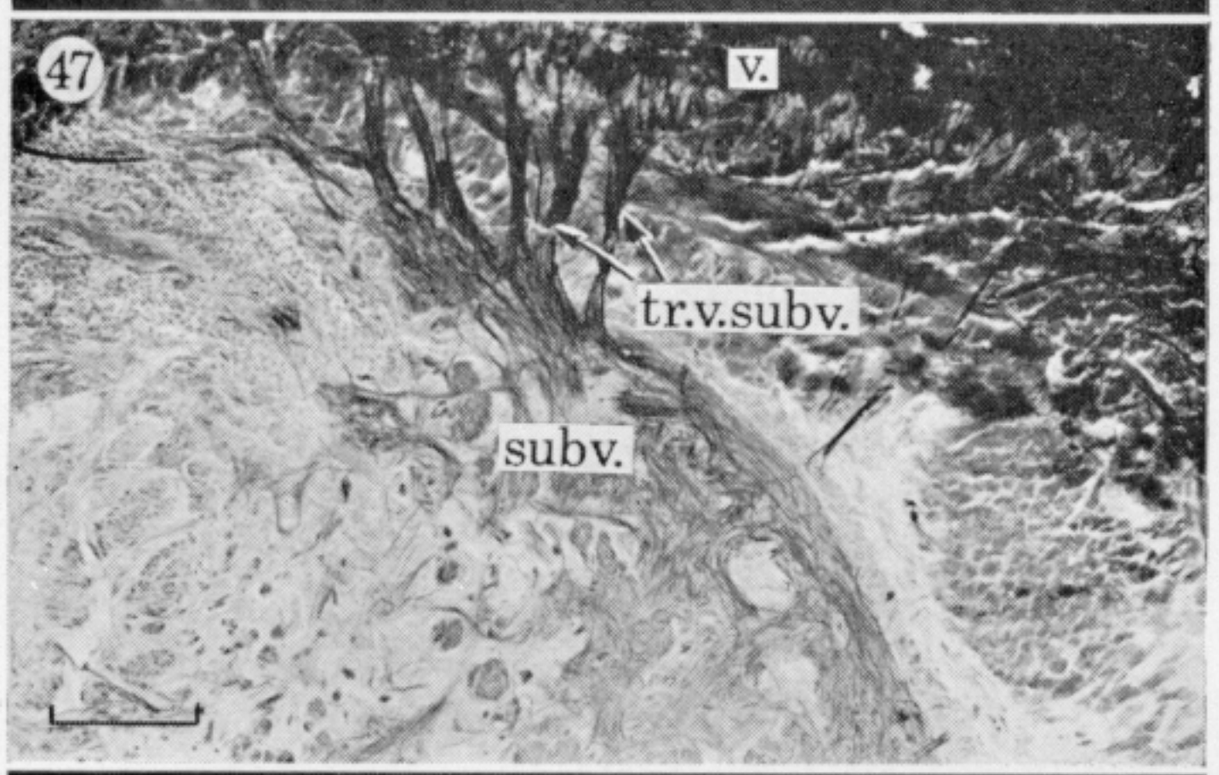


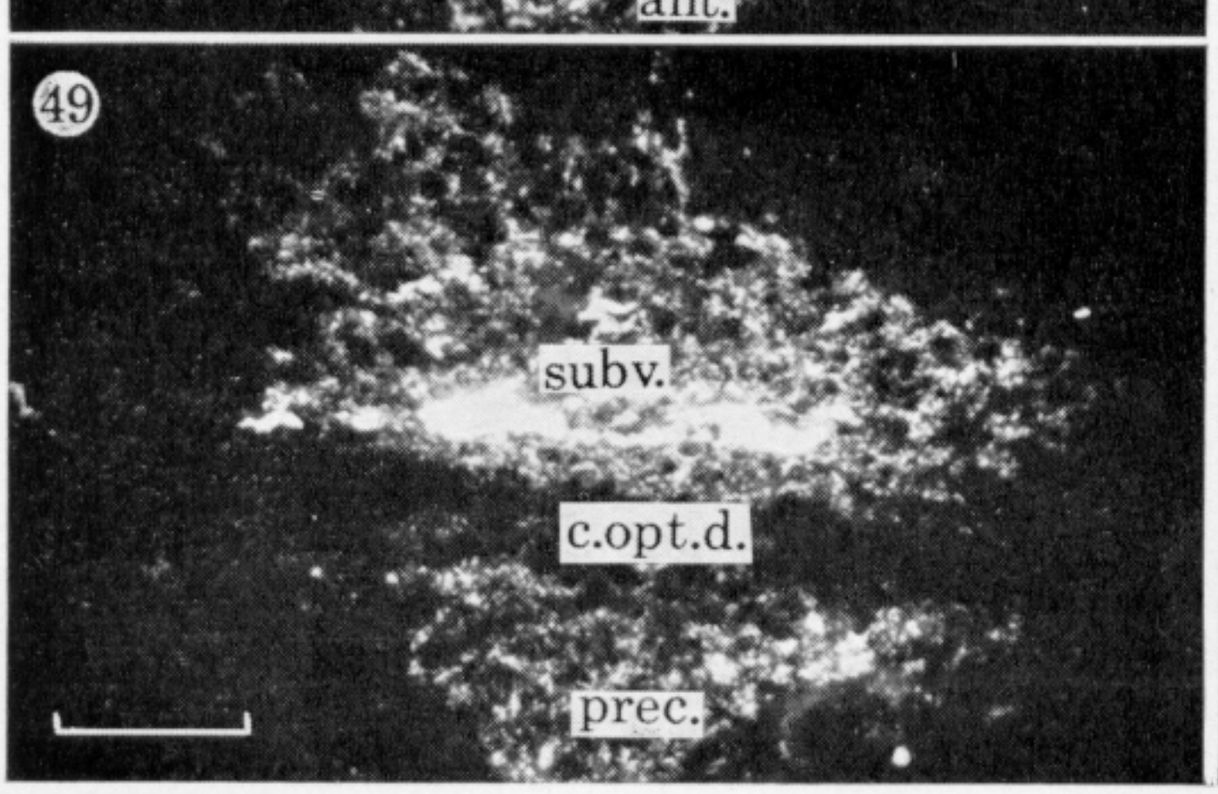


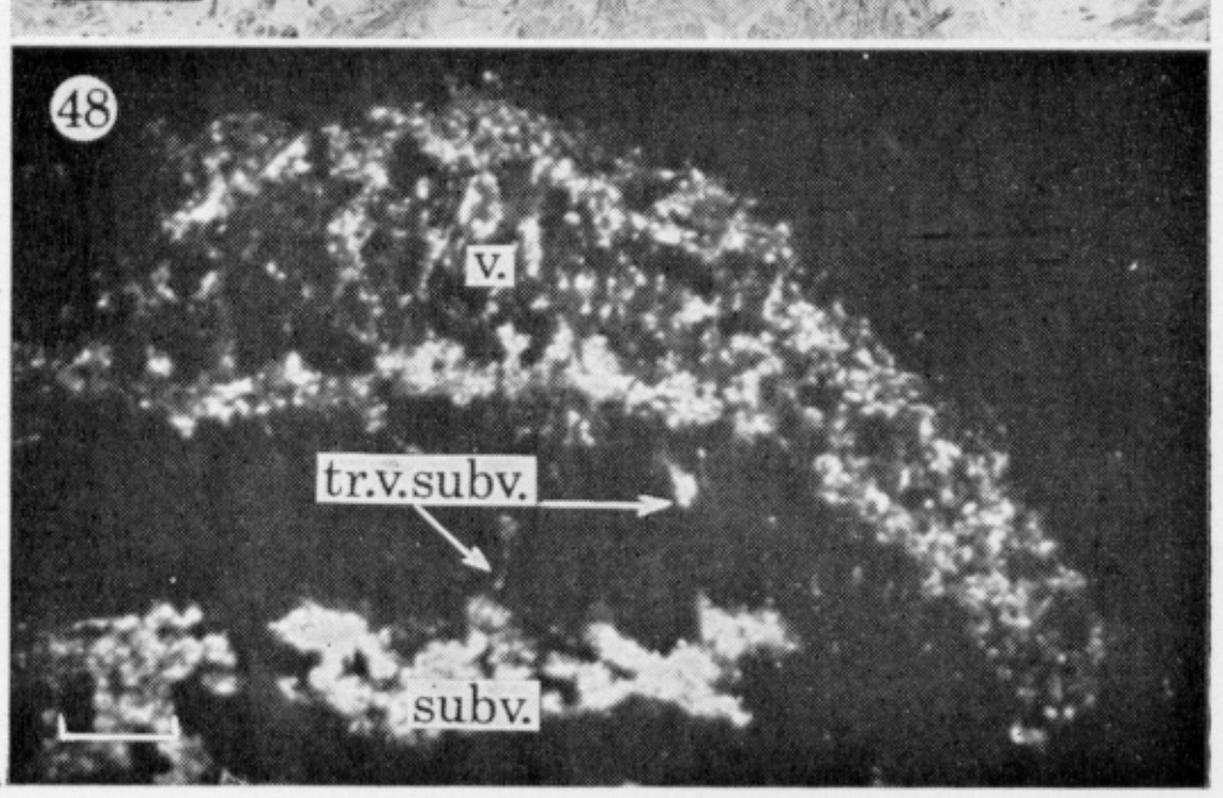




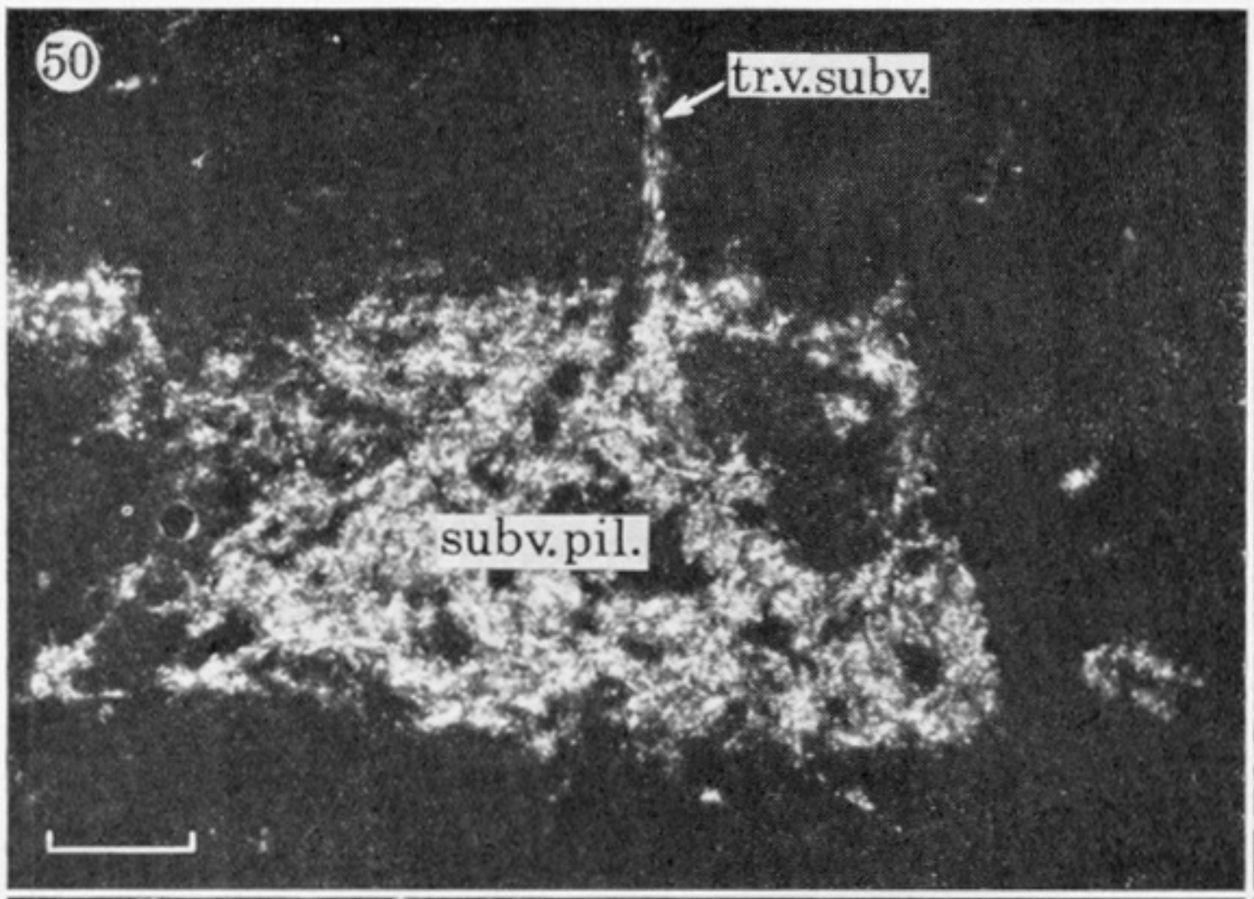


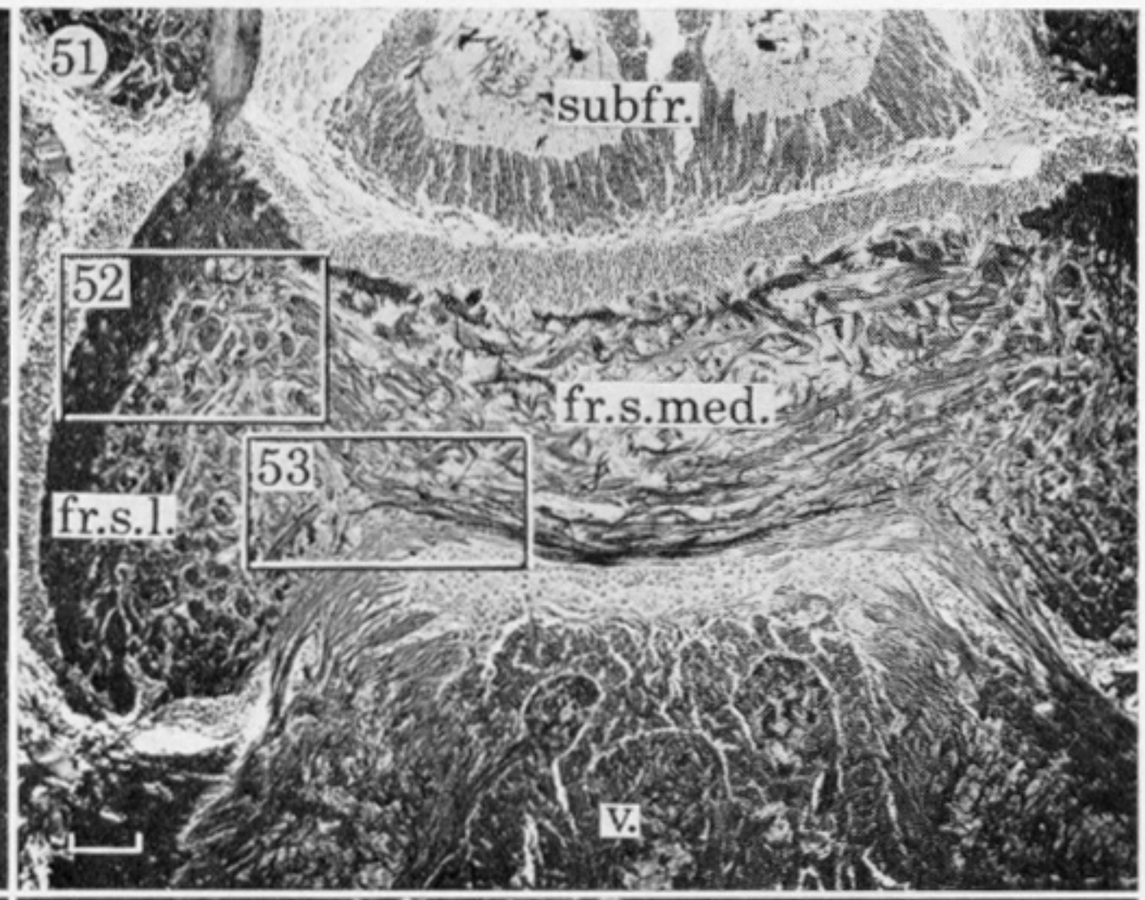


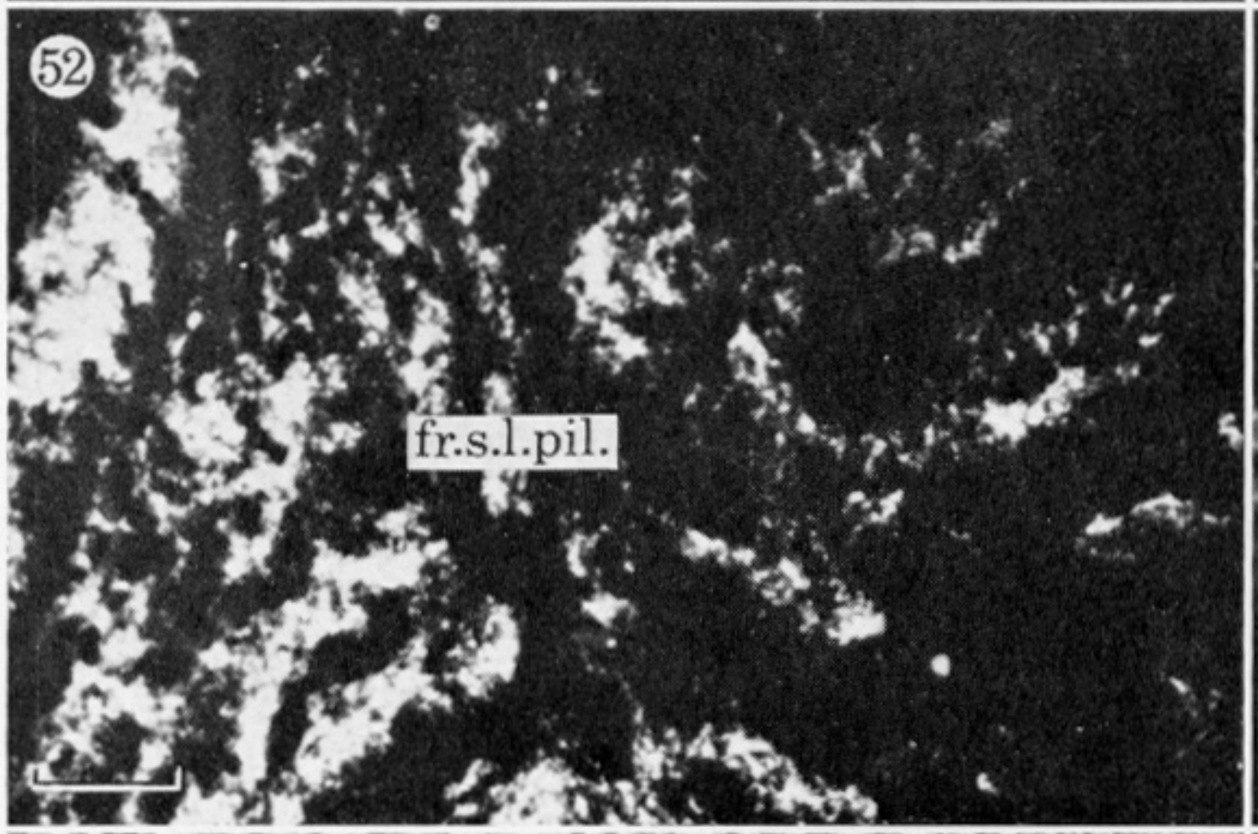


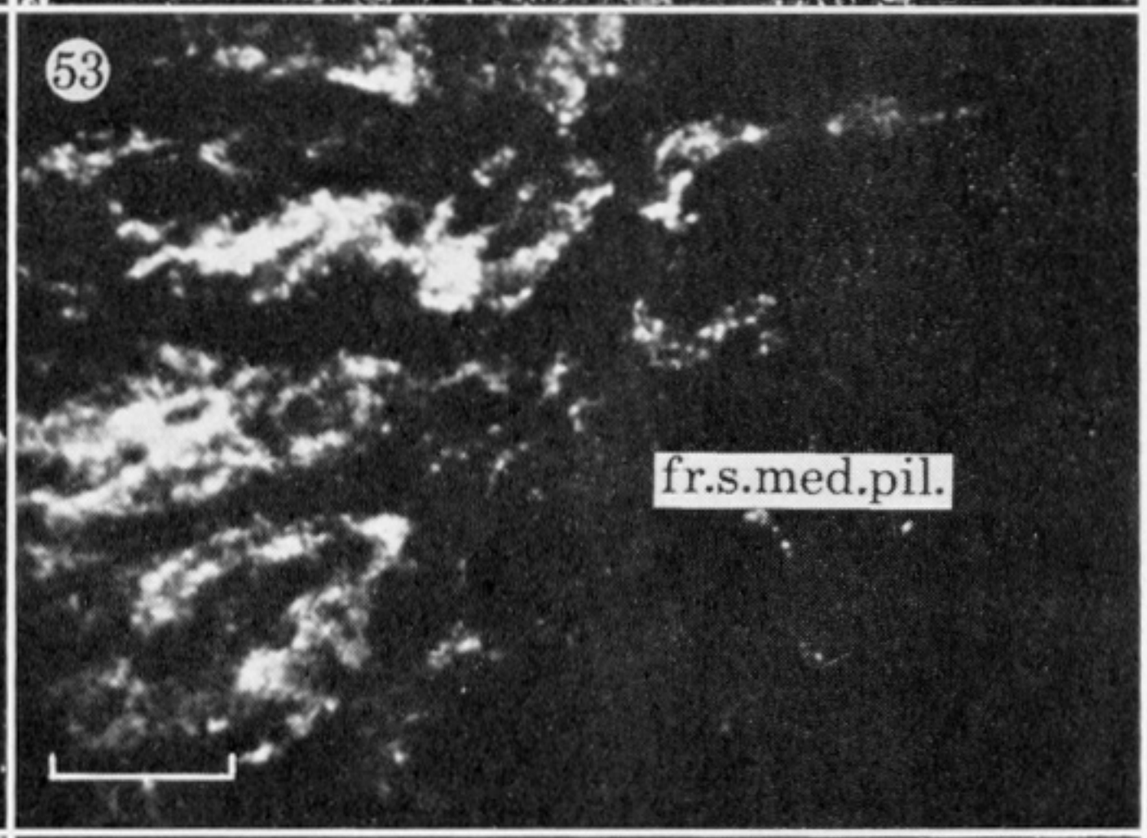


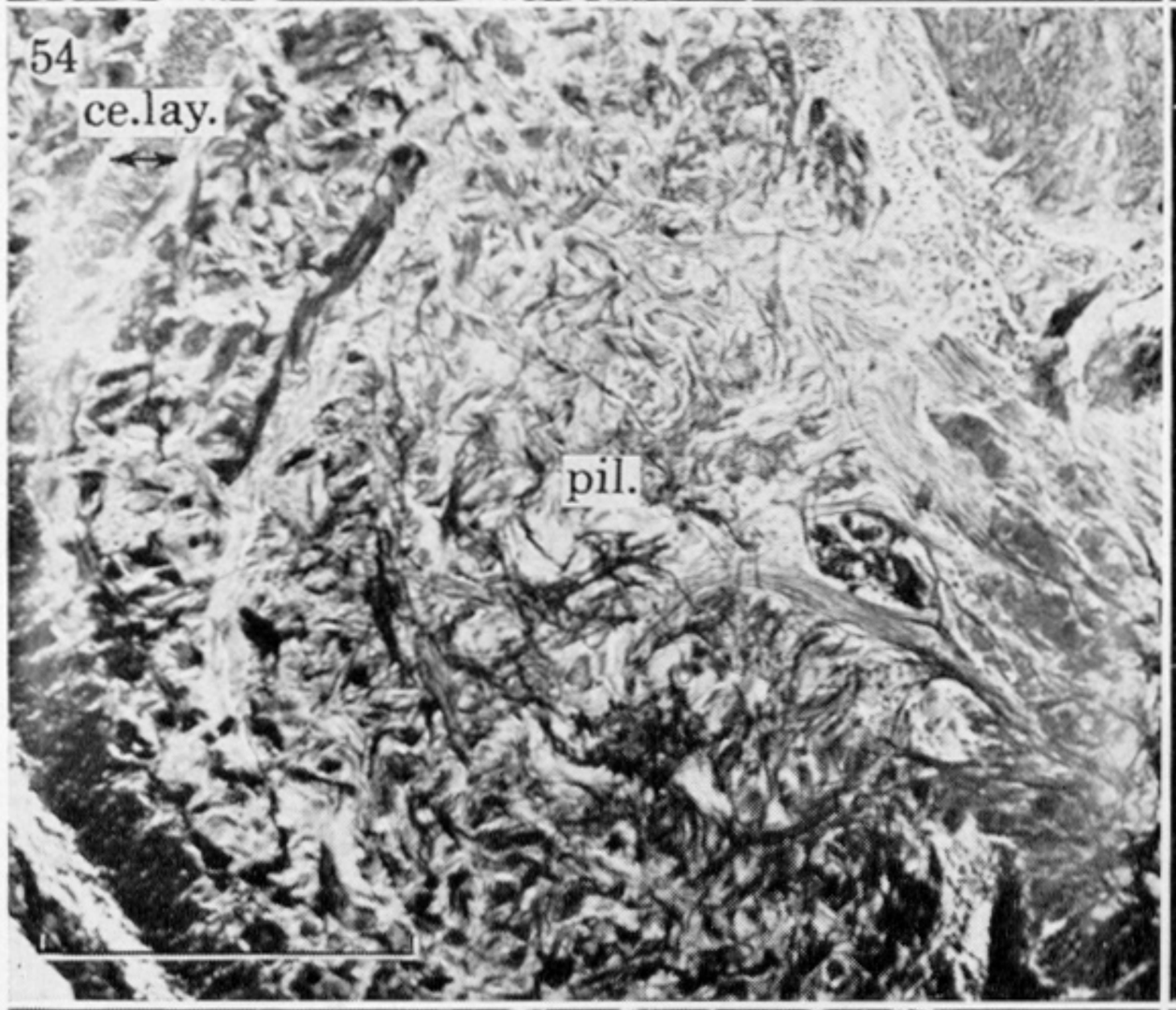
FIGURES 40–49. For description see opposite.

