THE CORPORA PEDUNCULATA OF *SPHINX LIGUSTRI* L. AND OTHER LEPIDOPTERA: AN ANATOMICAL STUDY

By LUCY PEARSON
School of Biological Sciences, University of Sussex, Falmer, Brighton, Sussex

(*Communicated by J. Z. Young, F.R.S.—Received* 16 *June* 1970)

[Plates 31 to 35]

CONTENTS

The corpora pedunculata, or mushroom bodies, are paired lobes of neuropile present in the protocerebrum or dorsal brain of all insects. They are divisible into three parts: calyx, stalk and roots. The latter usually comprise two simple lobes, the α and β lobes.

The corpora pedunculata of a variety of Lepidoptera were examined. All had a double calyx-cup. Each 'cup-cavity' is composed of 'globuli' cell bodies. The broad stalk, a tract of fibres and neuropile, leads from the calyx to the complex 'roots'—α, β and γ lobes. A third group of globuli cells near the calyx gives rise to a tract leading to a second lobe-system—the tripartite Y-lobe—in the roots. As neither the Y tract nor the Y lobe has been described before in any insect, their possible homologues are unknown. The two lobe systems in the roots are closely intertwined, yet have no interaction except in the γ lobe.

A number of different neuron types with branches in the mushroom bodies has been described from Golgi preparations. Some (intrinsic cells) divide in the calyx and again in the roots, but do not pass out of the mushroom bodies. Others (extrinsic cells) branch in the mushroom bodies and in other areas of the brain, thus connecting two regions.

Intrinsic cells arise from cell bodies in the calyx-cups or posterior to them. There are two types: one has extensive spine-covered branches in the calyx, while the second has claw-like terminals covering a narrow cylindrical field. Processes from these cells run to the α, β and γ lobes via the stalk. A wide-field accessory cell, which arises from the third group of globuli cell bodies, also has claw-like endings in the calyx. A process of this cell runs in the Y-tract to the Y-lobe. Extrinsic terminals in the calyx arise

Vol. 259. B. 833 (Price £1.10*s* (£1.50); U.S. $3.90) 36 [Published 11 February 1971

from cells branching in the antennal lobe, in an accessory optic area in the protocerebrum, in the 'undifferentiated' protocerebral neuropile, or in the suboesophageal lobes. The antennal terminals in the calyx are knob-like. It is proposed that they form the centre of the 'glomeruli' typically present in calycal neuropile. The claws of the bunched intrinsic and accessory cells probably fit around these knobs.

Within the stalk, different subvarieties of intrinsic cells have been distinguished on the basis of the distribution of the side-branches and spines which they bear. The stalk is thought to be the site of extensive postsynaptic interaction between intrinsic cells. Fibres in the stalk run in bundles or groups. All the fibres in one bundle are of the same subvariety.

In the roots, the subvarieties of intrinsic cells have different branching patterns. The α and β lobes are not homogeneous, but are divided into sublobes. Extrinsic fibres ramify only within one sublobe generally, though some have very large fields. The connexions of the roots are obscure. Some extrinsic fibres branch again in the 'undifferentiated' protocerebral neuropile; others, from the β lobe, may run to the suboesophageal lobes.

There are profound differences between the internal organization of the mushroom bodies in Hymenoptera (Kenyon 1896; Goll 1967) and Lepidoptera. The functional implications of the Lepidopteran form are discussed.

Introduction

With regard to the structure of the insect brain, Kenyon (1896) wrote: 'no-one seems to have worked at the subject. Such being the case, my endeavour...to bring this subject into line with what is now known relative to the structure of the central nervous system of several other *invertebrates* and more especially of the *vertebrates* will doubtless be appreciated.' Over seventy years later, these words are not inappropriate as an introduction to the present paper: an account of the anatomy of the corpora pedunculata of some Lepidoptera, based primarily on material stained by Golgi methods.

Insect brain anatomists have paid more attention to the optic lobes than to other areas (Zawarzin 1913; Cajal & Sanchez 1915; Strausfeld & Blest 1970; Strausfeld 1970; Trujillo-Cenoz & Melamed 1963; Melamed & Trujillo-Cenoz 1967). As for the rest of the brain, there have been a number of general anatomical surveys—those dealing with Lepidoptera include Bretschneider (1921, 1924), Hanstrom (1928) and Ehnbom (1948)—but few Golgi studies. Kenyon's (1896) 'Brain of the bee' is still the most detailed and comprehensive account of the brain of a single species, although selectively stained material was also described in Sanchez's (1933) account of the corpora pedunculata of the cockroach, Pflugfelder's (1936) survey of the brains of Hemiptera, and Goll's (1967) paper on the brain of *Formica*. Because of the paucity of previous data, therefore, the present description is presented in some detail.

The corpora pedunculata, or mushroom bodies, are paired lobes of striking appearance, present in the protocerebra of all insects (figure 1). The name 'mushroom body' derives from the shape of these areas of neuropile, which are divisible into three regions. The names of these regions—calyx, stalk and 'roots'—continue the fungoid analogy. The use of the term 'roots' is confusing to most neuroanatomists, for in this case it does not have the usual anatomical connotations. The 'roots' of the mushroom bodies are in fact areas of neuropile like the calyx or the central body. Vowles (1955) attempted to replace this inappropriate nomenclature by the terms α lobe and β lobe for the two subdivisions of the 'roots' in Hymenoptera. Unfortunately, the 'roots' in Lepidoptera include four other lobes in addition to the α and β lobes. Thus, the term 'roots' has been retained here, partly because its use, if misleading, is at least traditional. It is felt that the introduction of another term for the whole complex would be confusing, and might prove equally inappropriate in the light of further investigations.

The calyx of each mushroom body lies dorsally in the protocerebrum, in the cell-body layer. The fine neuropile of the calyx takes the form of a cup in many insects. The stalk or pedunculus is a broad tract of fibres running downwards from the calyx. In the ventral protocerebrum it branches to form the 'roots'. In most insects there are two of these, running at right angles to each other and to the stalk. The α lobe (posterior root, dorsal stalk, *tubercule extérieur*, or *ruckläufiger Steil*) projects anteriorly; the β lobe (median root, inner root, *tubercule interne*, or *Balken*) extends to abut against its partner in the midline, in front of the central body. Both lobes take the form of columns of neuropile, composed mainly of hundreds of fibres lying parallel to each other.

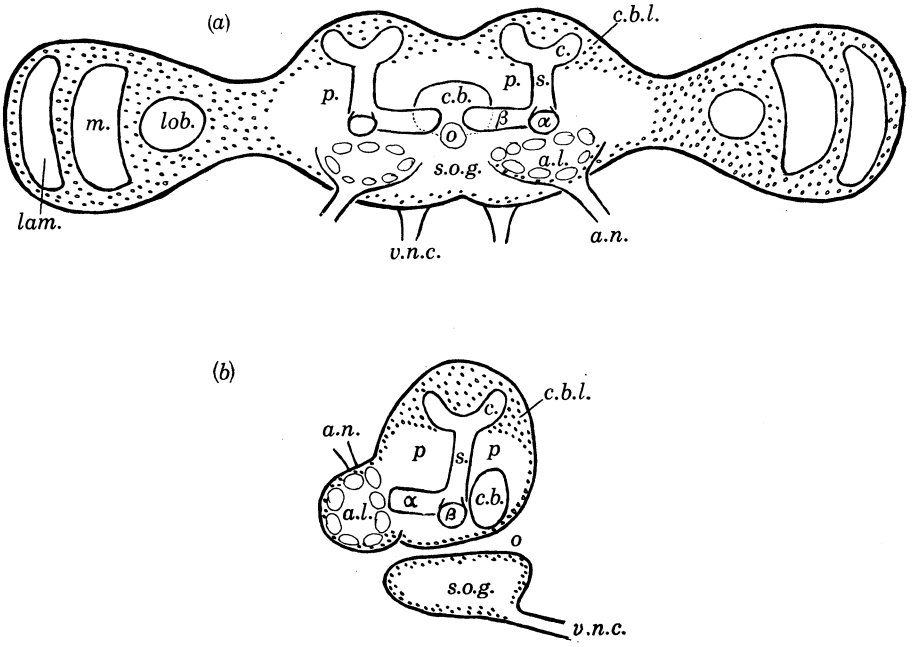


FIGURE 1. Schematic diagrams to show the mushroom bodies and other brain regions in a 'generalized' insect. (*a*) Frontal section of brain. (*b*) Sagittal section of brain. *Key*:

a.l.	antennal lobe	*lam.*	lamina
a.n.	antennal nerve	*lob.*	lobula (optic lobes)
c.	calyx	*m.*	medulla
s.	stalk (corpora pedunculata)	*o.*	oesophagus
α	α lobe	*p.*	protocerebrum
β	β lobe	*s.o.g.*	suboesophageal ganglion
c.b.	central body	*v.n.c.*	ventral nerve cord
c.b.l.	cell body layer		

The calyx and the 'roots' have numerous connexions with other areas of the brain by means of fibre tracts. The large antenno-glomerular tract from the sensory antennal neuropile to the calyx is obvious in all insect brains studied. Other tracts are less clear, and have been the subject of considerable controversy. For instance, in *Formica*, Vowles (1955), using Holmes-stained sections, claimed the following connexions: to the calyx from the medulla, the lobula, the optic tubercle, the antennal lobe (six tracts), and the suboesophageal ganglion (three tracts); from the α lobe to the contralateral α lobe, the medulla, the lobula, the optic tubercle, the antennal lobe, and the suboesophageal ganglion; from the β lobe to the central complex, the antenno-motor centre, and the suboesophageal ganglion. Goll (1967), in Golgi studies

on the same species, found the following: to the calyx from the medulla, the lobula, the protocerebral lobes, and the antennal lobe (two tracts); from the stalk to the protocerebral lobes; from the α lobe to the protocerebral lobes and the antennal lobe; from the β lobe to the central complex, and the protocerebral lobes on both sides of the brain.

The mushroom bodies have been subjected to a certain amount of physiological investigation. Vowles (1964) established that impulses in the stalk pass from the calyx to the roots, and that the calyx receives visual input. Maynard (1962, 1967) stimulated the antennal nerve electrically and recorded activity in the calyx and stalk. He found that, after a strong input volley or brief trains of repetitive input, a single large 'spike' was recorded some 70 ms or more later in the stalk. The 'spike' was thought to be due to the activity of a number of stalk fibres discharging in synchrony. This synchrony was thought to be achieved by the postsynaptic interaction of the stalk fibres. Huber (1962, 1965, 1967) stimulated the corpora pedunculata of Orthoptera, and elicited complex, integrated patterns of behaviour—or the inhibition of such patterns. Rowell (1964) obtained similar results. Yet, apart from the fact that they appear to play an important part in the control and coordination of complex acts, the function of the mushroom bodies has remained somewhat of an enigma.

Lack of information has not hindered speculation, however. Dujardin (1850) thought that the mushroom bodies were the *corps d'intelligence* of insects, and Flogel (1878) pointed out that they are larger and better developed in insects whose behavioural repertoire includes highly complex acts. Vowles (1954, 1955, 1964) proposed that the sensory control of motor acts in general is mediated through the mushroom bodies. His sophisticated model included the passage of information *from* the mushroom bodies (α lobe) back to the sensory centres, so that feedback control of motor patterns was achieved. Huber (1965, 1967) concluded that the bodies are responsible for the selection and coordination of complex behaviour patterns. Finally, Horridge (1965), intrigued by the patterned array of the neurons in the mushroom bodies, suggested that they provide the means by which an insect, if it is able, forms a conceptual map of its surroundings and orientates itself relatively to that map.

Materials and methods

The brains of several hundred Lepidoptera were examined. Most preparations were of the privet hawk moth, *Sphinx ligustri* L. Other species used were: several noctuids, including *Triphaena pronuba* L., *T. interjecta* Hubner, *T. comes* Hubner, and *Catocala nupta* L.; *Antheraea pernyi* Guérin; *Automeris aurantiaca* Weymer and *Hylesia* sp., from preparations kindly lent by Dr A. D. Blest. Several dozen butterflies, *Pieris brassicae* L., were also used. Most of the descriptions given below apply to *Sphinx*. The other species are mentioned for their similarities to, and differences from, the hawk moth.

For gross anatomy, the Holmes (1947) reduced silver method, as modified by Blest (1961), was followed (6 ml of lutidine were used in the impregnating bath). For detailed anatomy of cell types, two variants of the Golgi method—Colonnier's (1964) version of the Kopsch method, and the Golgi rapid—were adopted. In the latter methods, most of the procedure was standard, but some variations were introduced.

(*a*) *Colonnier method.* The animals were killed by injection with 6% phosphate-buffered gluteraldehyde. Their brains were removed and immersed in buffered gluteraldehyde for up to one day. The rest of the procedure was standard. Within limits (6.8 to 7.2), the pH of the

prefixative had little effect; at lower pH values, the fibres appeared swollen, and the impregnation defective. Temperature variations seemed to have no consistent effect, though there were indications that temperatures of 25 °C and above improved the quality of impregnation.

(*b*) *Rapid method.* The animals were killed by injection with 2% potassium dichromate solution. Their brains were removed and immersed for 5 to 7 days in a solution containing three parts of 2% potassium dichromate and one part of 1% osmic acid. They were then immersed in 0.75% silver nitrate for 2 to 4 days.

(*c*) *Differences between the two methods.* The frequency of staining of any particular cell type varied, sometimes dramatically, according to which method was used. All cell types, however, maintained the same appearance irrespective of the method used to stain them (i.e. branching pattern, form of terminals, and so on, looked identical in both types of material).

In Colonnier-stained material, there was a tendency for impregnation to be incomplete. The fibres frequently appeared beaded. Sometimes this was thought to be due to a fixation artefact—namely, that the fibres shrink and the mitochondria swell in gluteraldehyde. At other times beading was thought to represent a regional synaptic specialization of the fibre. Beading was termed 'artefactual' if it was not always present on the fibre type concerned, or if it varied in appearance. This point will be further discussed in the relevant sections. Crystalline deposits of various types were fairly common.

In rapid-stained material, incomplete impregnation of stained cells was less common. Stained elements were more evenly distributed throughout the brain. The fibres tended to look swollen at times, though beaded fibres were less common. Crystalline deposits were rare, but the background tended to be darker than in the Colonnier preparations.

The measurements given in the text apply to Colonnier-stained material.

(*d*) *Factors affecting the success of the stain.* It has previously been noted (Cajal 1911; Boycott & Dowling 1969) that young (vertebrate) animals tend to give better results than old. This was found to be true of the moths used in the present study. It was consistently found that brains which had been torn, or extensively handled during dissection, showed deposits and scrappy, incomplete impregnation (see also Boycott & Dowling 1969). In some areas of the brain (e.g. the optic lobes), cells stained more frequently, more completely, and more clearly, than in other regions (e.g. the suboesophageal lobes).

(*e*) *Sectioning, and care of sections.* After staining, the brains were washed in distilled water for 6 h and dehydrated. They were then rapidly embedded in celloidin (B. D. H. Necol) which was hardened in chloroform. After a few hours, the blocks were sectioned at 80 to 100 μm on a sledge microtome. During sectioning, the block and knife were kept wet with chloroform. The sections were removed one by one as they were cut, dipped briefly in xylene, and stacked between pieces of tissue paper moistened in xylene. The sections were then mounted in Permount, and cover-slipped. This technique allows serial sections through large blocks of tissue to be prepared quickly and easily. These sections appear identical to those prepared in the conventional manner (i.e. cut in 70% alcohol, and re-dehydrated individually).

The sections keep well in the dark, but light, especially strong sunlight, causes rapid deterioration: the stain 'bleeds' from the fibres and the background darkens. One of the causes of this is that celloidin itself quickly turns brown when exposed to sunlight.