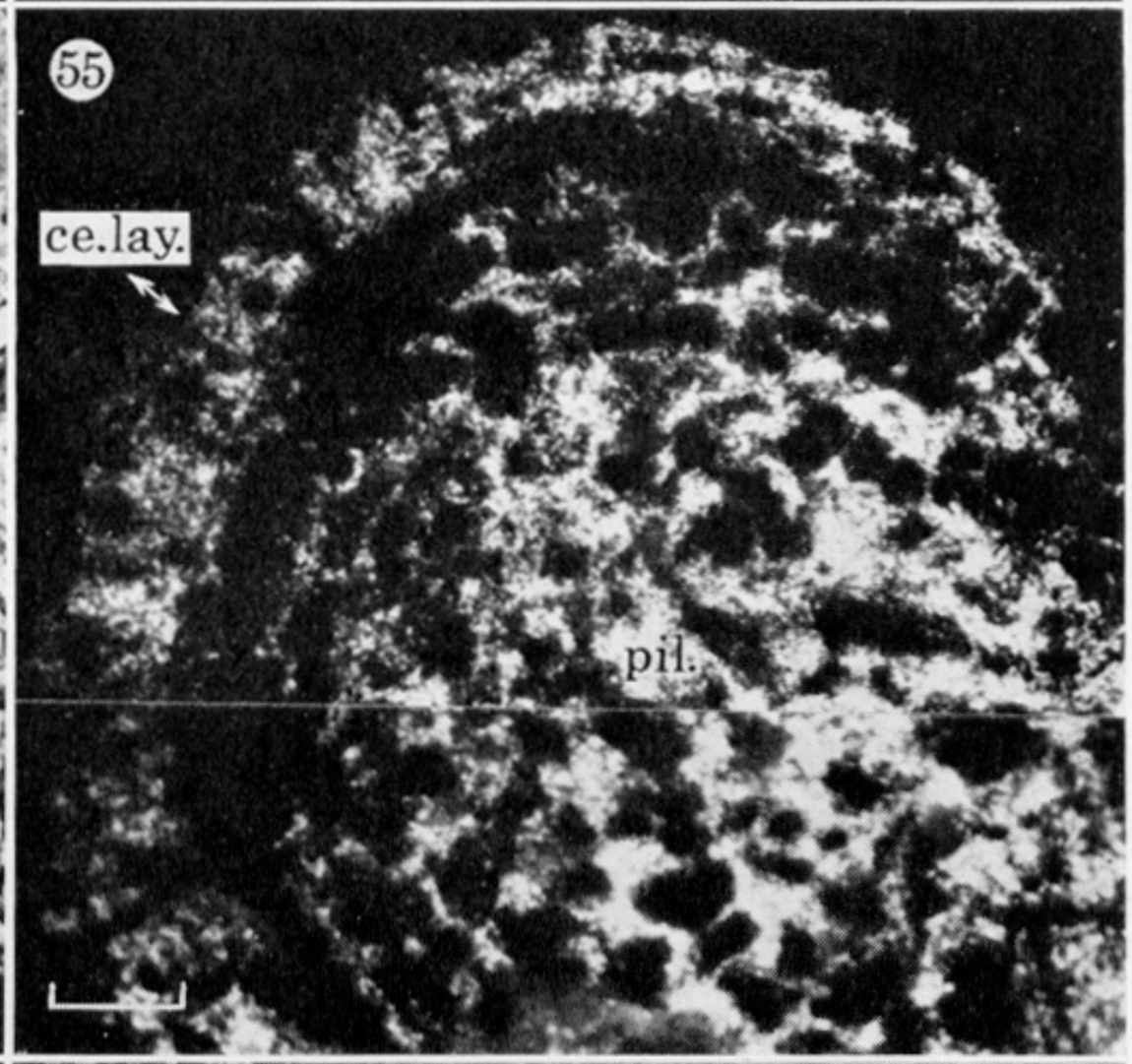


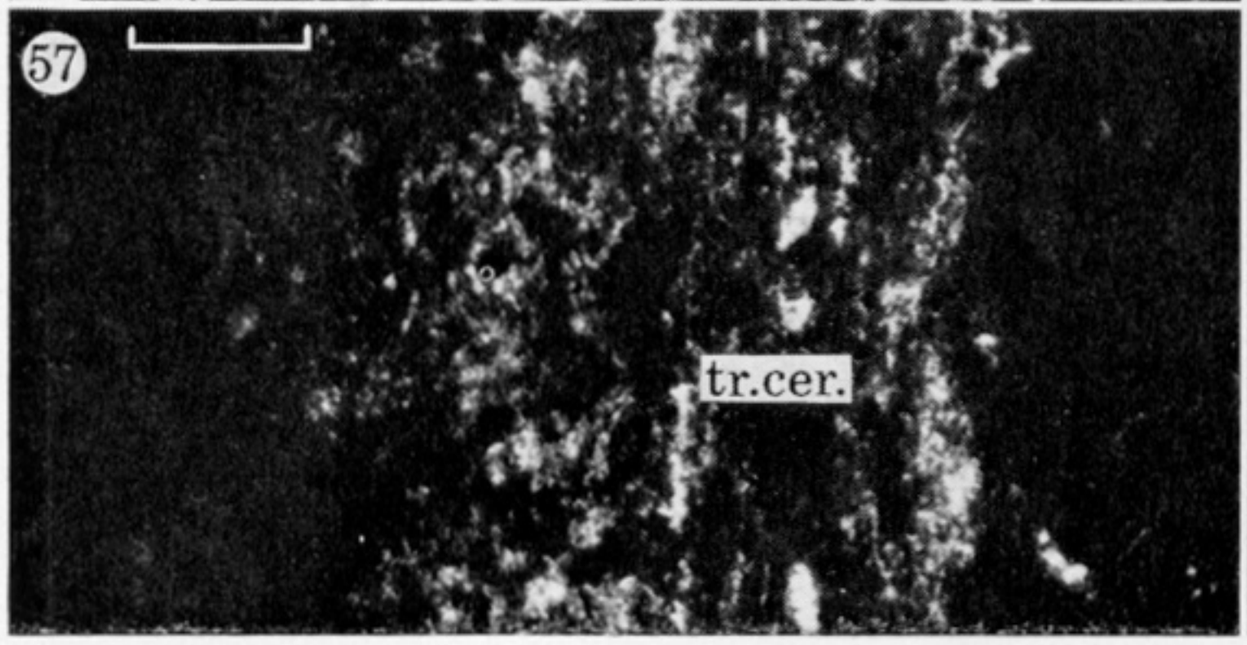


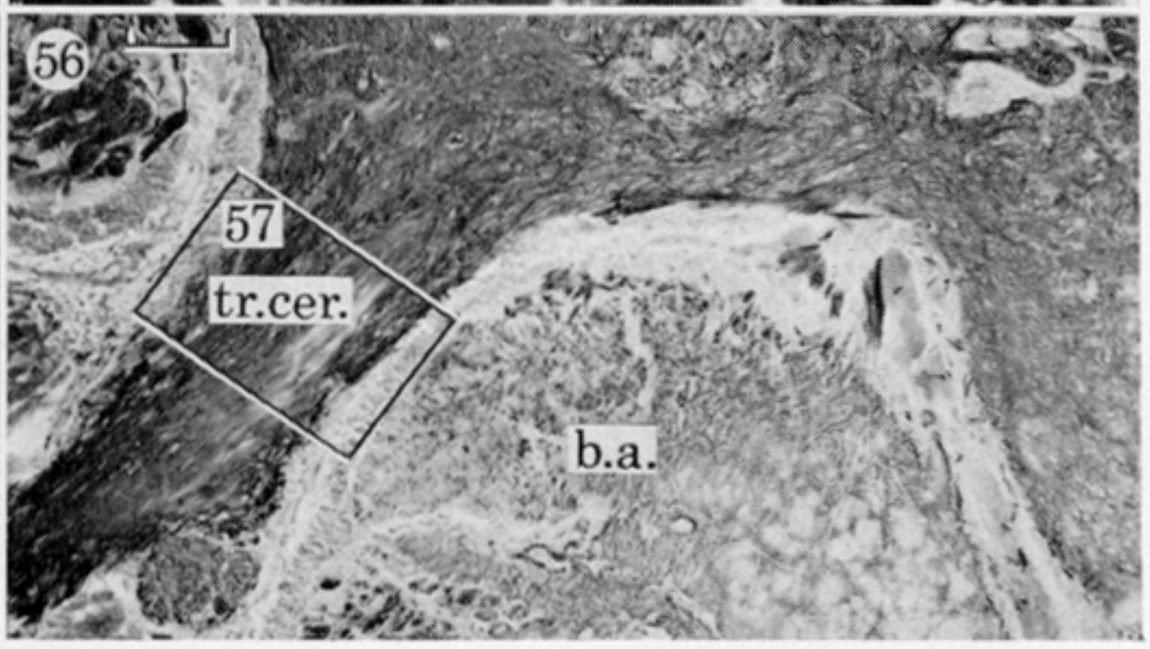




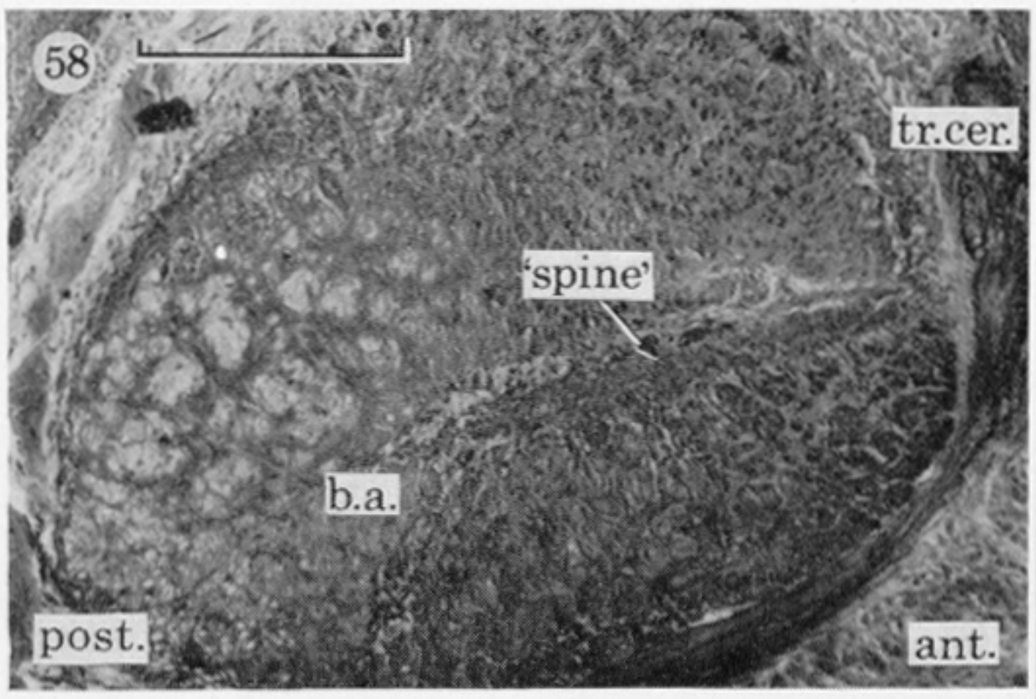


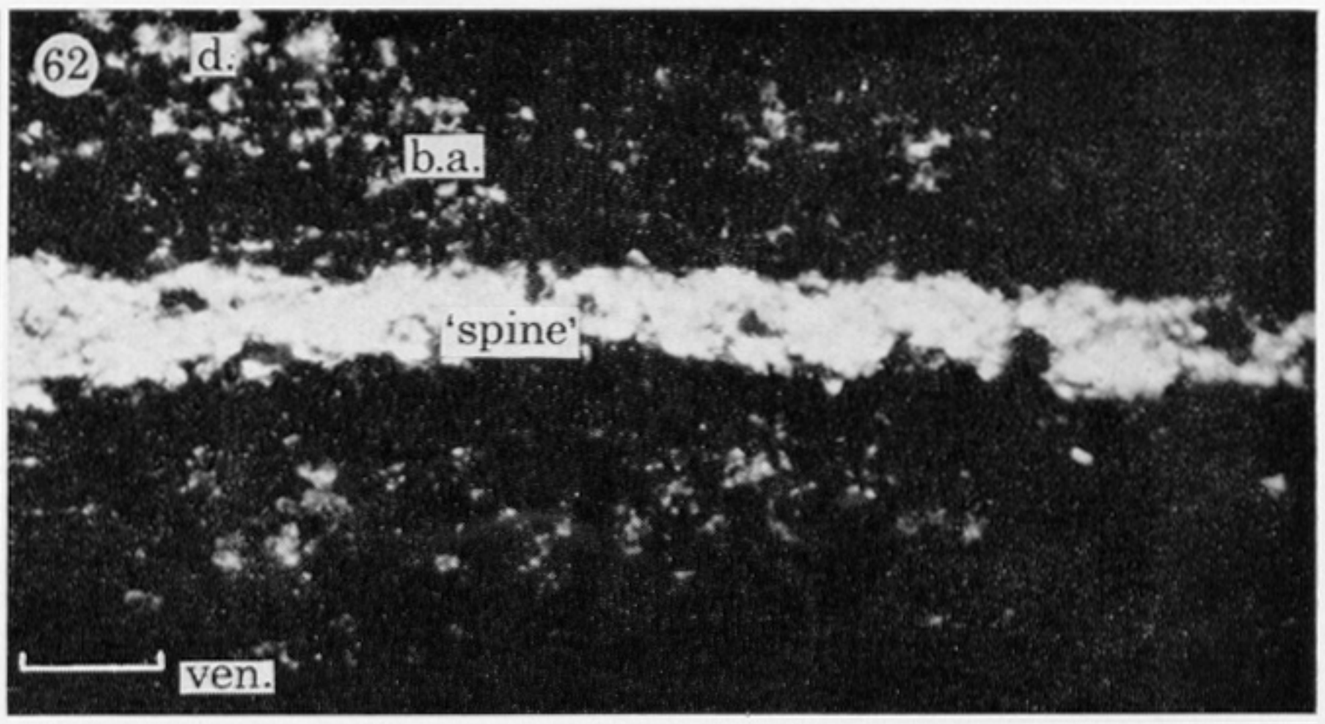


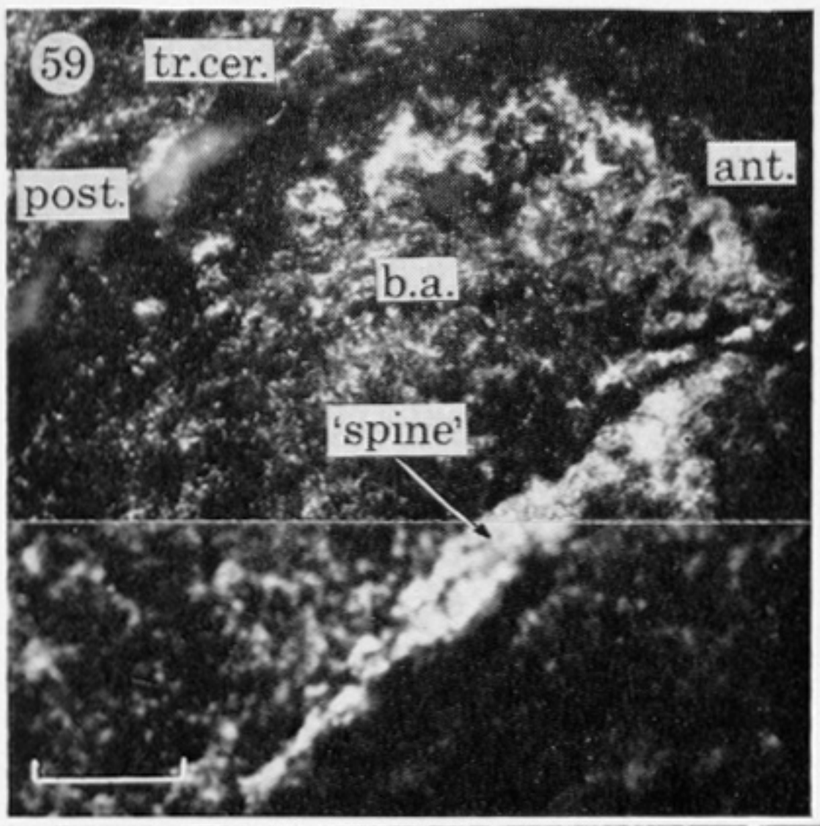


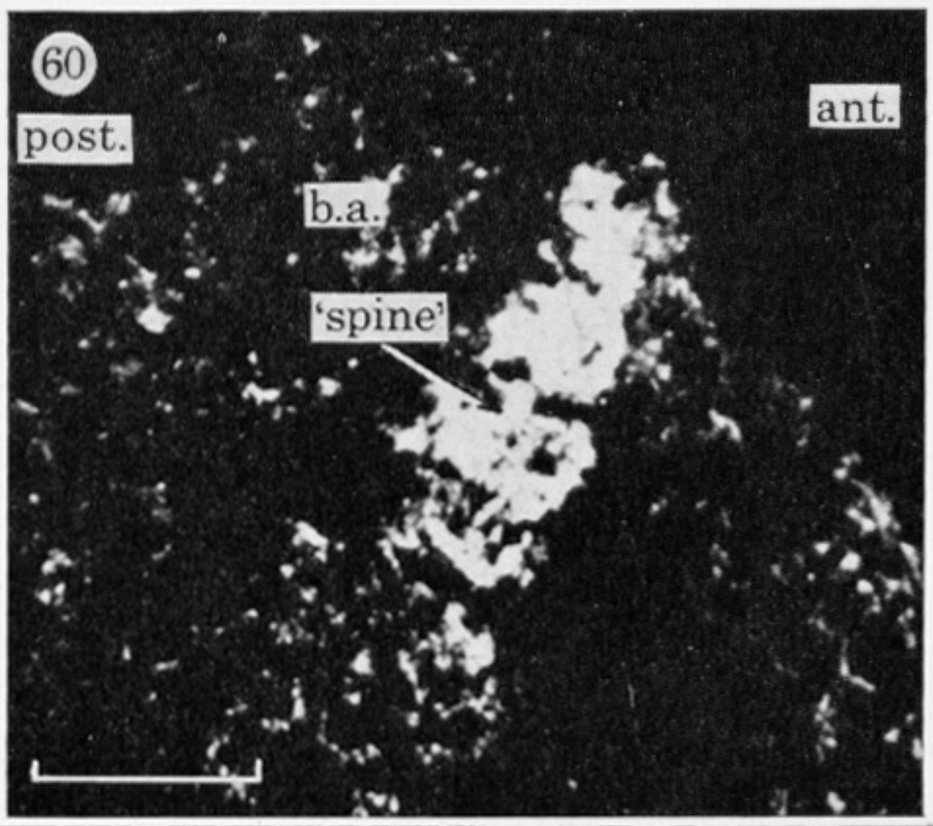


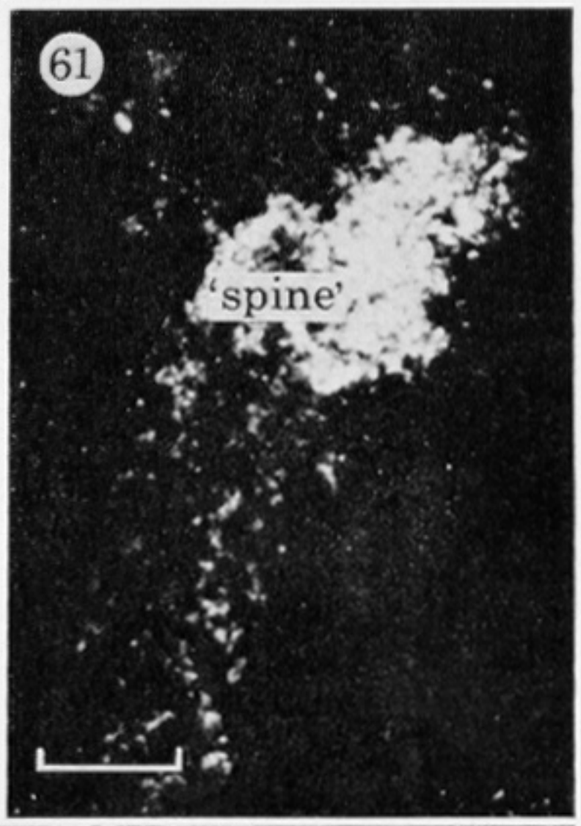
FIGURES 50–57. For description see page 134.

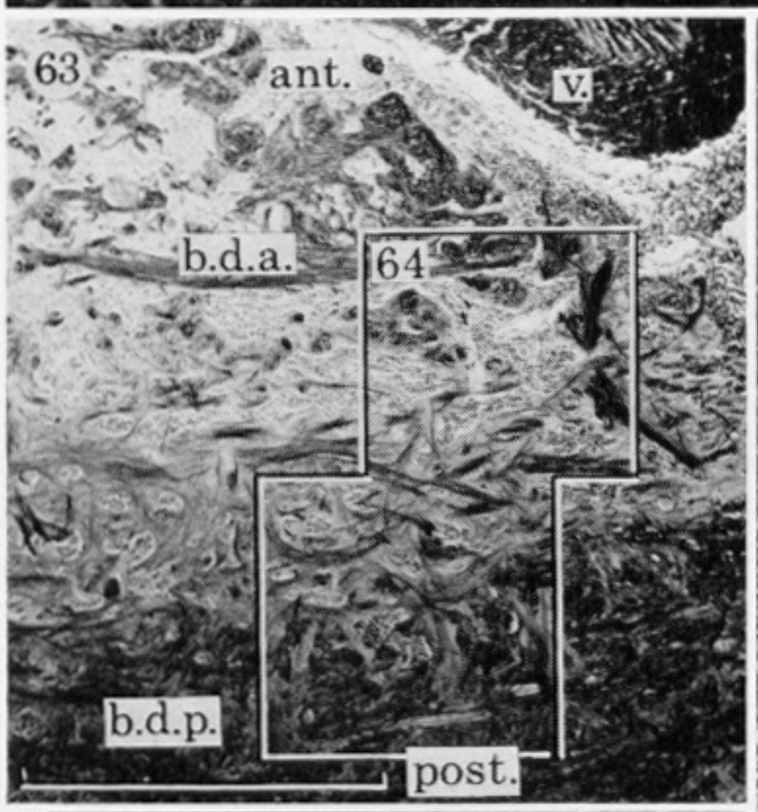


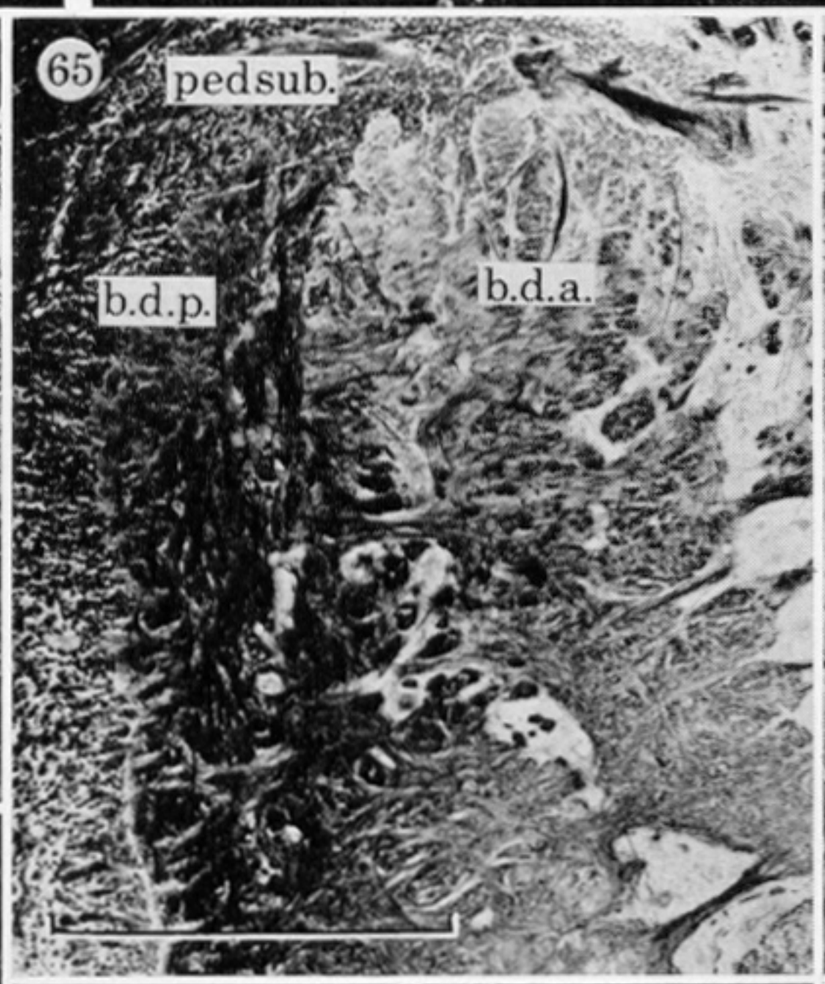


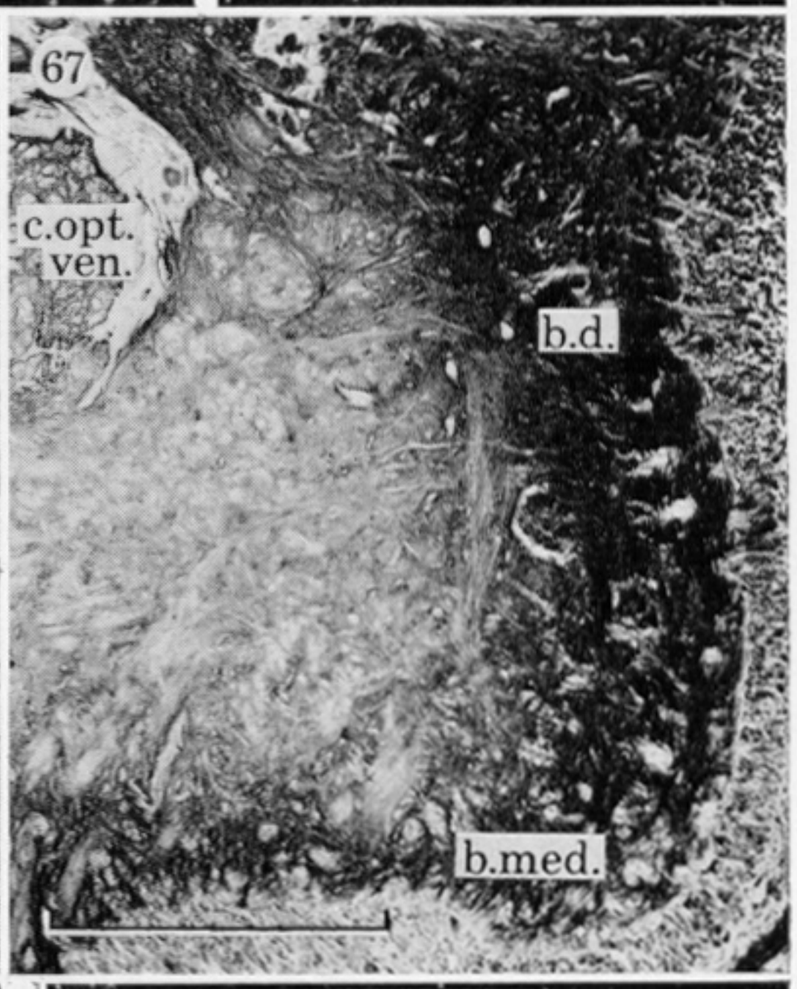


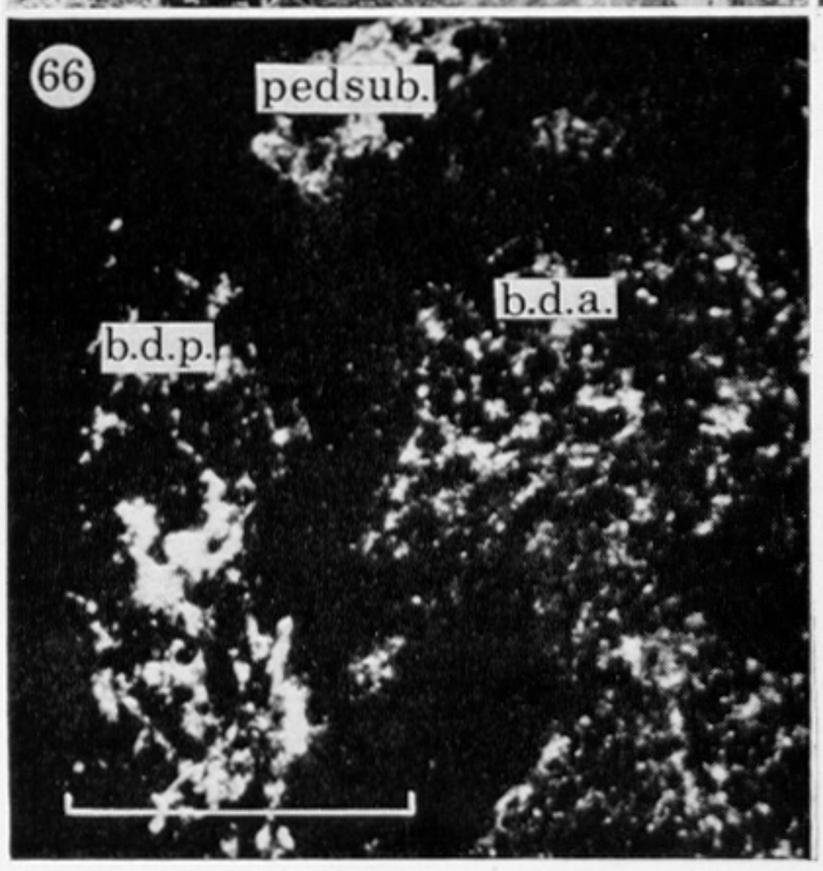


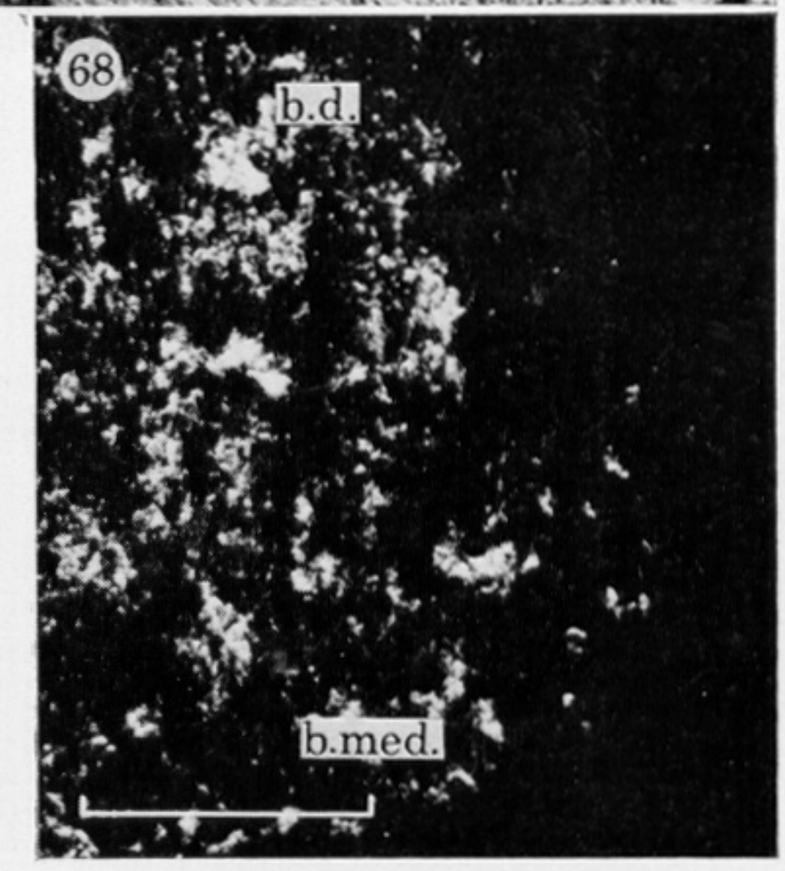




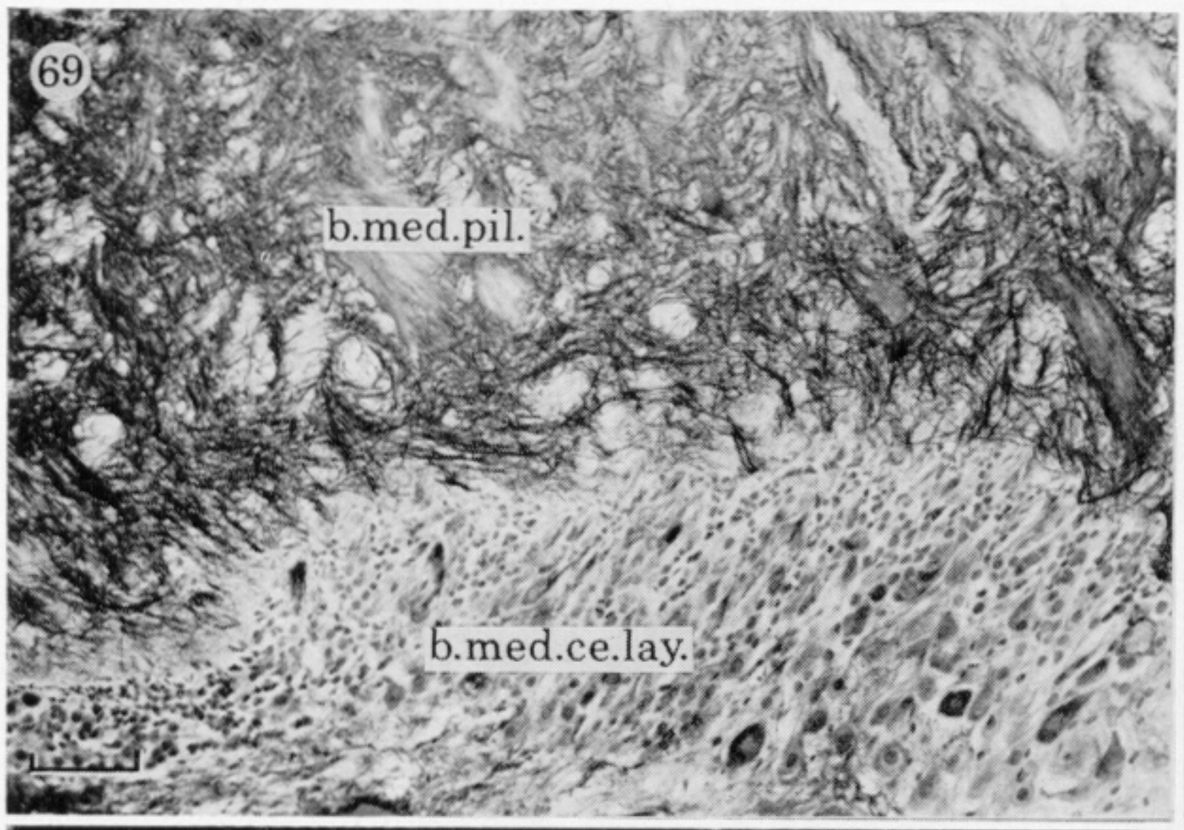


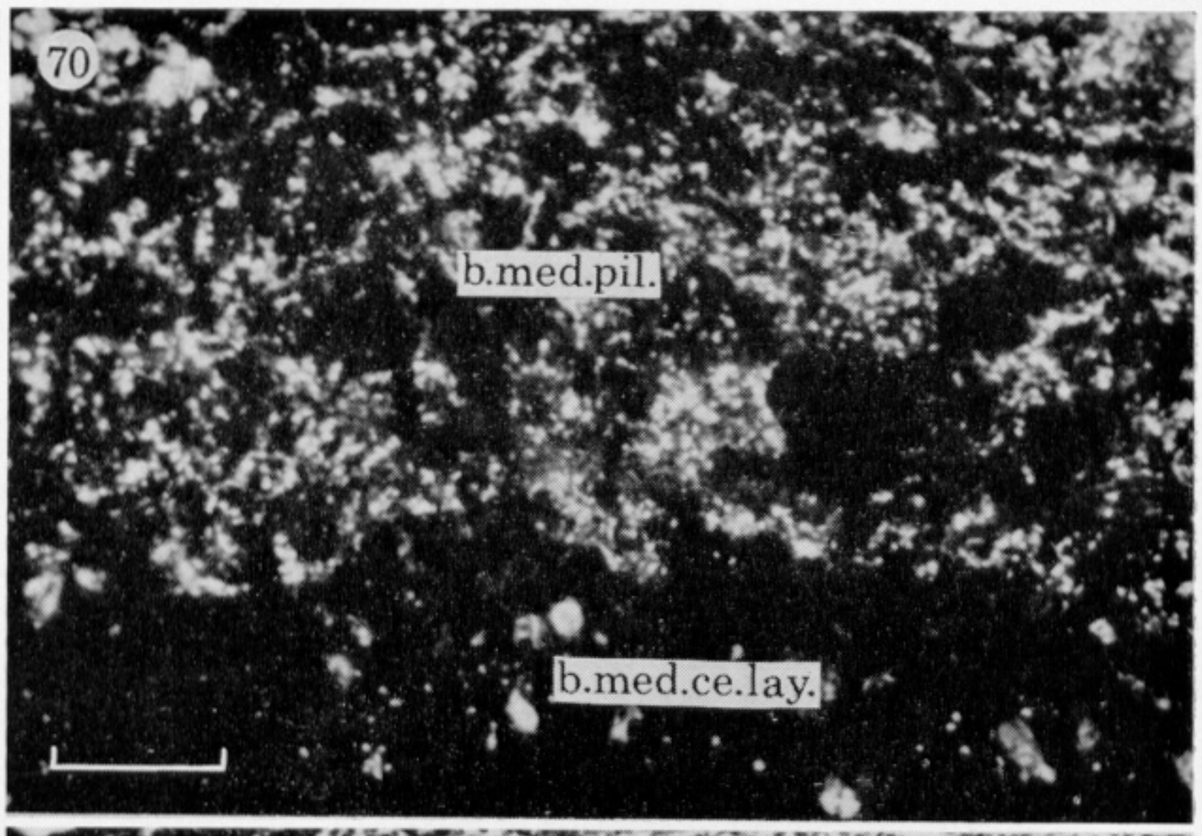


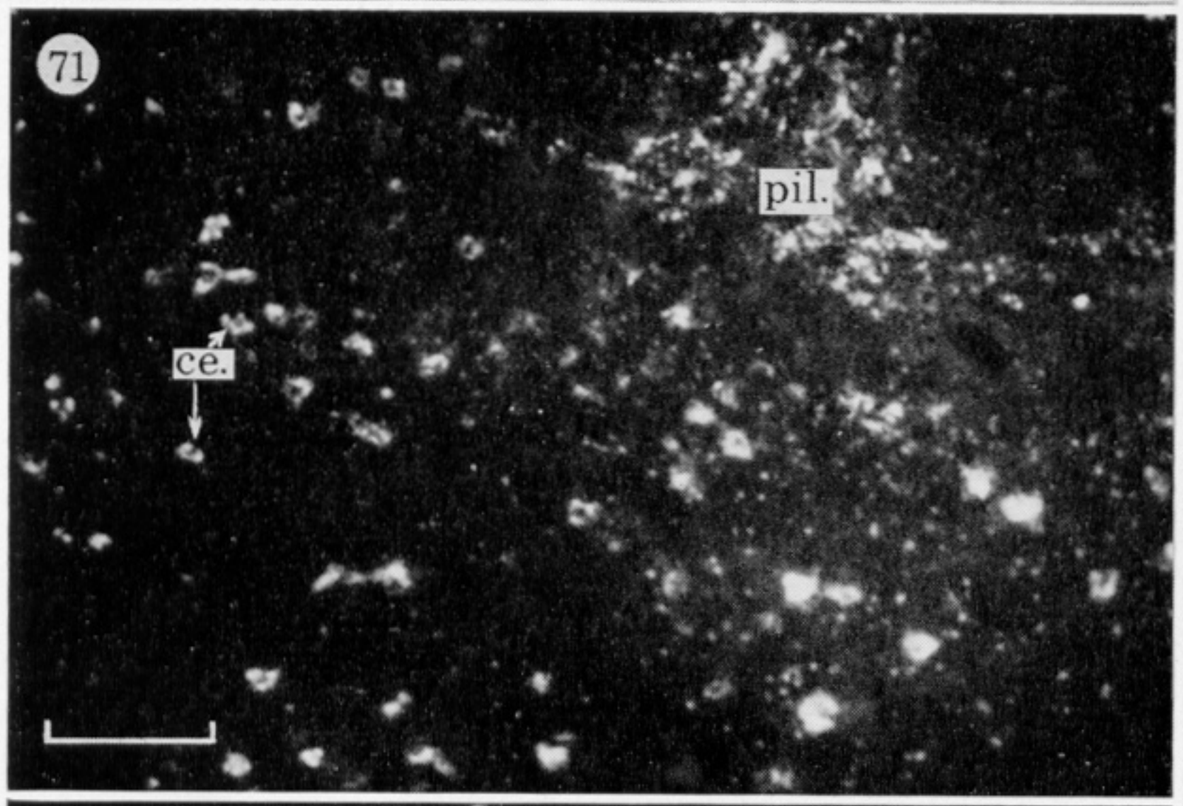


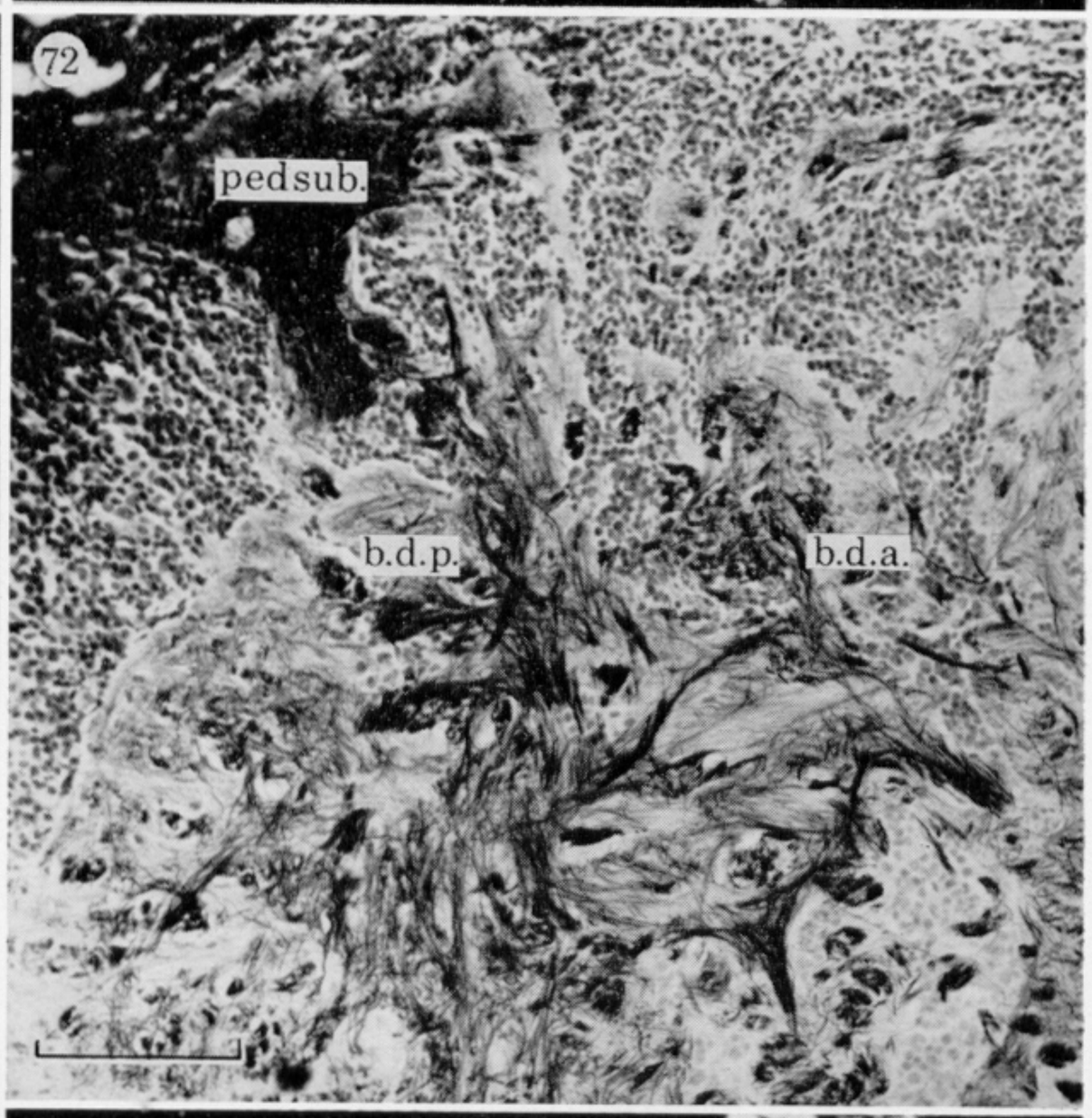


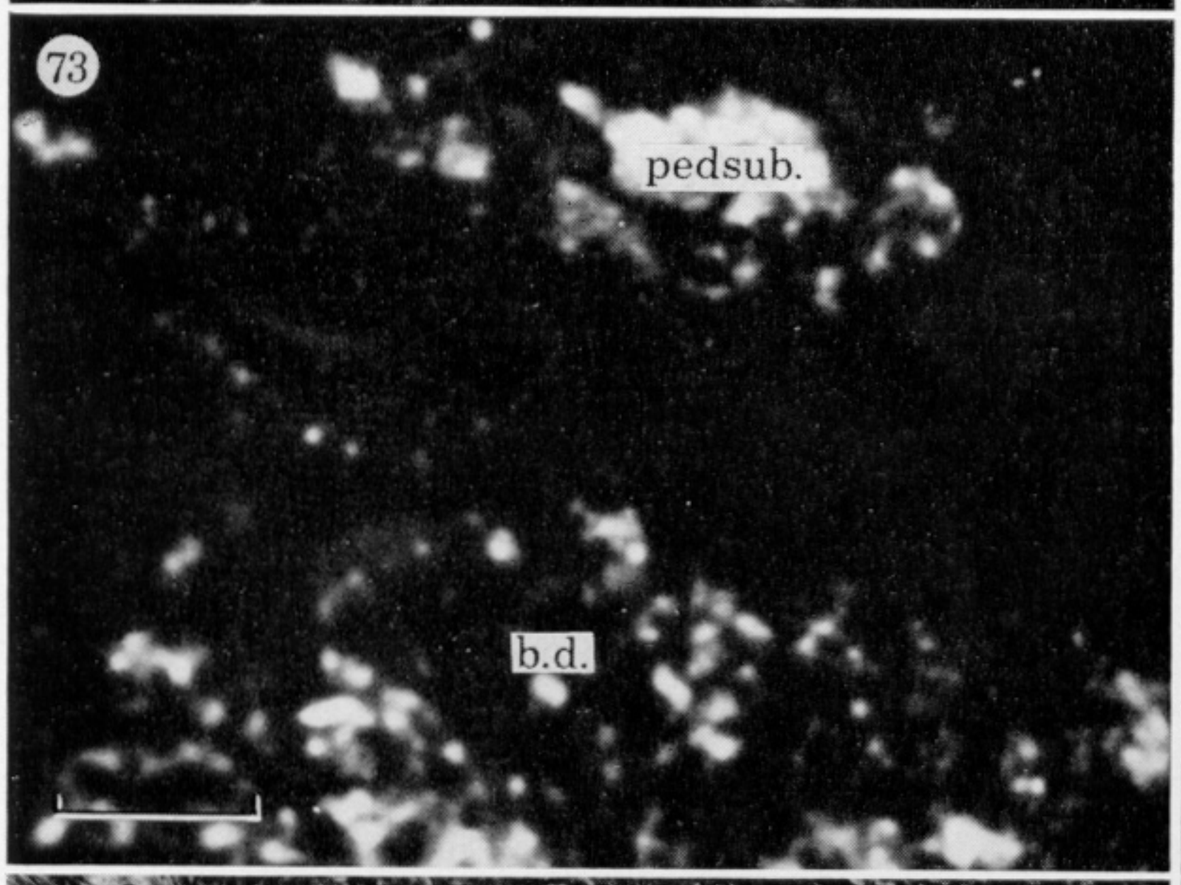
FIGURES 58–68. For description see page 135.