Interpretation of results

(*a*) *Holmes-stained material*

It is difficult, if not impossible, to trace tracts reliably through serial sections of non-selective reduced silver preparations. Different authors, working on the same region of the brain, frequently give totally different accounts of the tracts to be found. (Goll 1967, p. 163, gives a revealing summary of the connexions attributed to the corpora pedunculata of Hymenoptera by various workers.) Furthermore, it is impossible by these methods to ascertain the course and arborizations of the individual neurons in a tract. Here, connexions are described only if they have been seen in Golgi-stained, as well as in Holmes-stained, material. Any exceptions have been noted.

(*b*) *Golgi-stained material*

Differences in impregnation gave rise to difficulties in interpretation. It was found that, even in the best material, cells were sometimes stained so that a branch which was normally shown did not appear to be present. It was not easy to tell whether this represented a genuine anatomical difference (i.e. the branch in question was really missing) or a caprice on the part of the stain. The most economical assumption—that the impregnation was at fault—was usually adopted, unless there were other grounds for believing that two types of cell existed. In cases where branches were frequently unstained, the interpretation varied: this is mentioned in the text. The quality of impregnation of the spines of the spiny intrinsic cells, the most characteristic and frequently stained cells, was sometimes used as an approximate indicator of the success of the stain: that is, if this impregnation appeared defective, then the appearance of other cells might be interpreted accordingly.

The observations mainly comprise descriptions of the different cell types. The quality of the evidence on which each description is based varied. A rough indication of the reliability is given in table 1, where the scoring is as follows:

*** Entire cell seen fully impregnated on one section.

** Cell type traced through serial sections on several occasions. (The descriptions of cells in the first category were always supported by evidence of this calibre.)

* Rarely stained; or traced through serial sections in material which was thought to be unreliable (overstained, poorly stained); only seen in fragments, or on overstained sections, etc.

Table 1. An indication of the reliability of the evidence for the cell types described in the text

(*a*) Calyx		(*b*) Stalk	
spiny intrinsic	***	varieties of	most
bunched intrinsic	***	intrinsic fibre	**
bunched accessory	***	perpendicular extrinsic	*
branched accessory	*	parallel extrinsic	*
knobbed extrinsic	***	(*c*) Roots	
blebbed extrinsic	**	varieties of	most
branching extrinsic (varieties)	** or *	intrinsic fibre	**
		extrinsic fibres:	
		A(i), B(ii), C(i), E(i) and E(iii)	**
		all others	*

The same criteria were used in the optic lobe study of Strausfeld & Blest (1970).

Tracheae were occasionally stained by both Golgi methods, especially in *Pieris*, but they are easily distinguished from nerve cells (figure 66, plate 35). Glia did not appear to be stained in Colonnier material, but the Golgi rapid method did stain them. They were distinguished on the conventional, rather arbitrary, grounds: position, shape and size of the cell body; shape and extent of their processes; and type of impregnation—usually reddish and scrappy.

Drawings were made on squared paper, with a calibrated eyepiece grid in the microscope. At the magnification used, one square represented 6 μm. Each illustration is thus a scale drawing of a single, well-stained, characteristic example of that cell type.

Observations

(These apply to *Sphinx* unless otherwise noted.)

Gross structure (figures 2; 36 to 43, plates 31 and 32)

Introduction

Compared to its position in other insects, the Lepidopteran brain is tilted backwards, so that the paired corpora pedunculata lie with their calyces posterior and dorsal. The stalk runs horizontally forwards to the anteriorly placed roots. In *Pieris*, this tilting is less marked than in the moths.

Lepidoptera typically have rather small corpora pedunculata and, in moths, large antennal lobes.

The calyces (figure 2). These are composed of fine glomerular neuropile, and project backwards from the dorsal protocerebral neuropile (figures 36 to 40, plates 31 and 32). They are entirely surrounded by cell bodies, except at the point of origin of the stalk. Each calyx comprises two cup-shaped regions of neuropile lying side by side, with their adjacent walls completely fused. The 'cavities' of the cups, whose open ends face backwards, are composed of cell bodies. These cell bodies are smaller than those surrounding the calyx. The cytoplasm of these cells is scant, and the nucleus usually stains heavily with basic stains. They are generally referred to as 'chromatic' or 'globuli' cells. A third group of chromatic cells is found dorsal and rather lateral to the calyx; unlike the other two groups, it is not surrounded by a 'cup' of calycal neuropile. From the bottoms of the cups, two tracts emerge; they fuse at the anterior edge of the calyx, forming the stalk.

In a typical *Sphinx* brain measuring about $3\frac{1}{2}$ mm across (i.e. from retina to retina), the size of the calyx at its widest is 320 μm from side to side; 150 μm antero-posteriorly; and 180 μm dorso-ventrally. The walls are about 60 μm thick, and the central portion about 75 μm.

The stalk (*pedunculus*) (figures 40 to 42, plate 32). This arises from the base of the calyx, and runs anteriorly and slightly ventrally through the protocerebral neuropile. It is a broad band of fibres, about 65 μm in diameter. Blocks of neuropile lie wedged between the fibres. In transverse section, the stalk often appears divided into two: a latero-ventral portion and a medio-dorsal one. Further subdivisions may also be visible: these may correspond to the grouping of the bundles of stalk fibres described below.

The Y-tract (figures 36 to 38, plate 31). In addition to the stalk, there is a second tract which

connects the calyx to the roots. The fibres in the tract arise from the third group of chromatic cells described above. Running medially and anteriorly, the fibres pass close to the anterior border of the calyx, where they bifurcate. One branch enters the calyx via a short tract running backwards; the other branch passes onwards, medially and anteriorly, above the stalk, to run behind the tip of the α lobe. It then broadens to form the Y-lobe. The tract is 20 μm in diameter throughout its length; it consists solely of fibres and lacks neuropile blocks.

The 'roots' (figures 2; 39 to 43, plates 31 and 32). Anteriorly, the stalk swings towards the mid-line, and fibres issuing from it give rise to the roots. The α lobe, a narrow (40 μm) column of fine neuropile, projects dorsally from the swollen base of the stalk. The β lobe continues medially, and swings slightly backwards to abut against its contralateral partner in the mid-line, anterior to the central body. The β lobe is stouter than the α lobe—75 to 80 μm in diameter. Anterior to the β lobe, and originating at the same point, is a third lobe, the γ lobe. This is much narrower than the neighbouring β lobe, and projects only a short distance medially. It does not reach the mid-line.

The α and β lobes are further subdivided along their long axes. Each can be seen to consist of two fingers of neuropile closely apposed. Within this gross subdivision, the lobes (in particular the β lobe) show evidence of further regional differentiation. Groups of fibres emerging from the stalk divide only in certain regions of the roots: in other areas they are smooth and devoid of side branches.

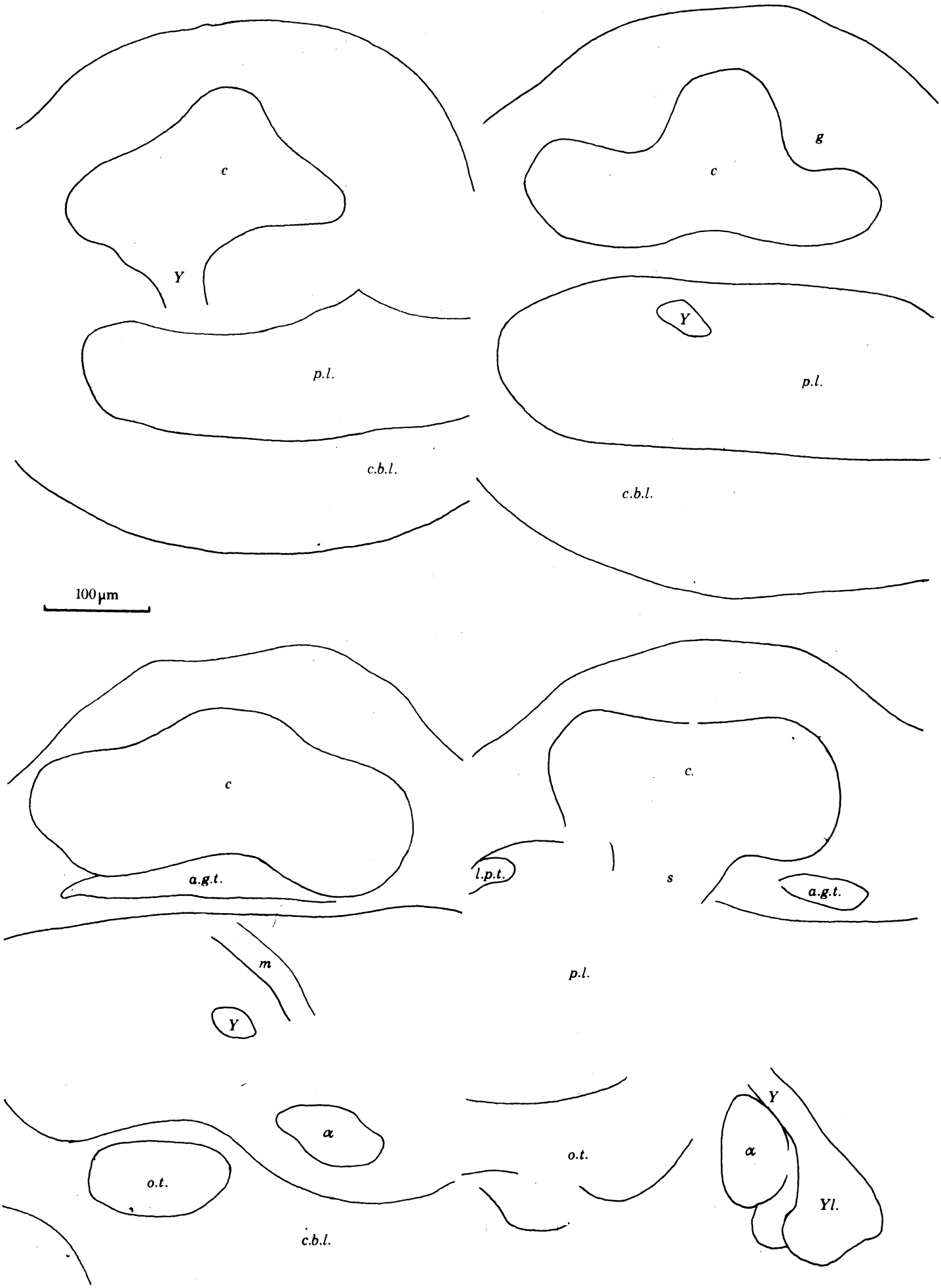
In addition, there is a second lobe system in the roots. This does not appear to have been described previously. It has been named the Y-lobe, because of its shape. Its associated tract (the Y-tract) leads from the calyx and forms the stem of the 'Y'. Just medial to the α lobe, the Y-tract broadens to form a region of neuropile wider than the α lobe (figure 39, plate 31). Below this, the Y-lobe divides into two. One arm plunges straight downwards through the base of the stalk. It remains entirely surrounded by the neuropile of this area, with which it apparently has no connexion (figures 41 to 43, plate 32). It ends abruptly, level with the ventral edge of the roots. The other arm of the 'Y' swings anteriorly across the top of the β lobe; it then turns ventrally and passes into the γ lobe.

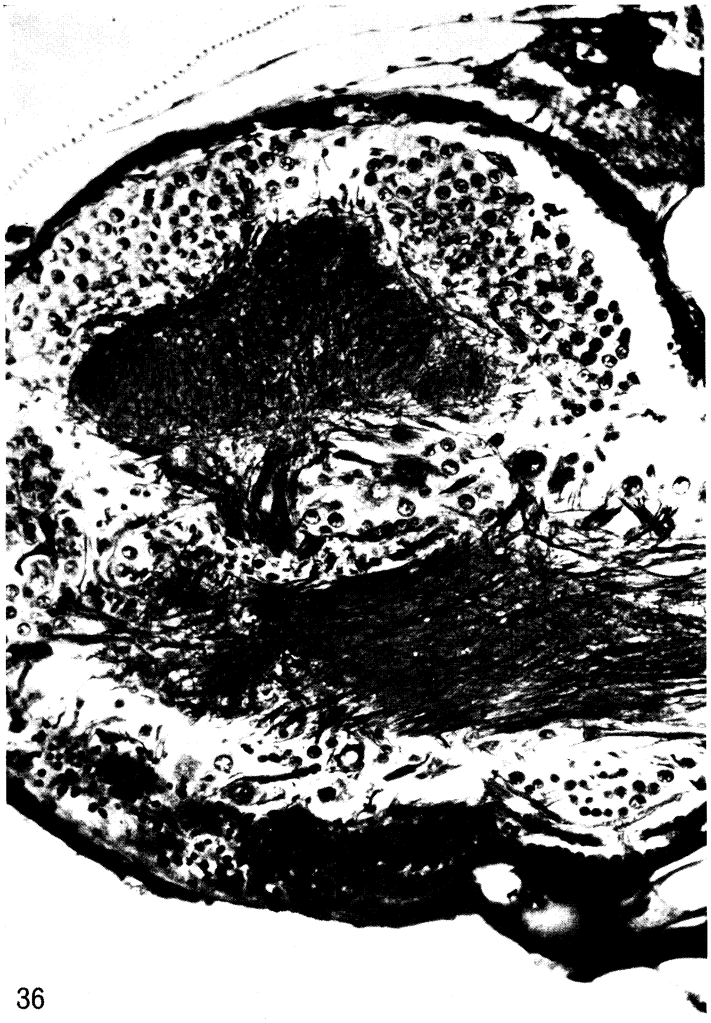
Description and key for plates 31 and 32

Figures 36 to 43. A series of horizontal sections through the protocerebrum of *Sphinx*, showing half the brain on each section. Posterior above, midline at right. Holmes (Blest) reduced silver stain. × 200.

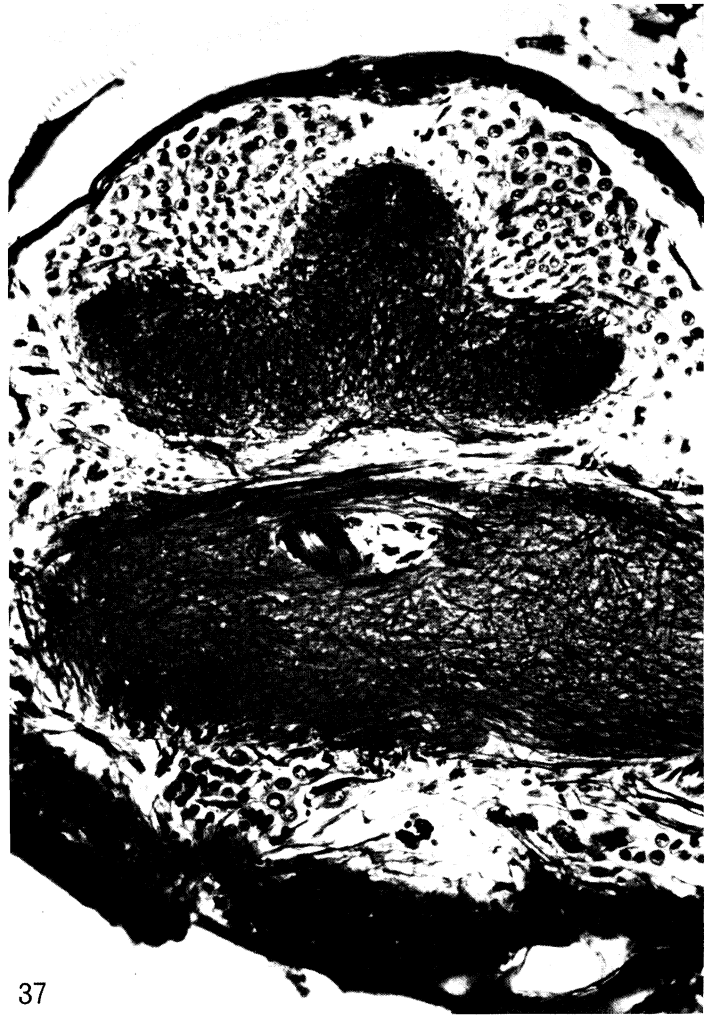
Figure 36. Section taken near the dorsal surface of the brain. The intervals between successive sections are as follows: figures 36–37: 30 μm; 37–38: 60 μm; 38–39: 60 μm; 39–40: 40 μm; 40–41: 20 μm; 41–42: 20 μm; 42–43: 30 μm. The most ventral limit of the roots lies 20 μm below the section shown in figure 43.