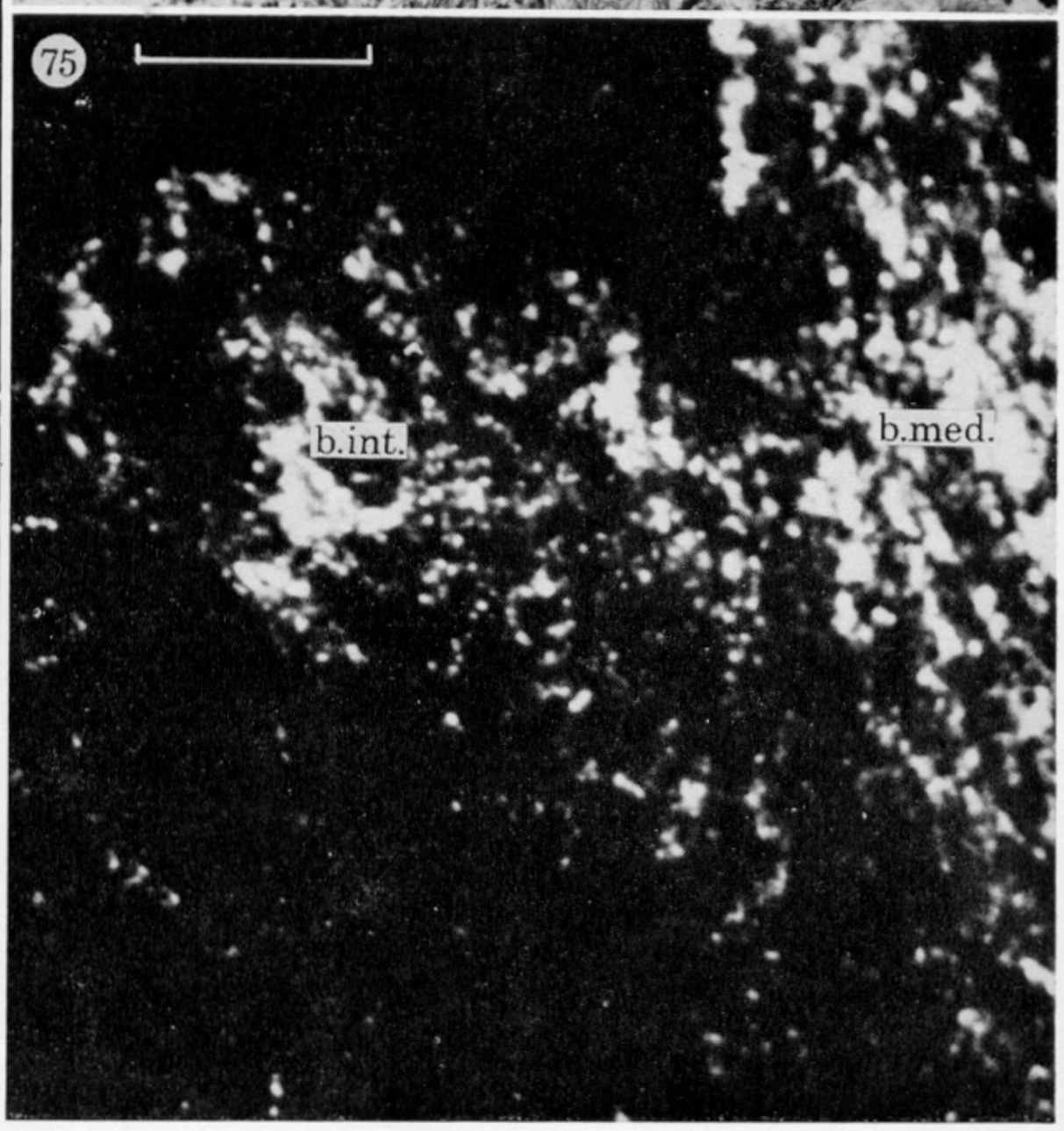


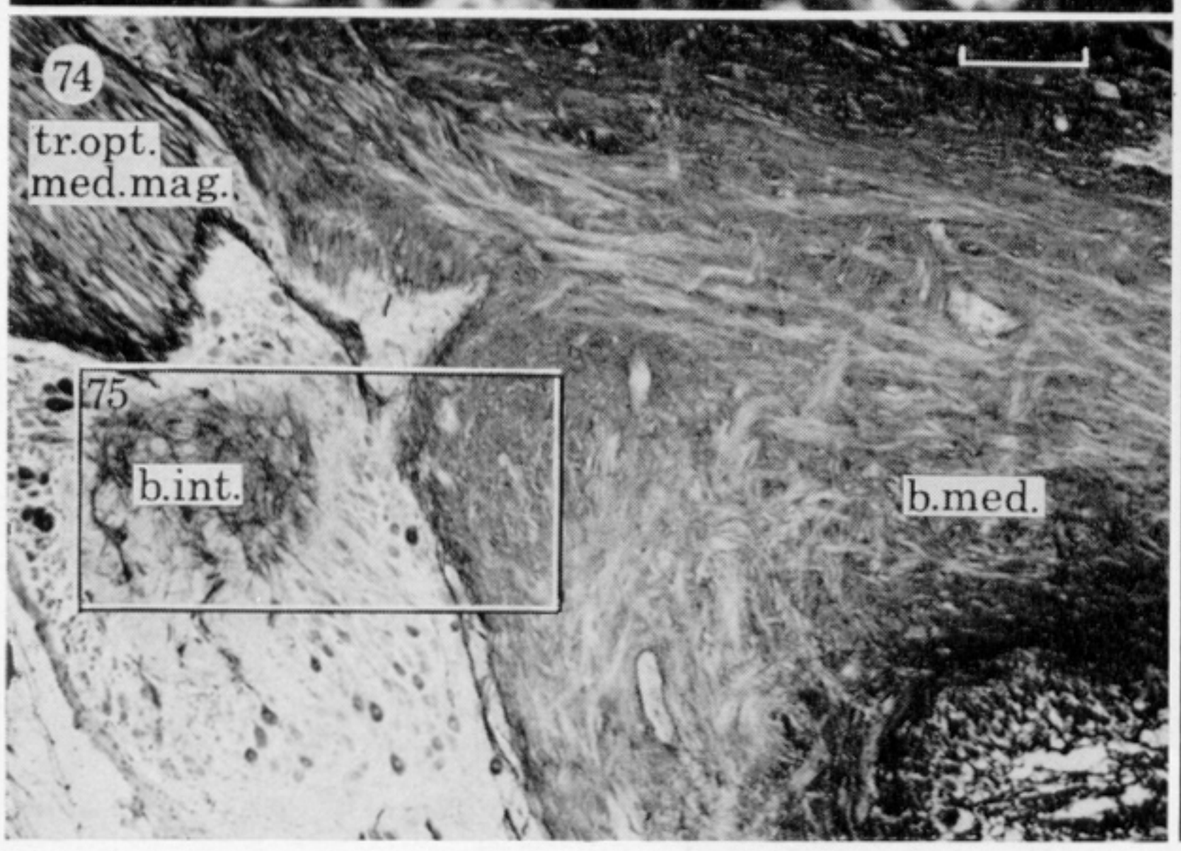




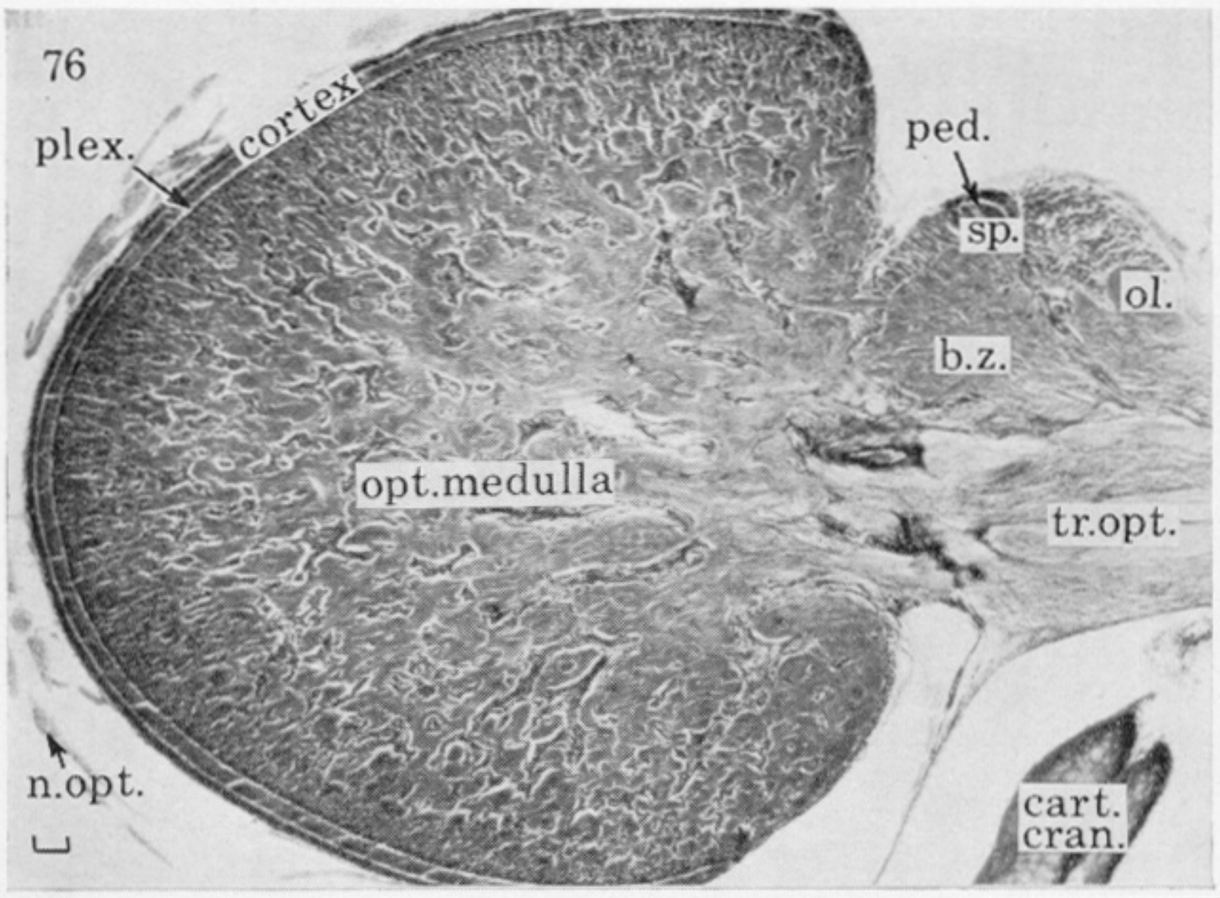


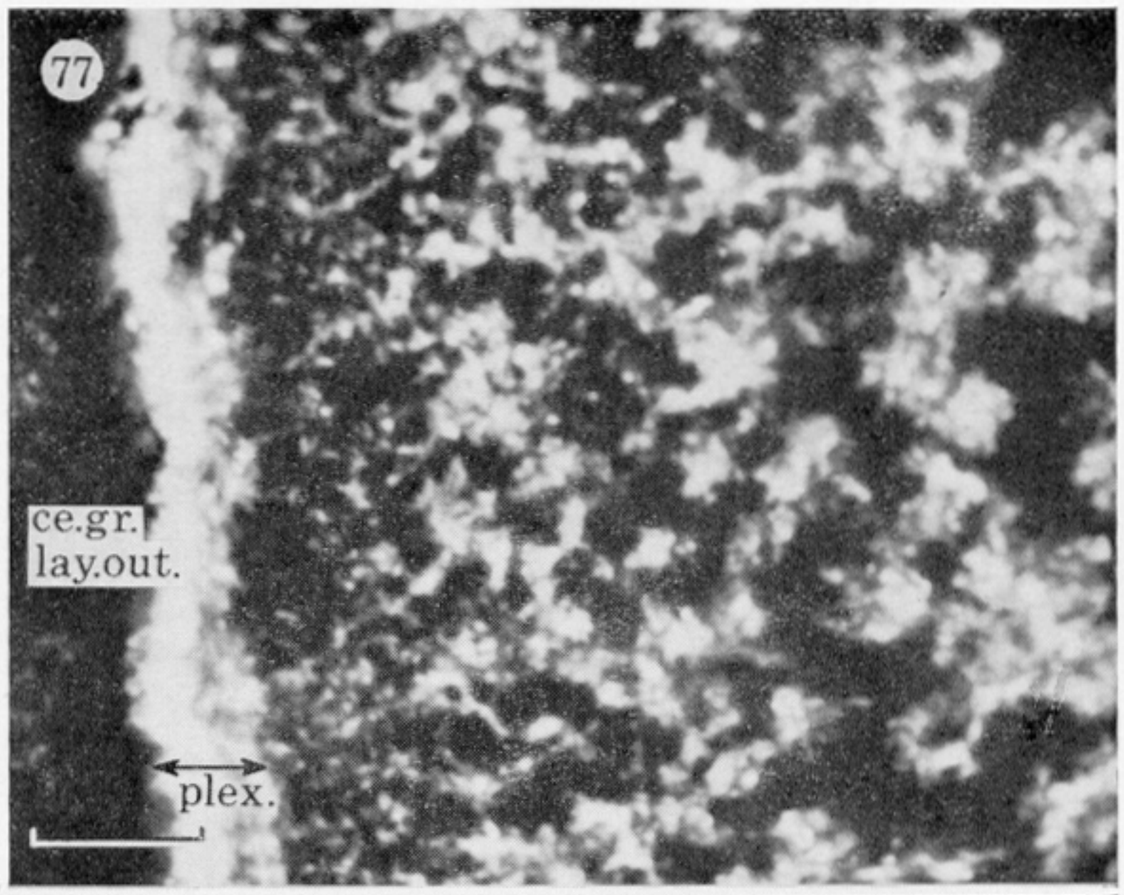


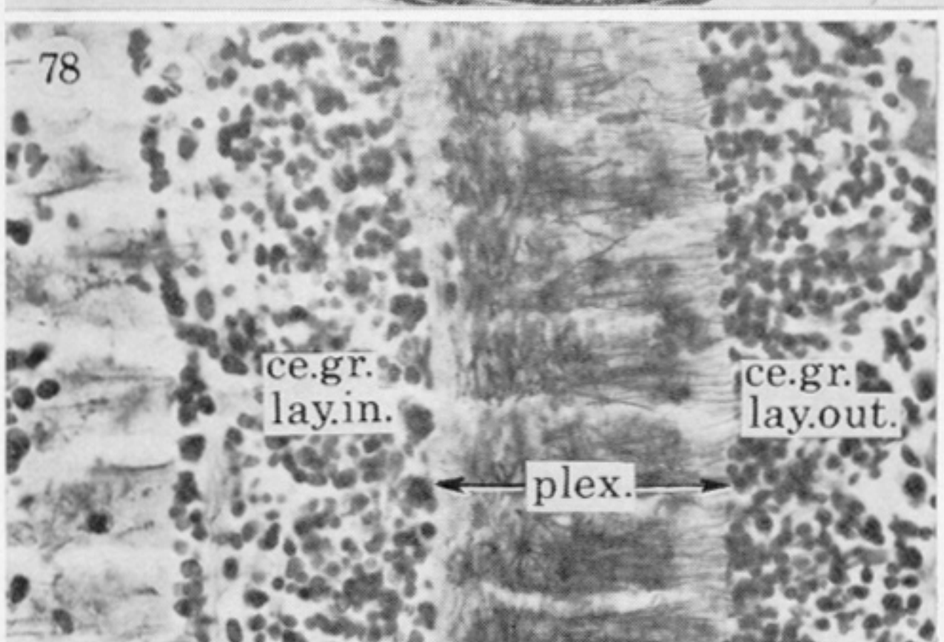


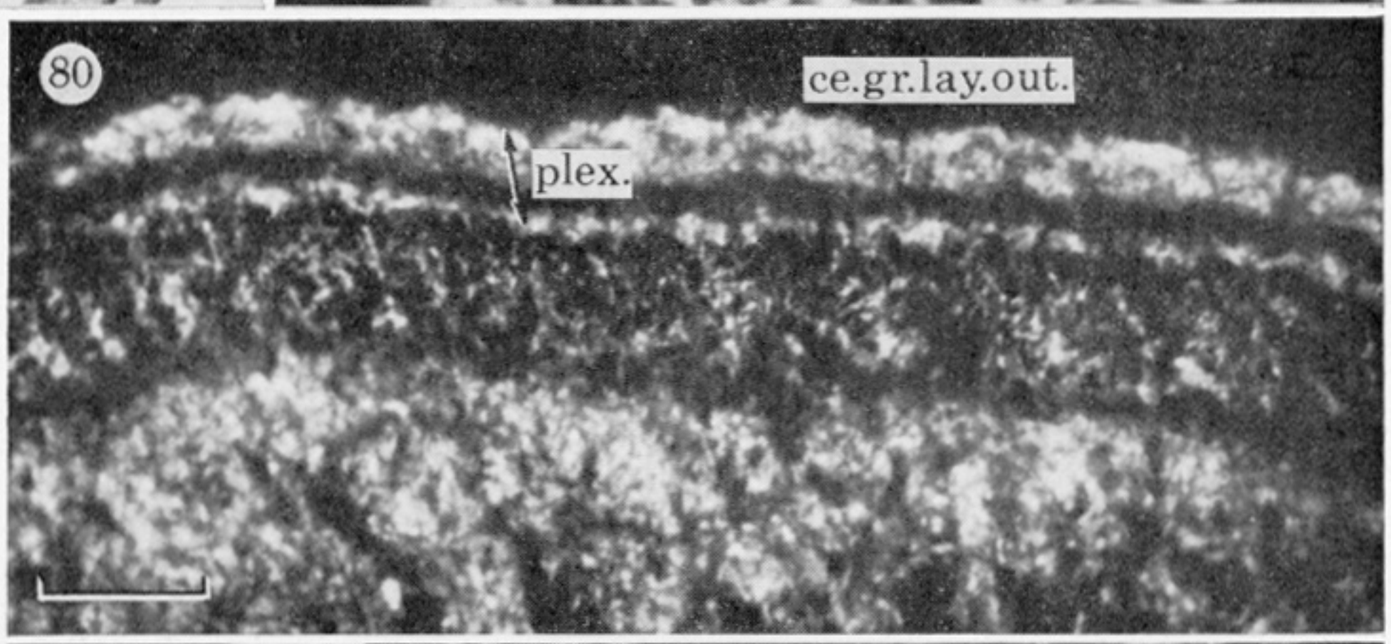


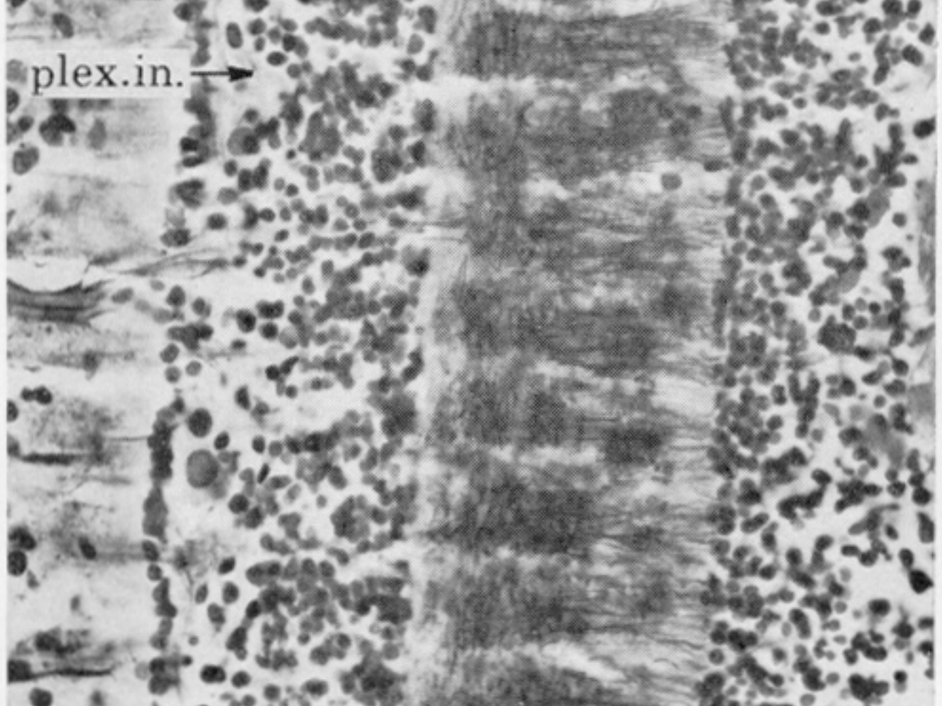
FIGURES 69–75. For description see opposite.