α	α lobe	*l.p.t.*	lateral protocerebral tract
a.g.t.	antenno-glomerular tract	*m.*	medial protocerebral tract entering calyx
a.l.	antennal lobe	*o.t.*	optic tubercle
β	β lobe	*p.c.b.*	protocerebral bridge
b.s.	base of stalk	*p.l.*	protocerebral lobes ('undifferentiated' protocerebral neuropile)
c.	calyx	*s.*	stalk
c.b.	central body	*v.Y.*	ventral limb of Y-lobe
c.b.l.	cell body layer surrounding proto-cerebral neuropile	*Y*	Y-tract
γ	γ lobe	*Yl.*	dorsal portion of Y-lobe
g.	globuli cells in 'cup-cavity'	*z.*	medial tract
l.l.	lateral lobes		

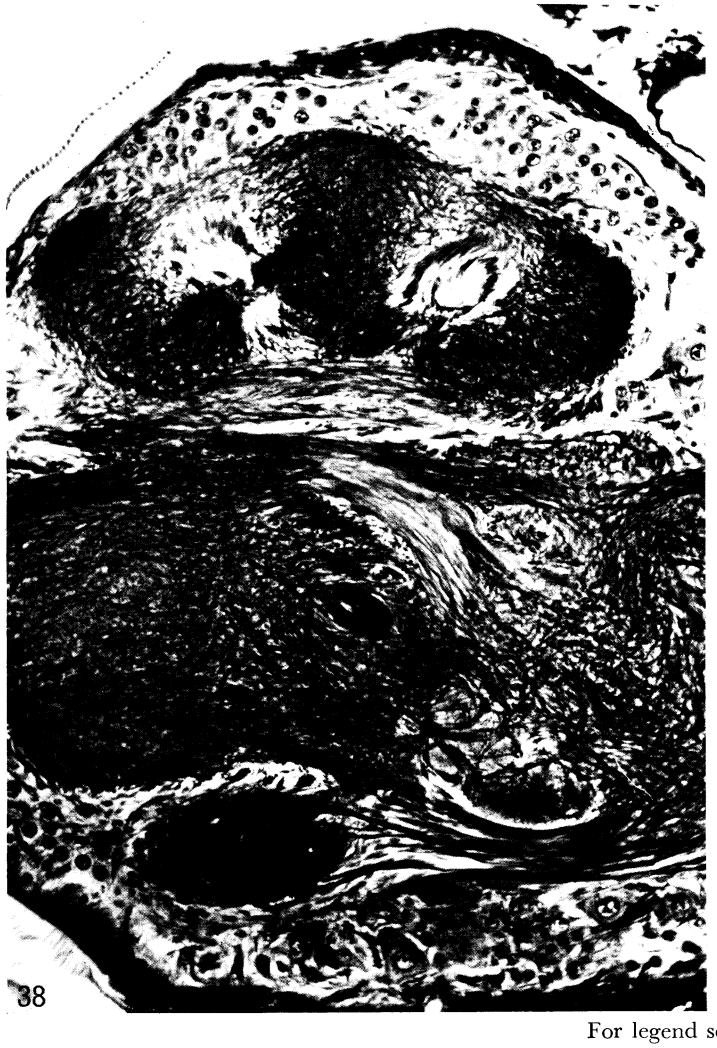

c
Y
p.l.
c.b.l.
g
c
Y
p.l.
c.b.l.
100 μm
c
a.g.t.
m
Y
c.
l.p.t.
s
a.g.t.
p.l.
α
o.t.
c.b.l.
o.t.
Y
α
Yl.



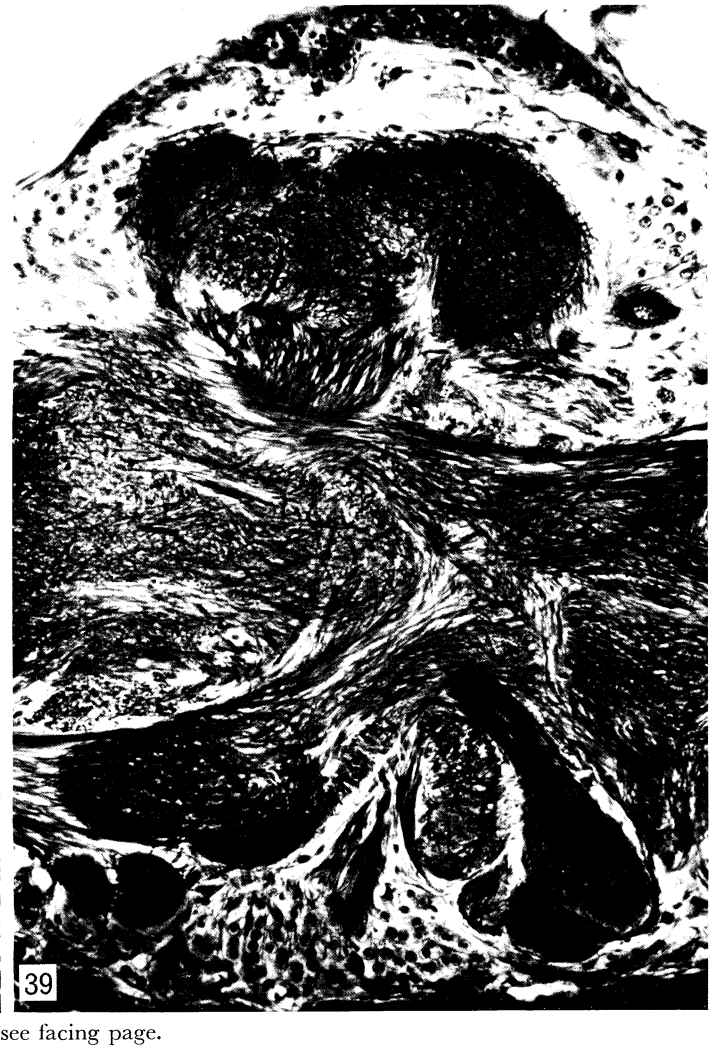
36



37



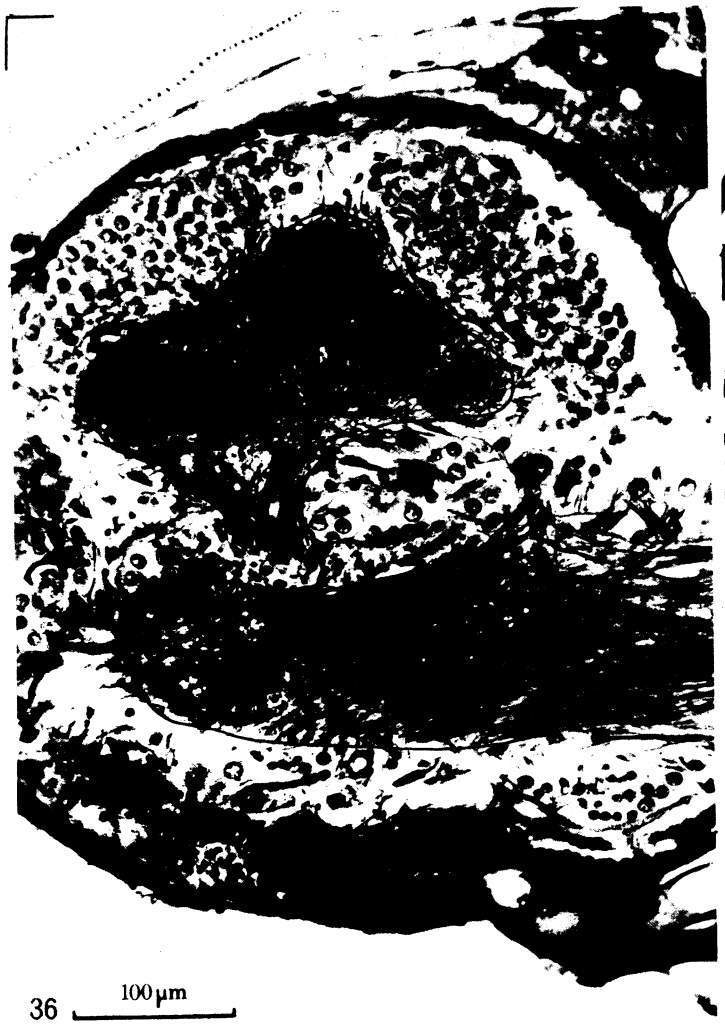
38



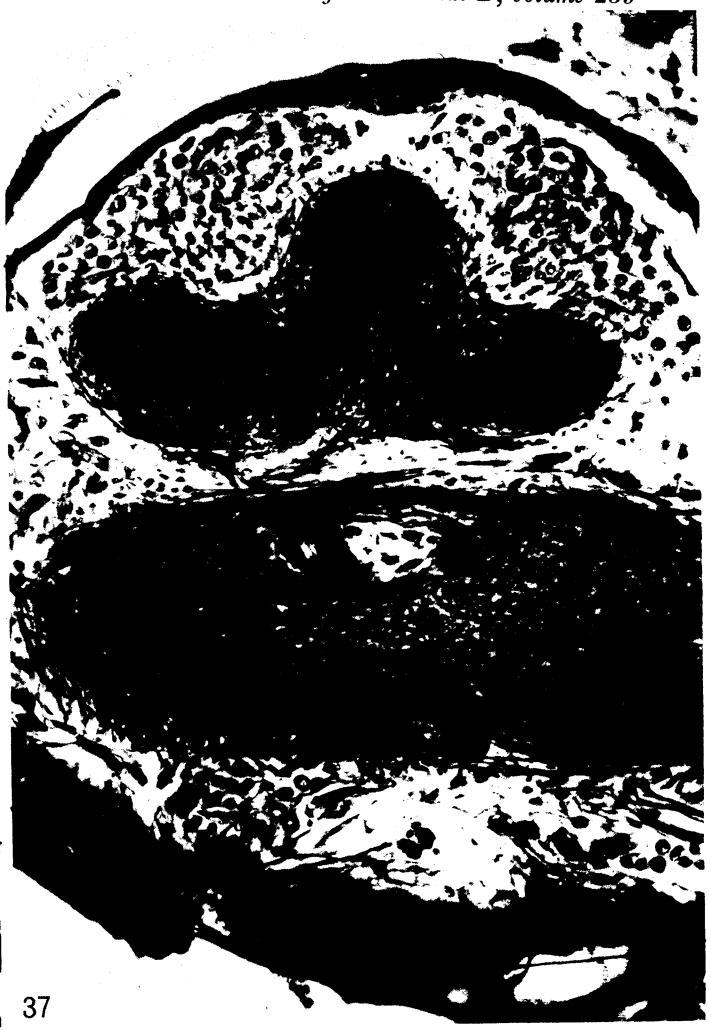
39

For legend see facing page.

(Facing p. 484)