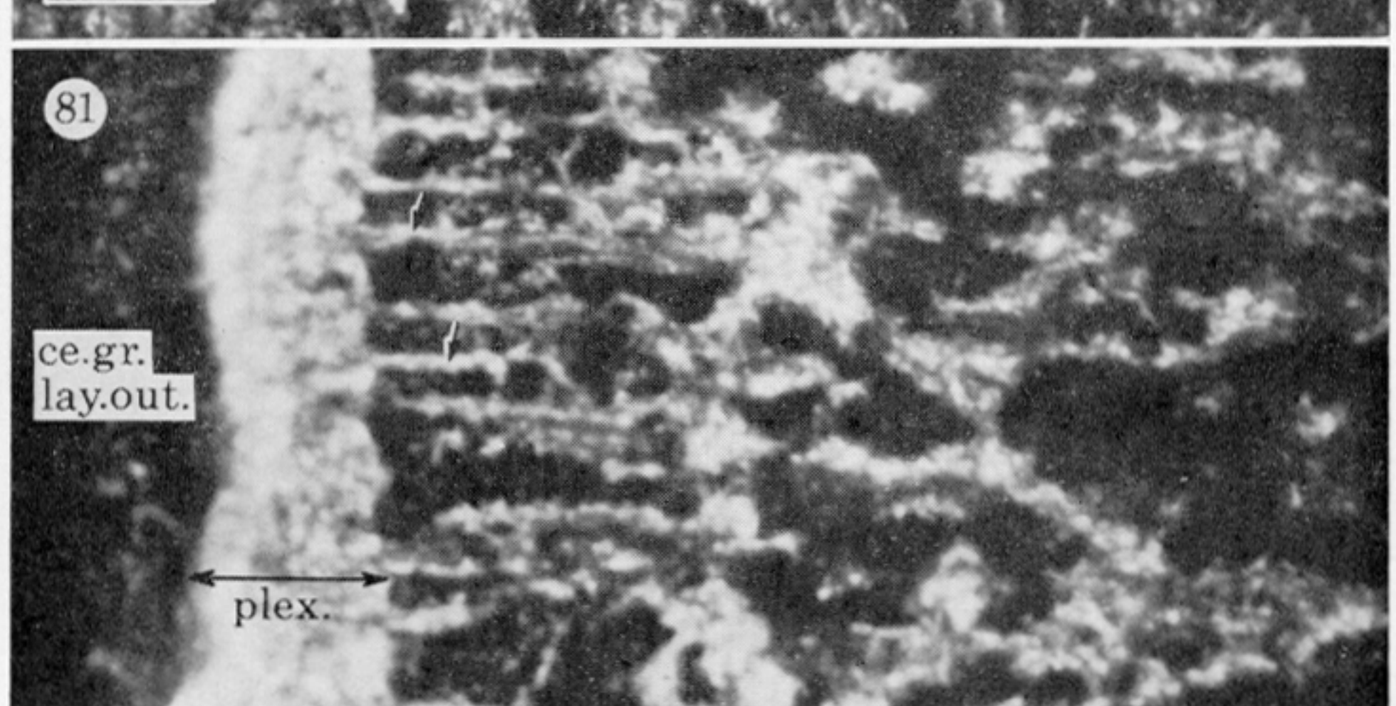




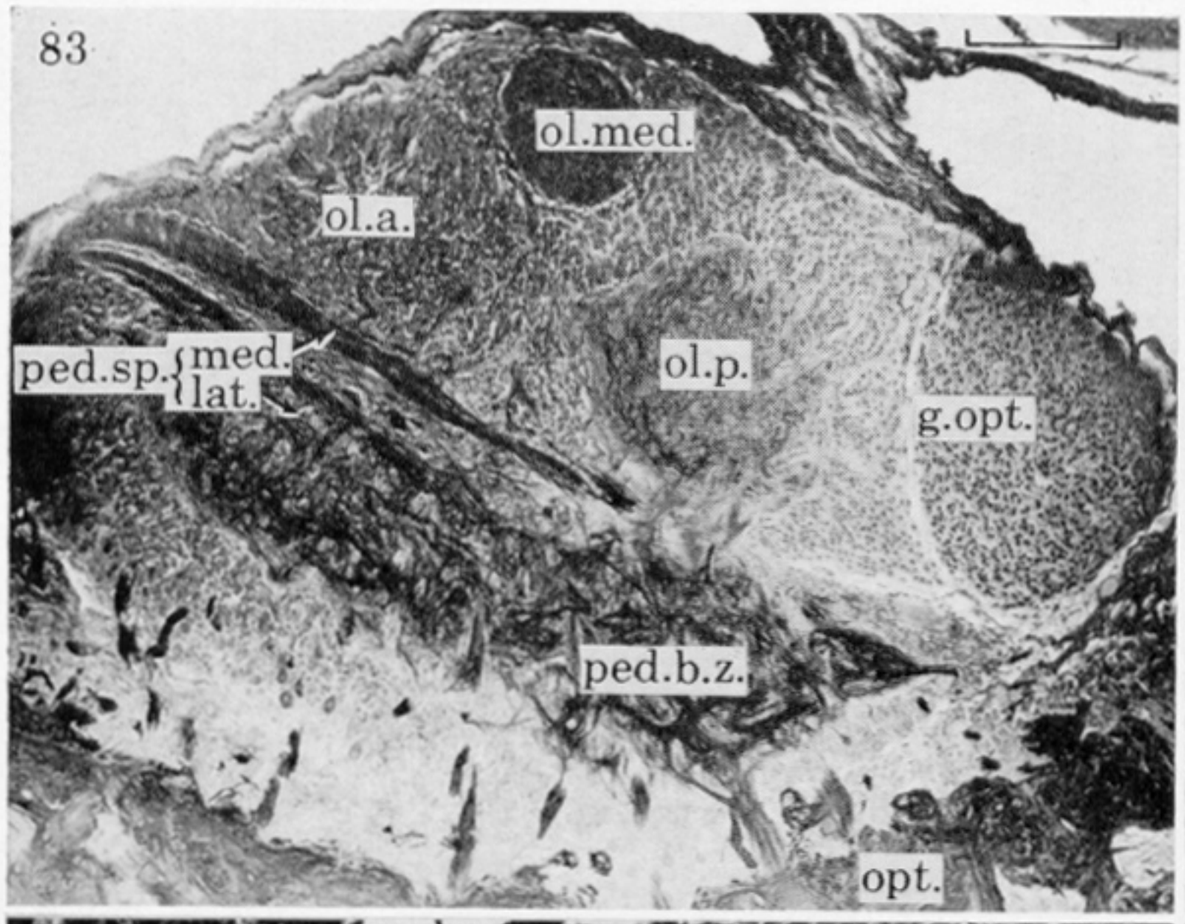


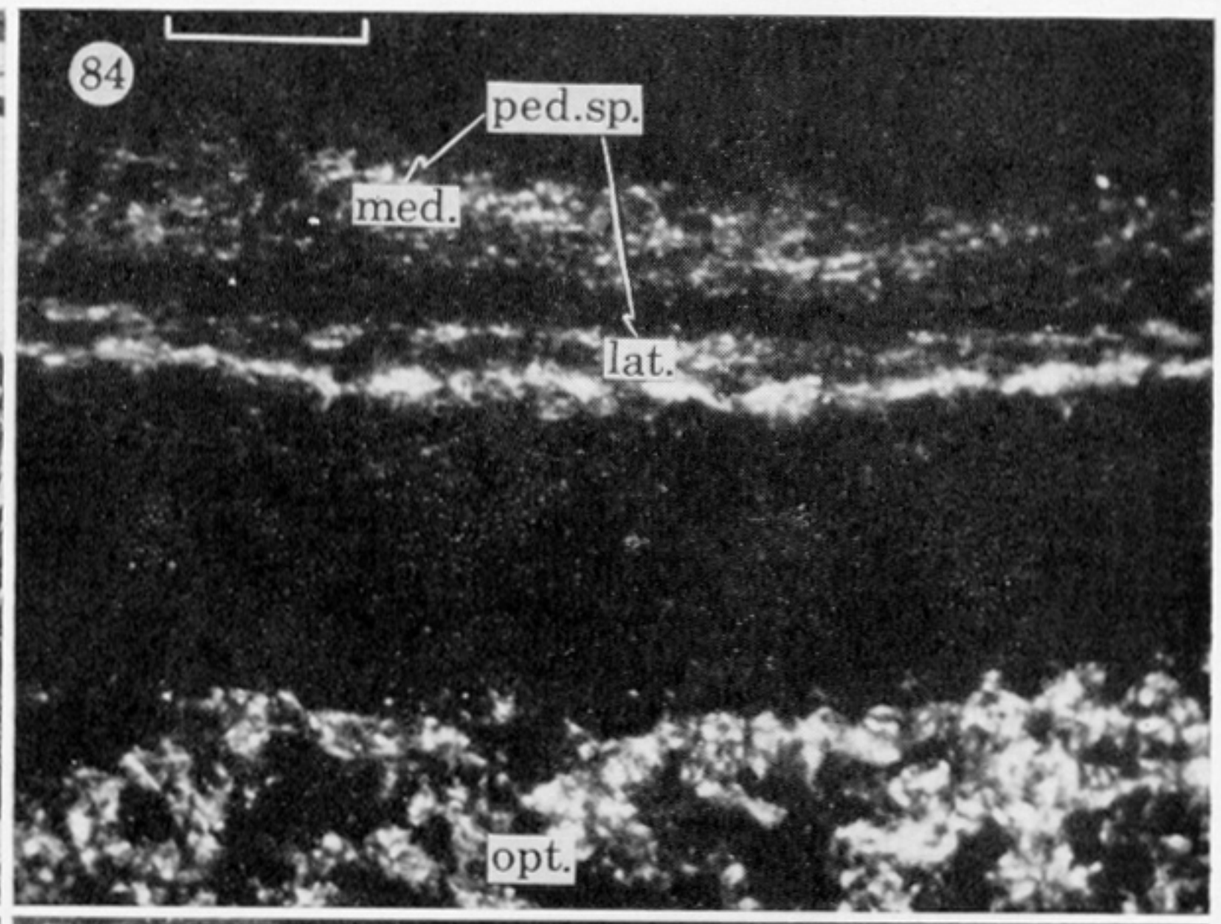


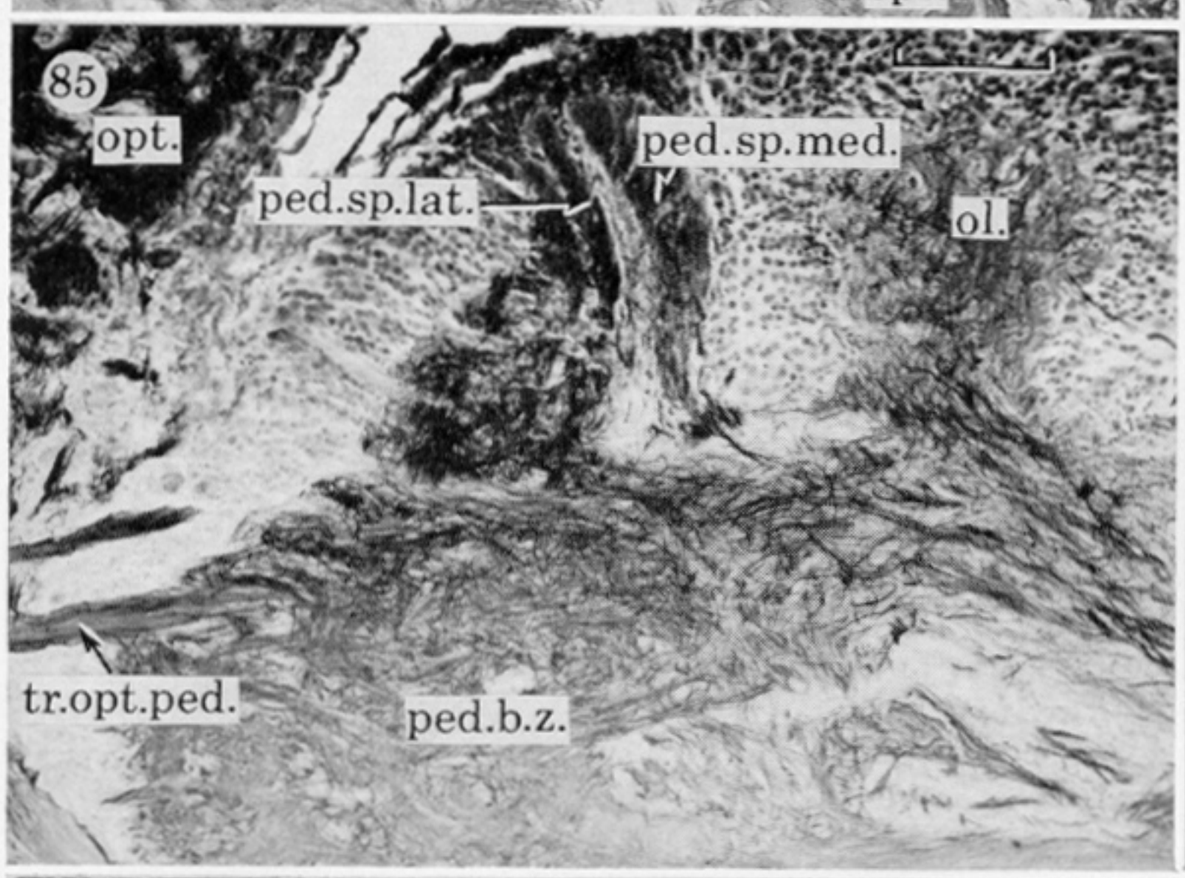


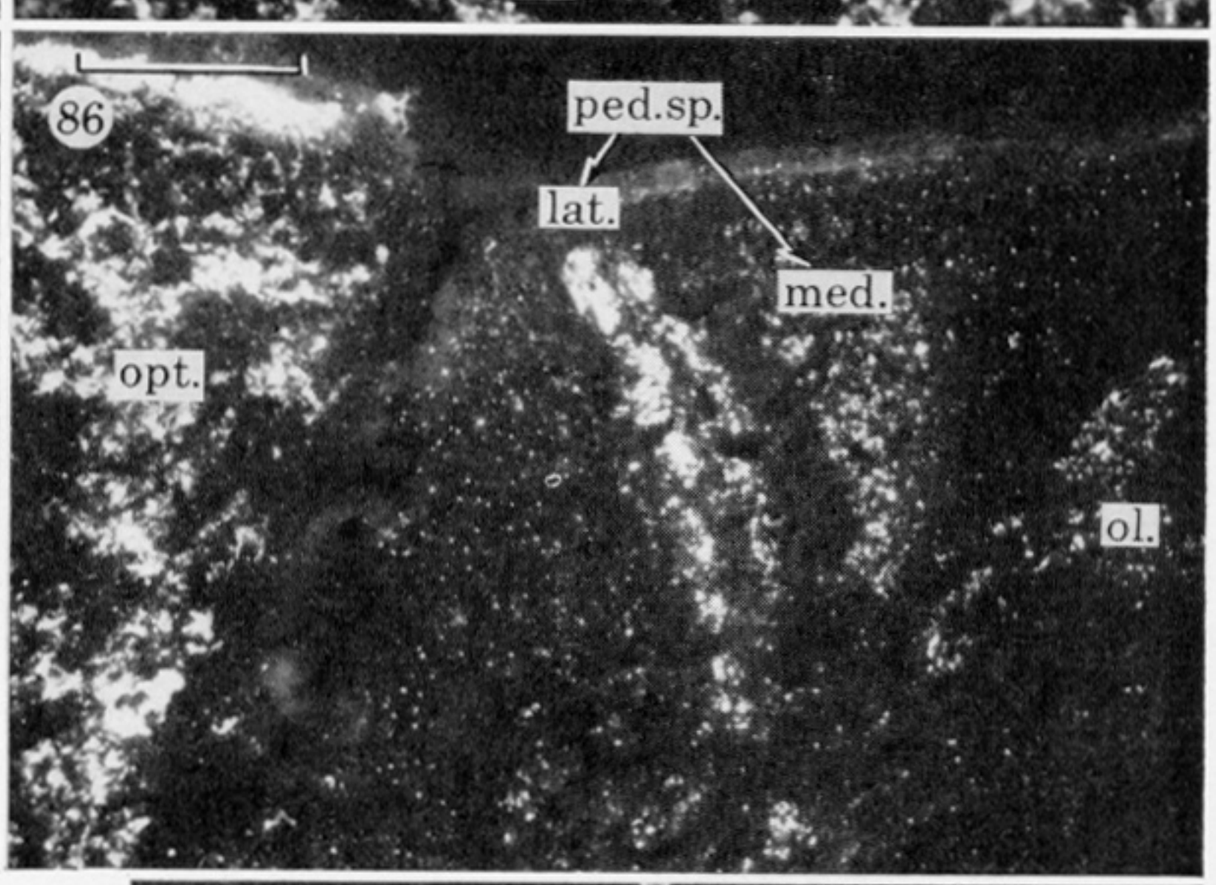


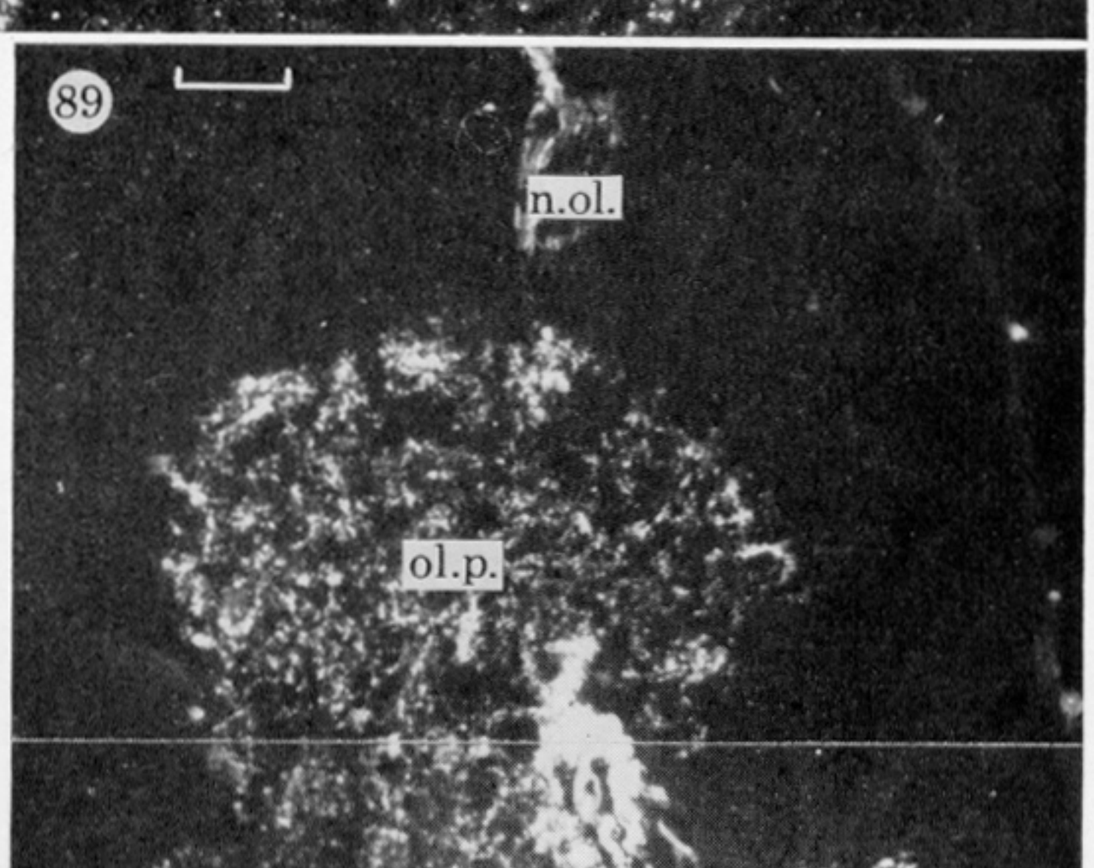
FIGURES 76–82. For description see opposite.