36 100 μm



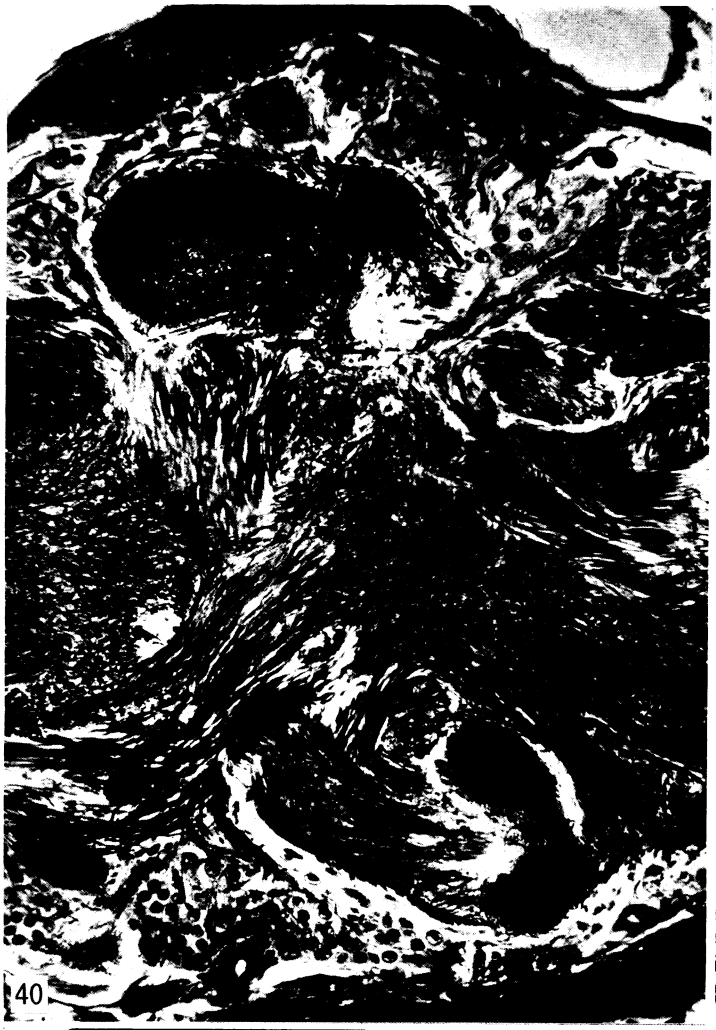
37

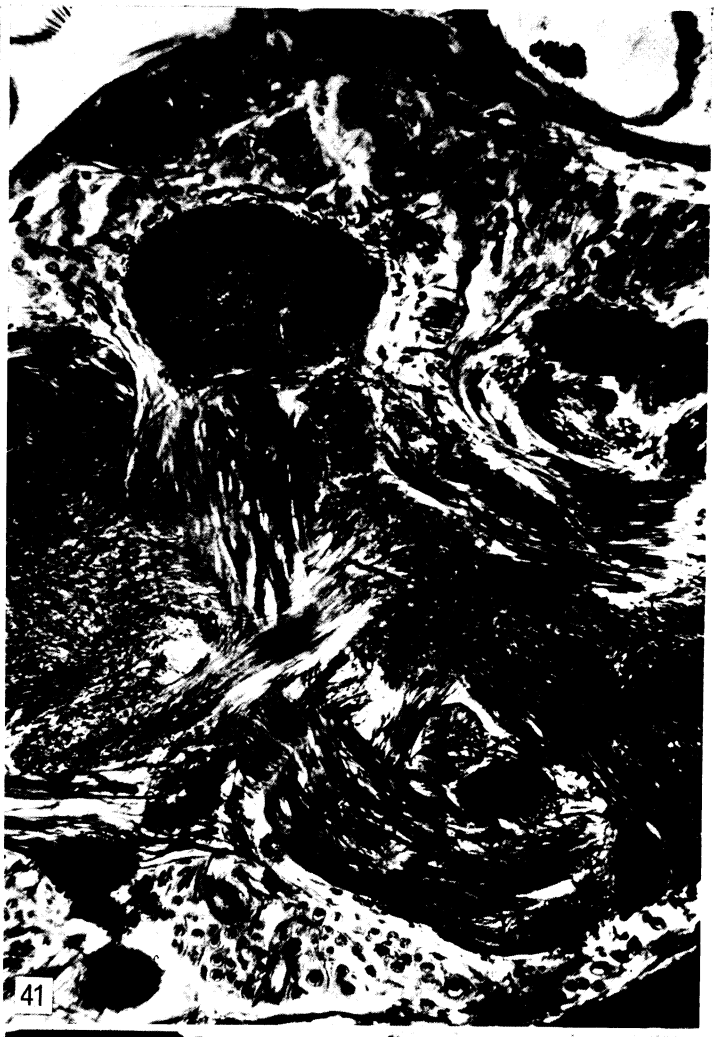
38

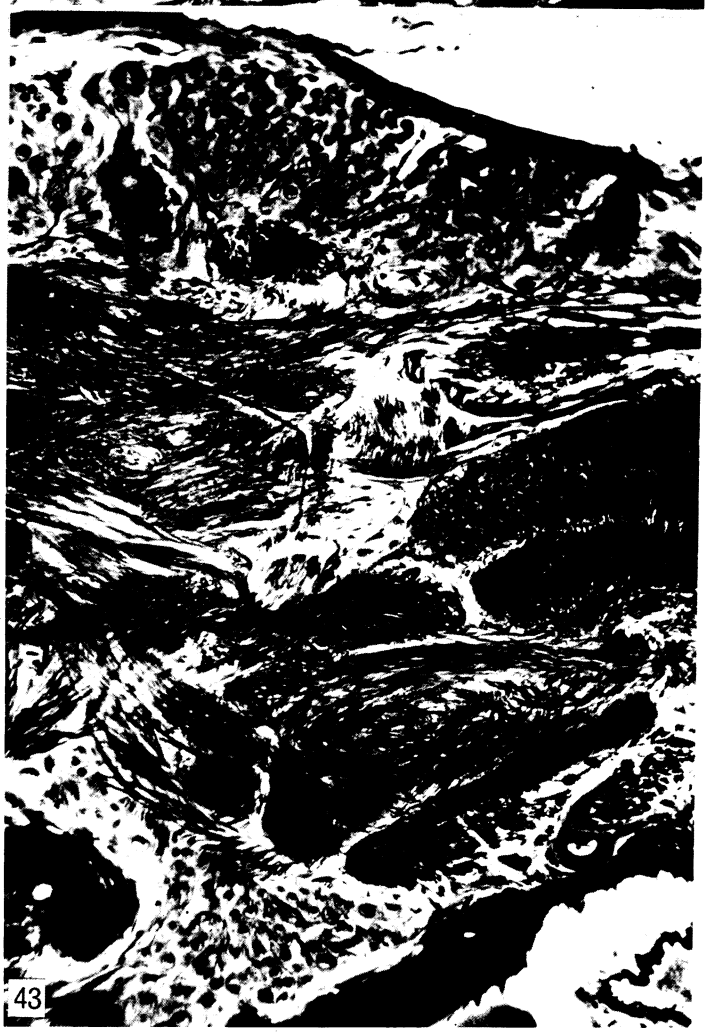
39

For legend see facing page.

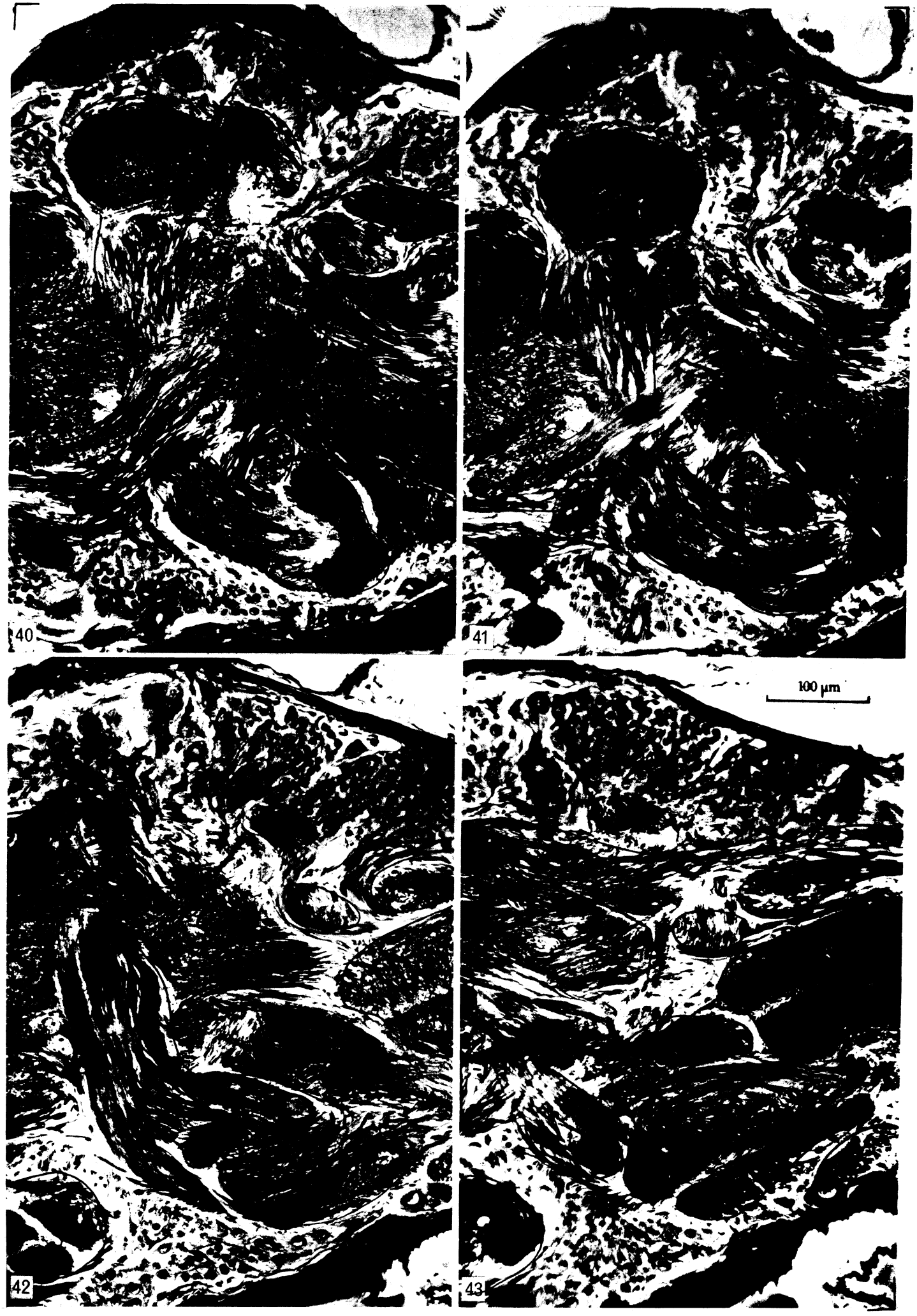

c
p.c.b.
a.g.t.
s
p.l.
Yl.
b.s.
c.b.l.
c.b.l.
c
p.c.b.
a.g.t
s
v.Y
b.s.
β
γ
100 μm
p.c.b.
a.g.t.
s
c.b.
b.s.
v.Y
β
γ
a.l.
p.l.
p.c.b.
a.g.t.
c.b.
l.l.
β
v.Y
γ
z
a.l.







For legend see previous page.



For legend see previous page.

The roots are entirely surrounded by 'undifferentiated' protocerebral neuropile, but they are clearly demarcated from it. No sheath separating the two is visible under the light microscope.