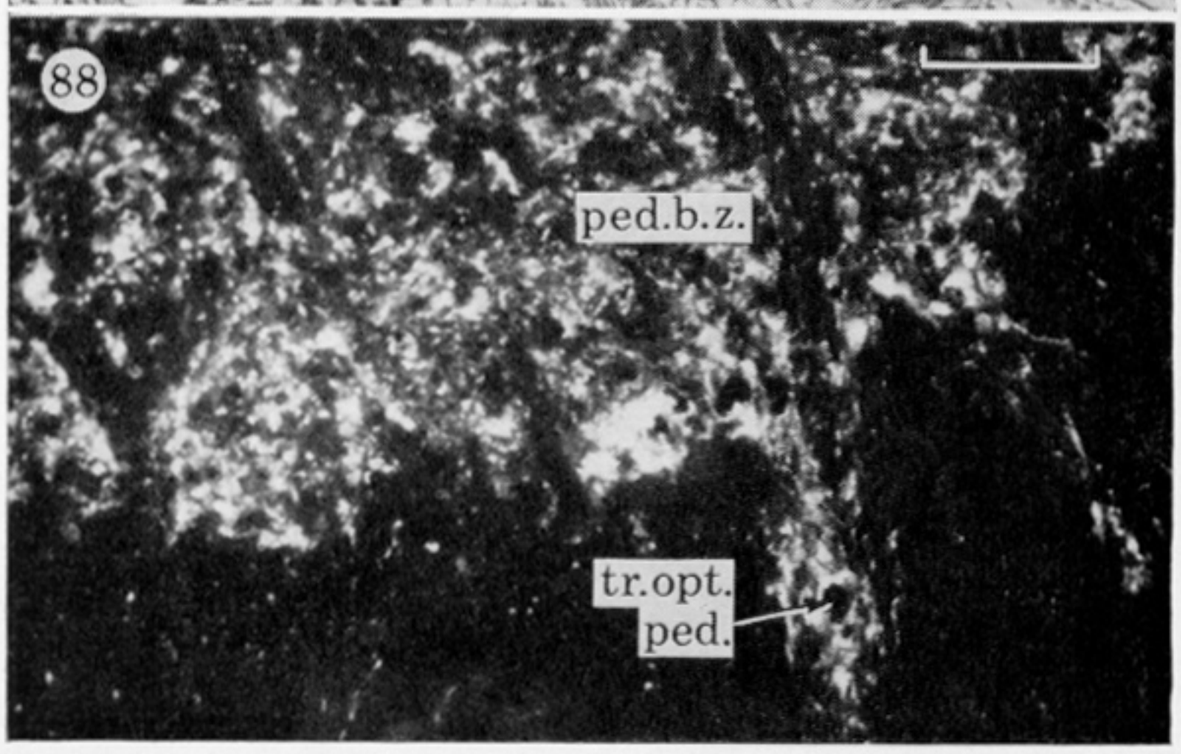




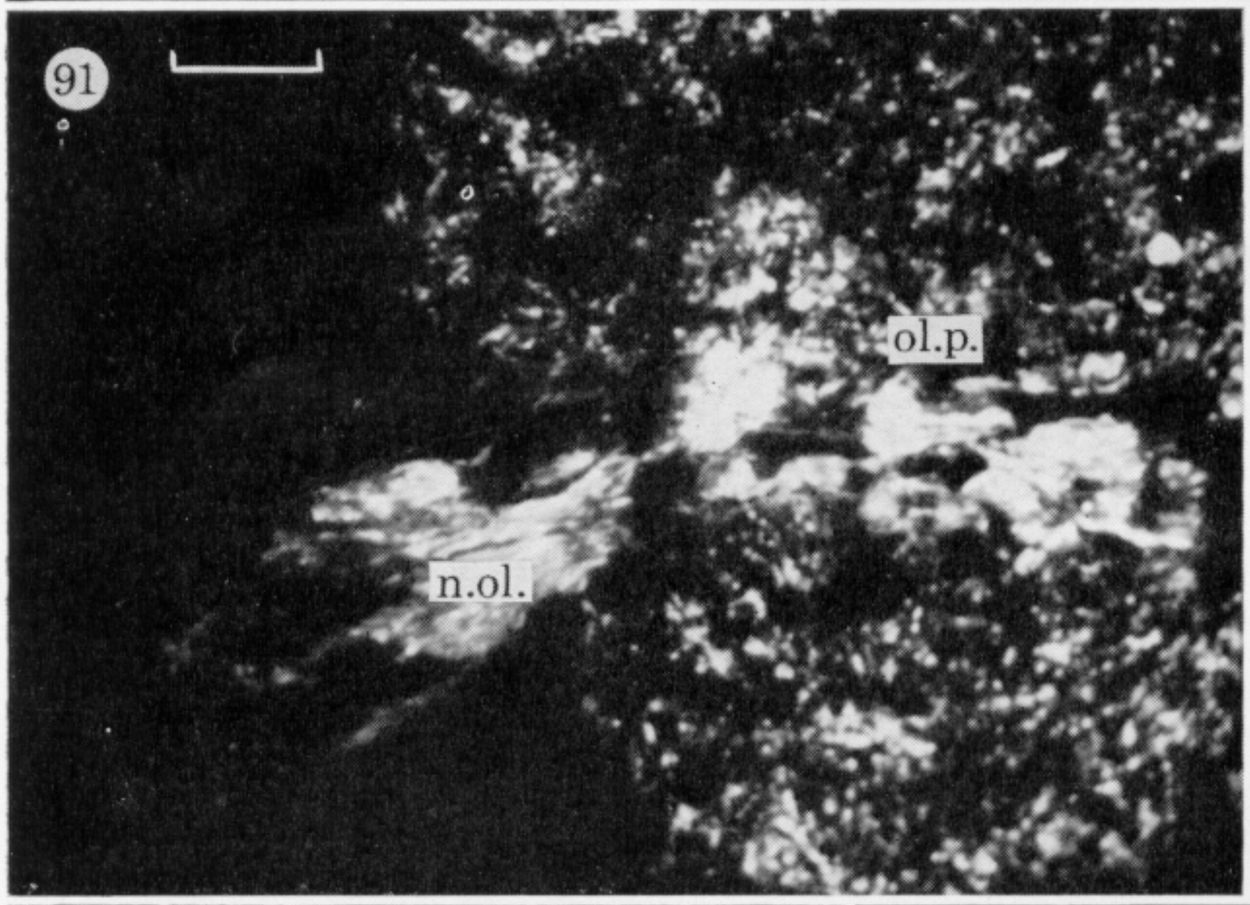


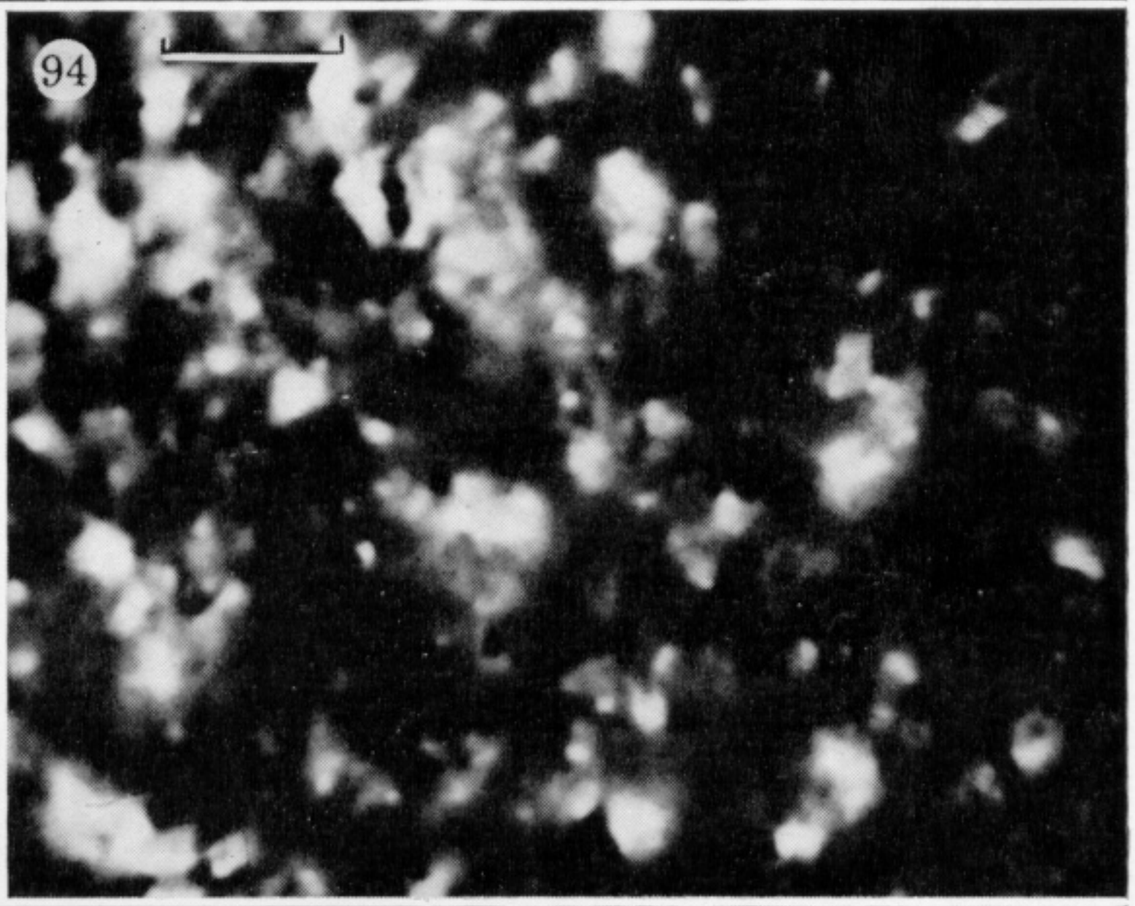


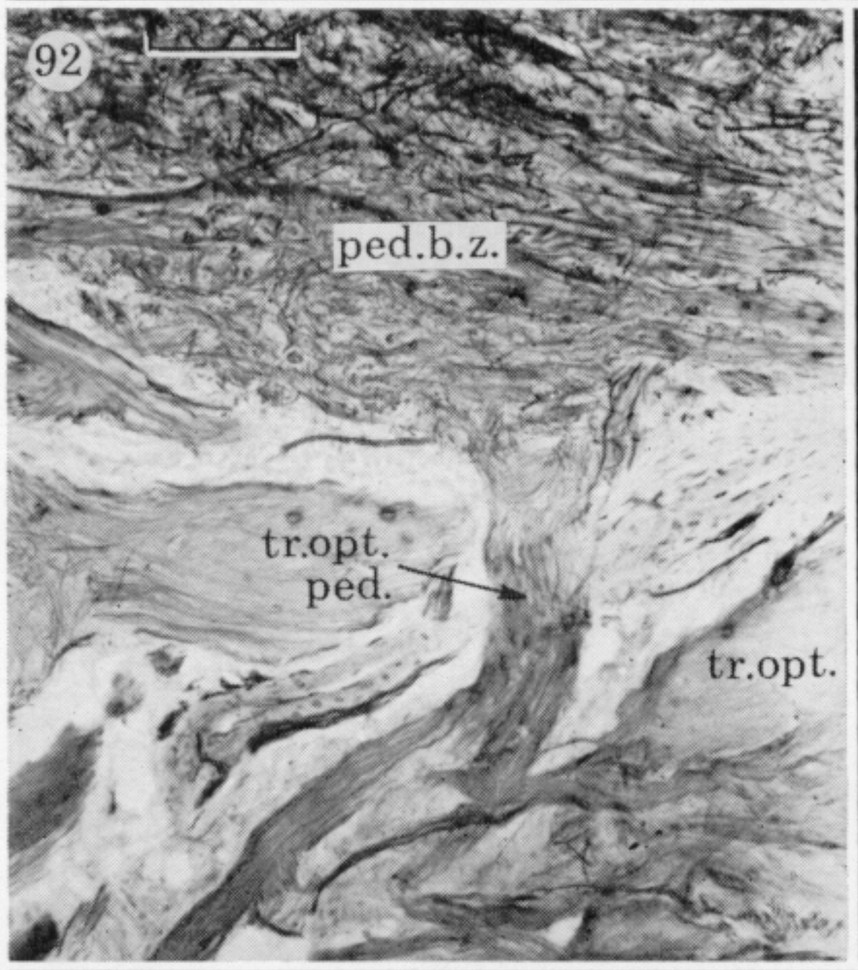


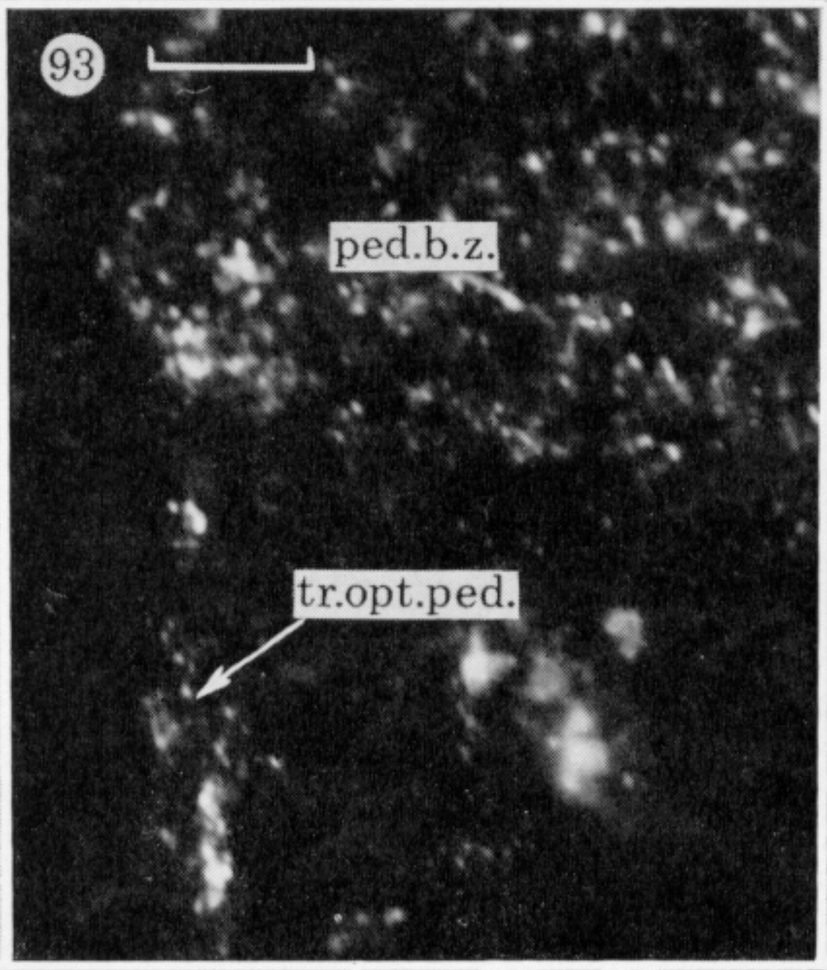


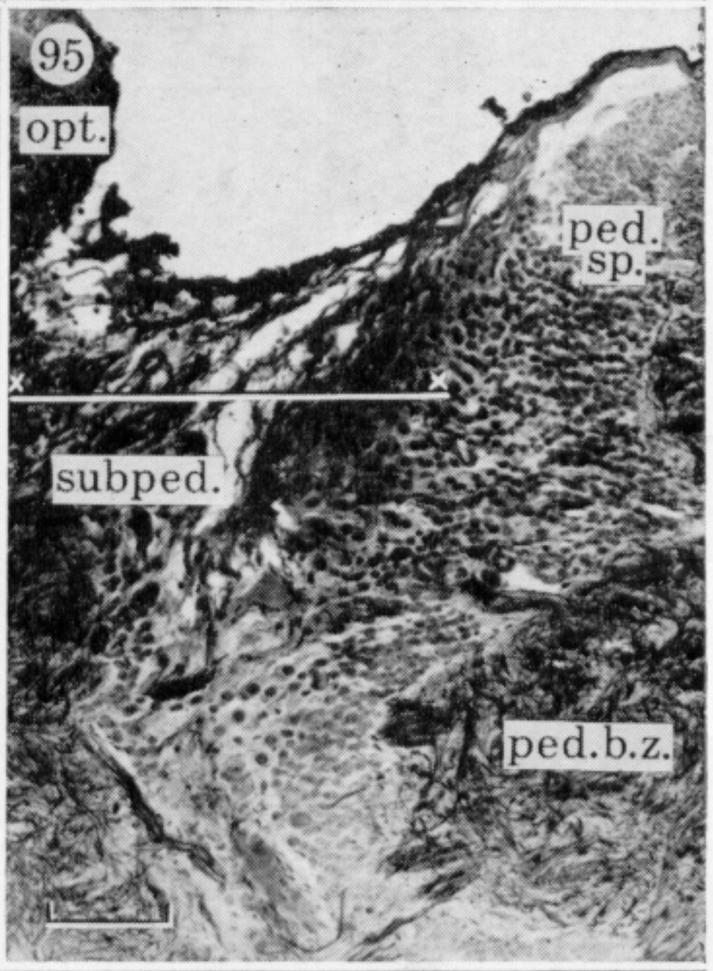
FIGURES 83–89. For description see page 138.

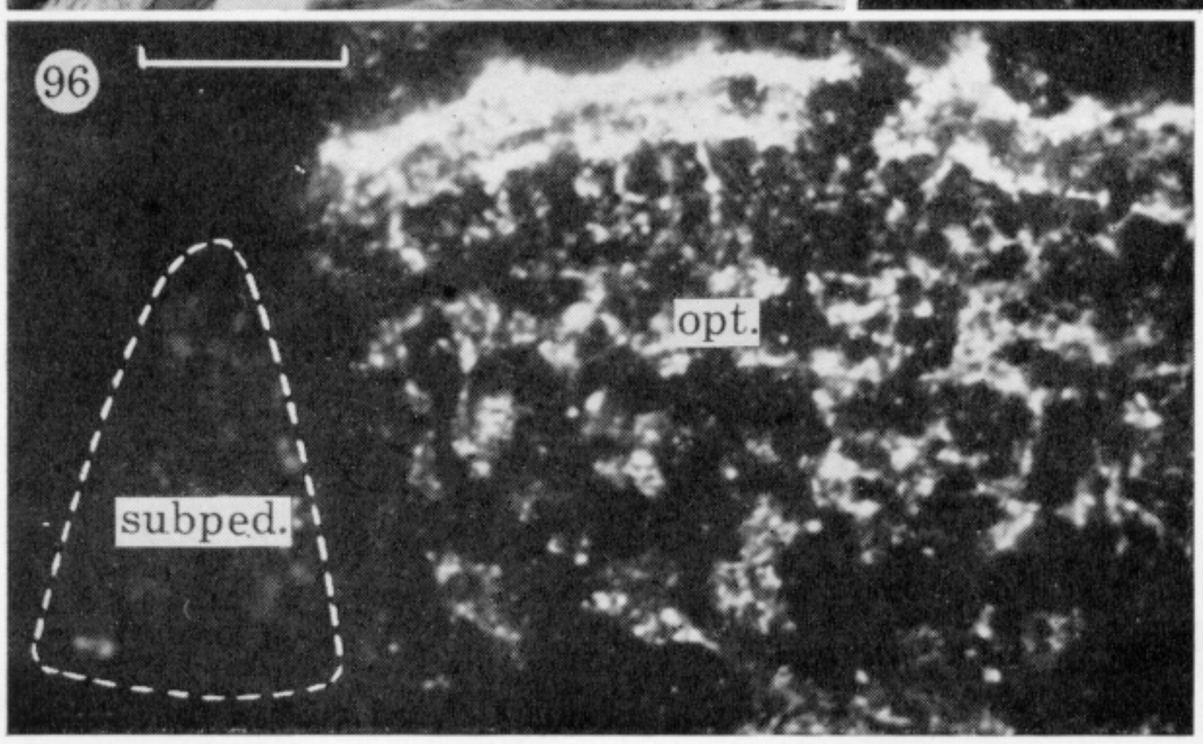


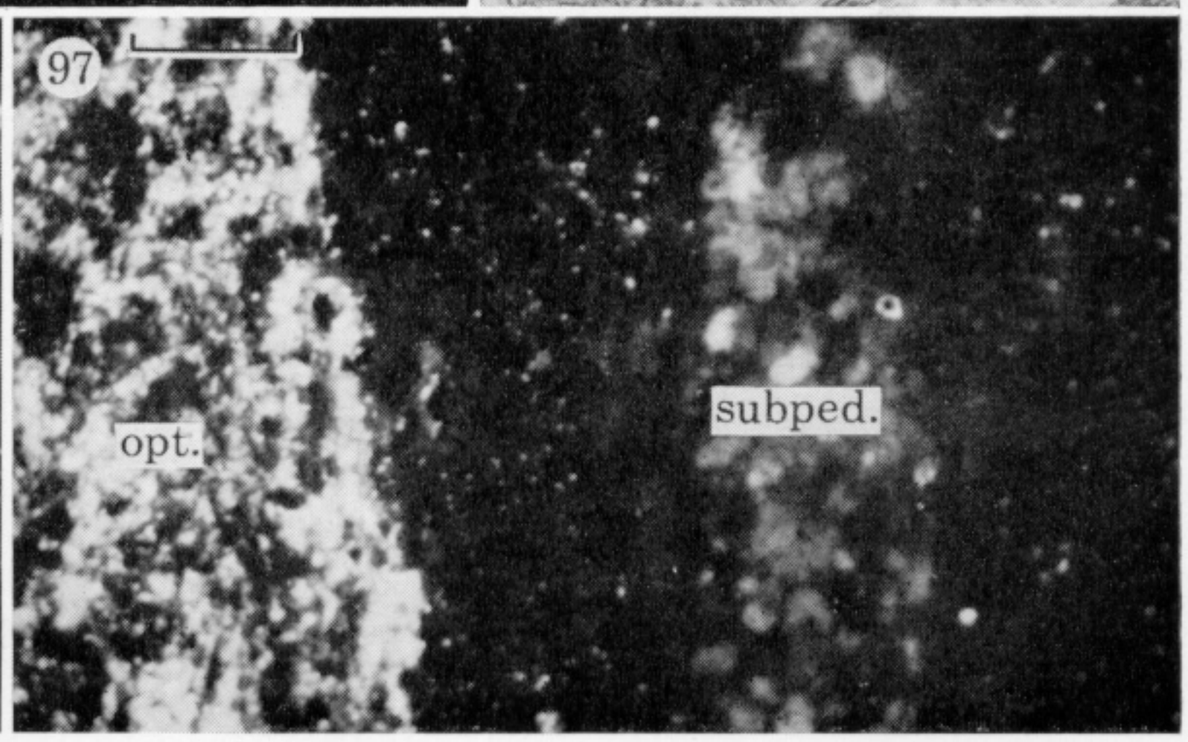




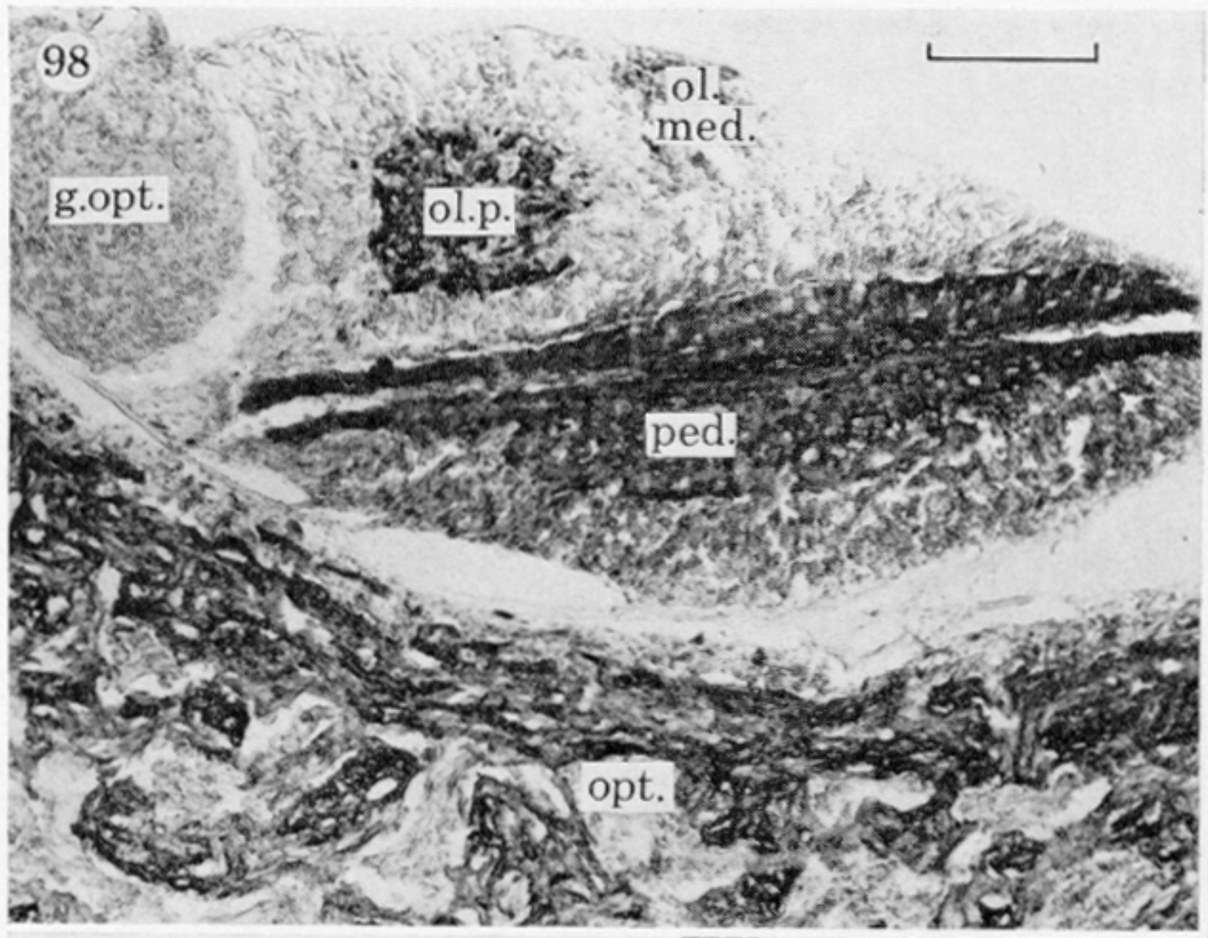


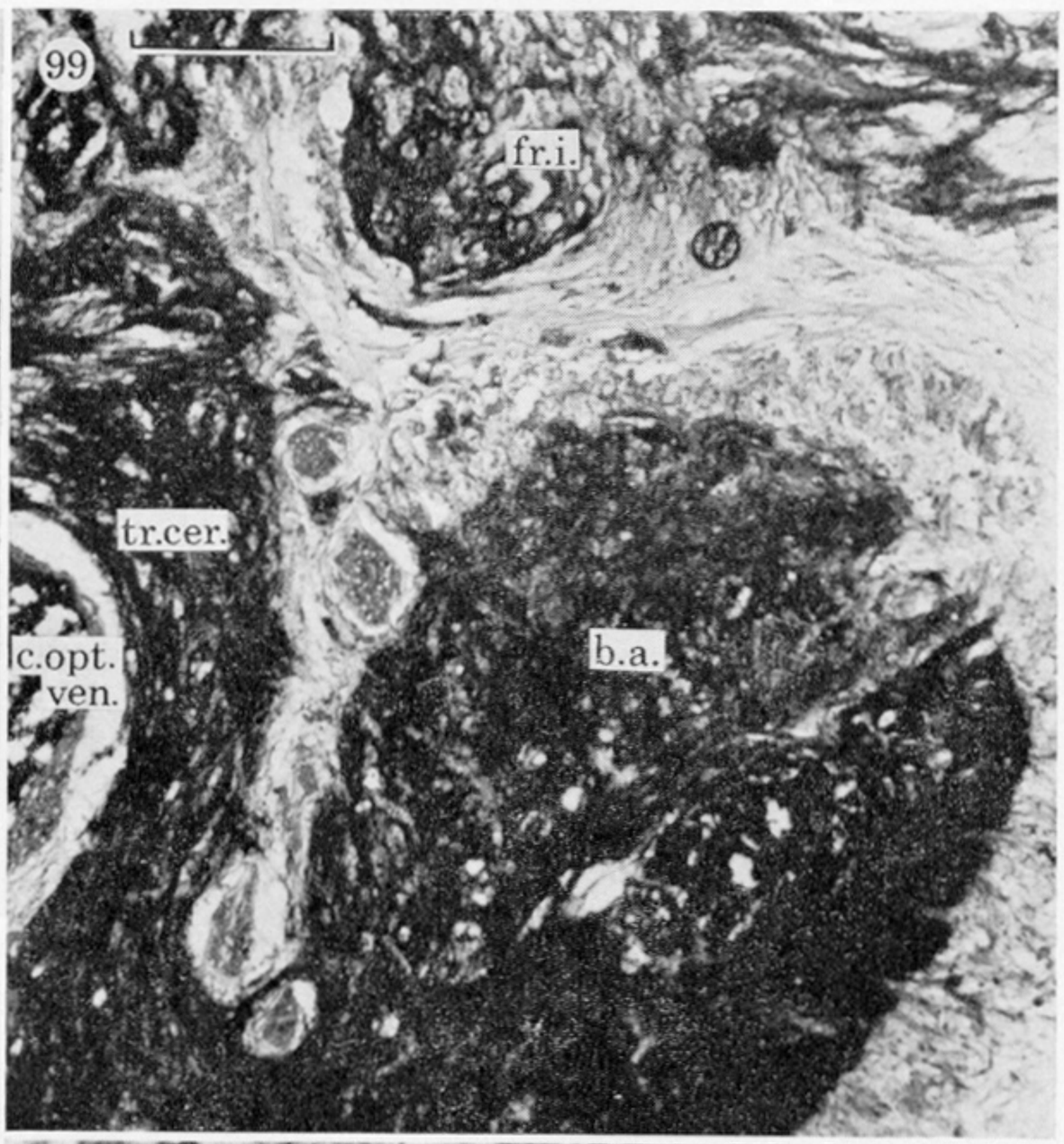


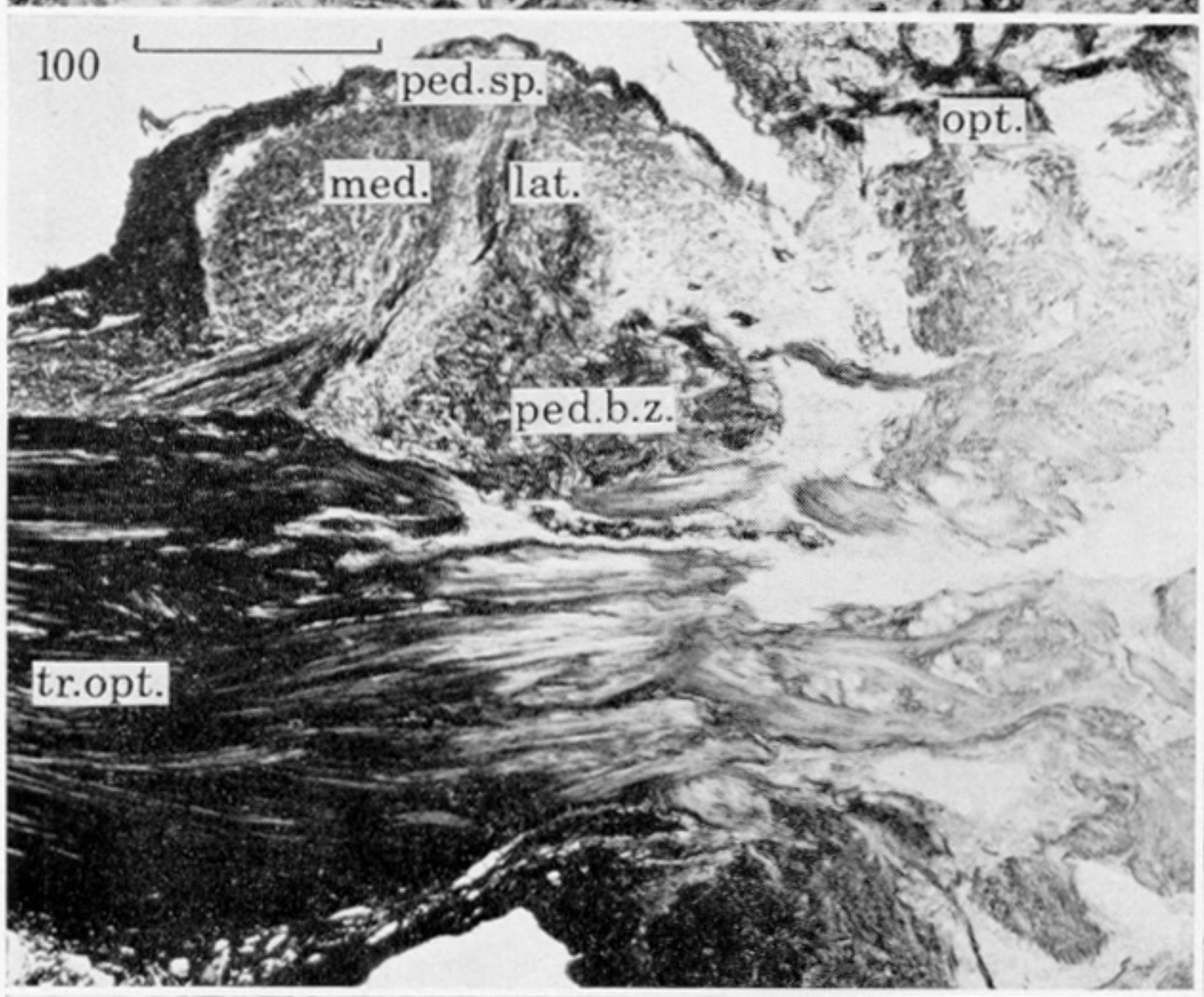


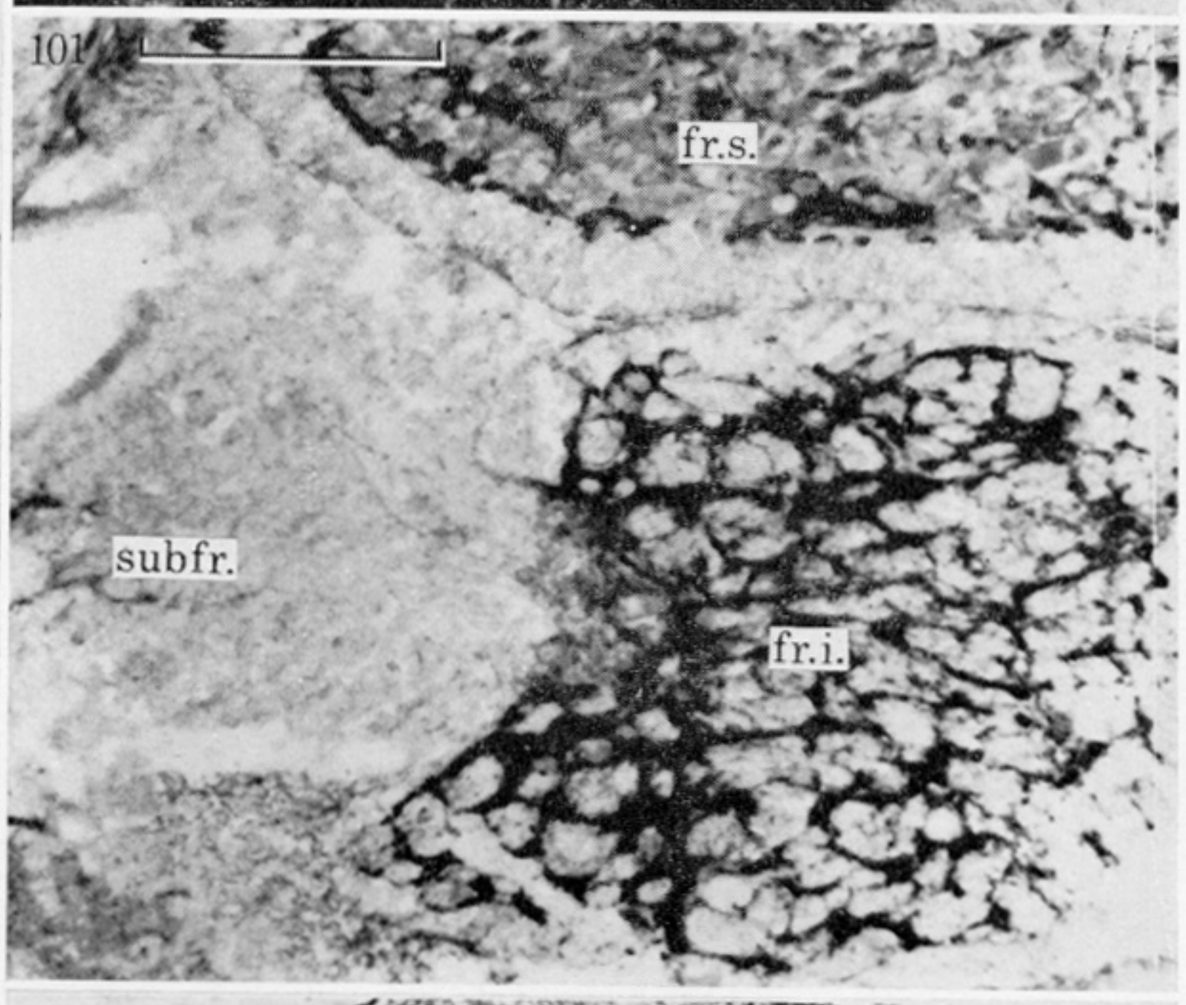


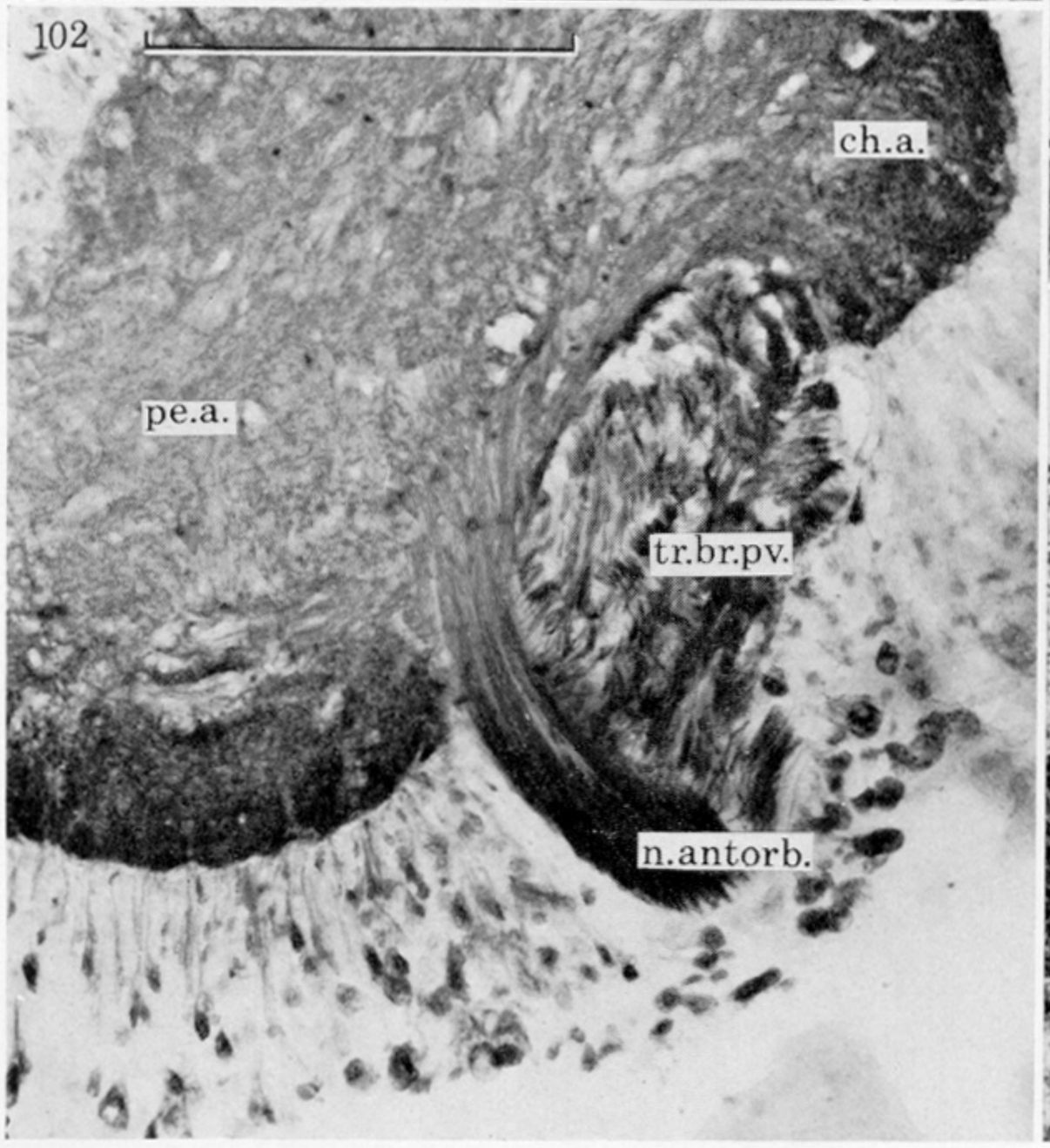
FIGURES 90–97. For description see page 139.

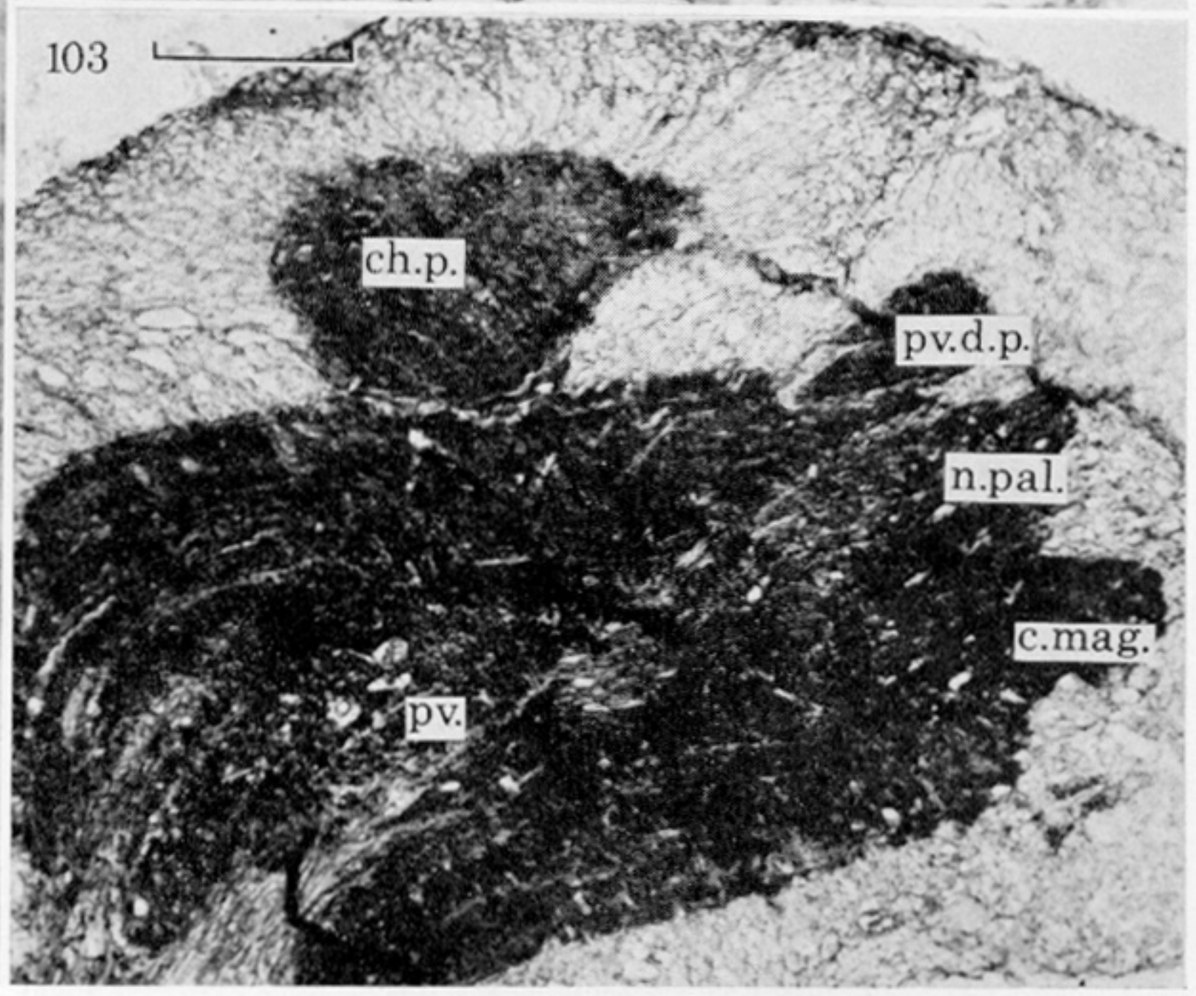












FIGURES 98–103. For description see opposite.