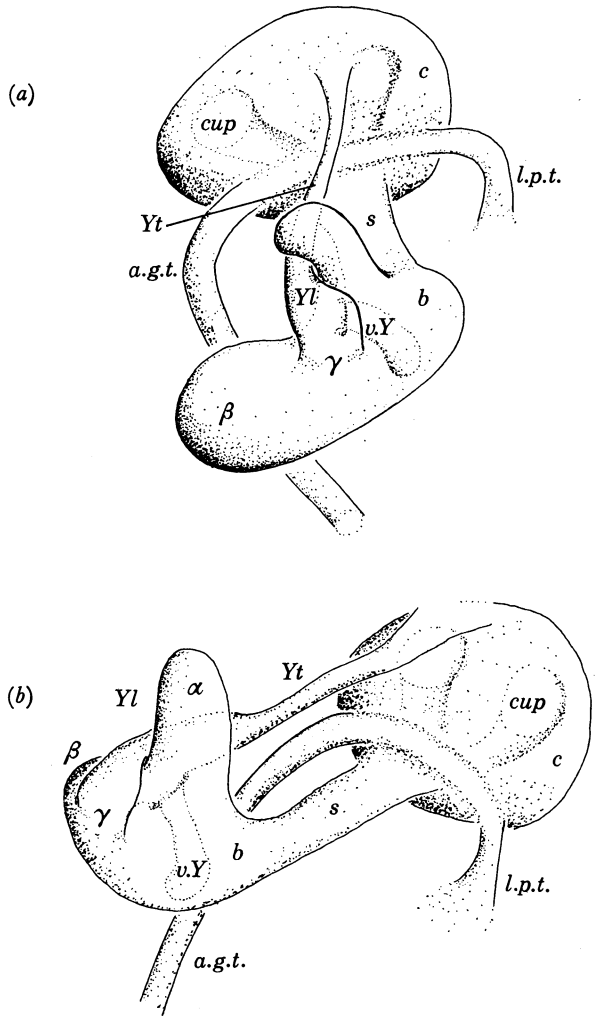


FIGURE 2. Scale drawings of the mushroom bodies of *Sphinx ligustri*. (*a*) As seen from the centre of the ipsilateral antennal lobe. (*b*) As seen from the anterior part of the ipsilateral optic lobe. These drawings were made by reconstructing serial sections through the mushroom bodies on perspective graph paper. *Key*:

α	α lobe	*γ*	γ lobe
a.g.t.	antenno-glomerular tract	*l.p.t.*	lateral protocerebral tract
β	β lobe	*s.*	stalk
b.	base of stalk	*v.Y*	ventral limb of Y-lobe
c.	calyx	*Yl.*	Y-lobe
cup	calyx-cup	*Yt.*	Y-tract

Other Lepidoptera. The other moths examined (*Automeris*, *Antherea*, *Hylesia*, *Triphaena* and a few other small noctuids) are all remarkably similar to *Sphinx* in the general form of their mushroom bodies, although their brains differ considerably in other respects (e.g. male *Antherea's* large antennal lobes; *Hylesia's* tiny optic lobes; presence or absence of ocelli). All have a double-cupped calyx, and the Y-tract and lobe are evident. Minor differences are as follows:

Automeris. The cavities of the calyx-cups are small and narrow, although the calyx itself is well developed. The stalk is slender. The α lobe is short and fat, and bends over towards the mid-line.

Hylesia. There is a partial division of the two calyx-cups, which have small cavities. The two cups are fused at the base only, showing some similarity to the Hymenopteran form. Similarly, the α lobe is large, and projects well into the dorsal cell-body layer. The Y-lobe is also large, and the β lobe particularly broad at the tip. The stalk is rather slender however.

Antherea is almost indistinguishable from *Sphinx*.

Triphaena spp. and the other noctuids. The calyx is well developed, and rounded. The stalk and α lobe are slender; the base of the stalk is swollen.

Deilephila, the hawk moth studied by Bretschneider (1921), was not examined. However, the basic similarities between the moths listed above make it reasonable to assume that *Deilephila*, like the moths described here, has only two cup cavities. Bretschneider described a 'third group of globuli cells'. He stated that, unlike the other two groups, they have no distinct stalk of their own, and that the third calyx-cup, to which the cells belong, is poorly developed. The position of the cells is identical so that of the Y-tract cell bodies, and it seems almost certain that he was in fact describing Y-tract cells. He does not mention the tract, however; this is possibly because he studied only frontal sections of the brain, in which the tract is difficult to trace. His descriptions of the contorted roots tally with the assumption that *Deilephila* does not differ much from the moths described here.

Pieris, the only butterfly examined, shows several interesting differences from the moths. The calyx-cup is double, as in moths. The stalk is slender and runs almost dorso-ventrally. Neuropile is not found between the fibres. There appears to be no Y-lobe or Y-tract. The α lobe is very small. The base of the stalk is swollen, and the β lobe is large. The distinct lobular appearance characteristic of the roots in moths is lacking. Evidence to be presented below suggests that the Y-lobe may be fused with the other lobes of the roots, and that the tract may be fused with the stalk.

Tracts connecting mushroom bodies with other areas

Introduction

Intrinsic, accessory and extrinsic cells. The cells branching in the mushroom bodies fall into three groups: intrinsic, accessory and extrinsic cells. Intrinsic cells have cell bodies in the calyx-cup or in the cell-body layer surrounding it. Their processes branch in the calyx, and each cell

---

Description of plate 33

Figure 44. Spiny intrinsic cell branches in the calyx. *Sphinx*, Colonnier, × 600.

Figure 45. Spiny intrinsic cell branches in the calyx. *Sphinx*, Colonnier, × 1800. This and figures 47–50 have been greatly enlarged to show the possible components of a calycal glomerulus and their size relations.

Figure 46. Bunched intrinsic cell in the calyx. The fibre at the bottom of the picture runs down the stalk. *Sphinx*, Colonnier, × 600.

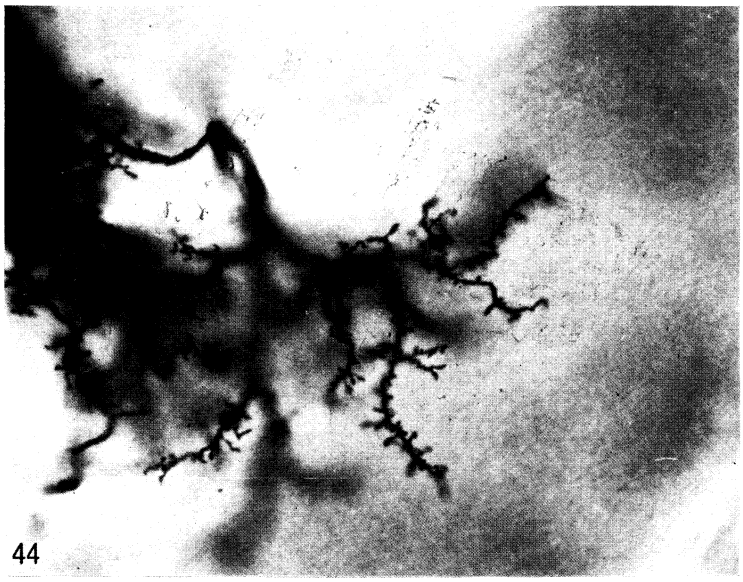
Figure 47. Portion of a bunched intrinsic cell. *Sphinx*, Colonnier, × 1800.

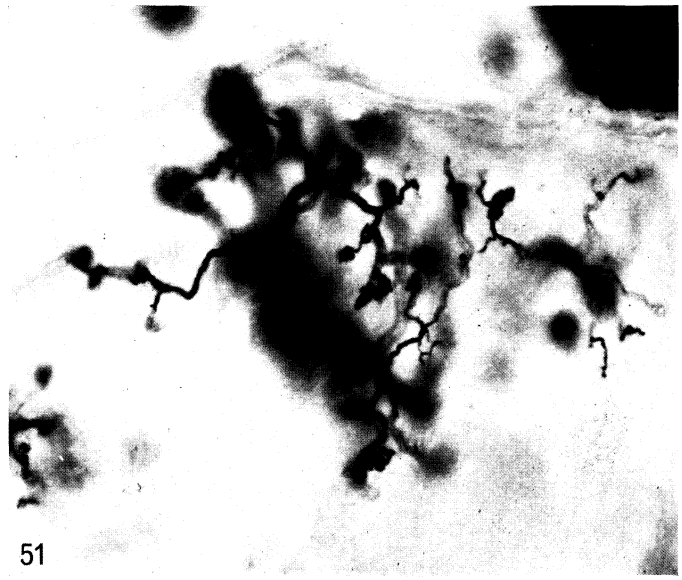
Figure 48. Clawlike terminal of a bunched accessory cell in the calyx. *Sphinx*, Colonnier, × 1800.

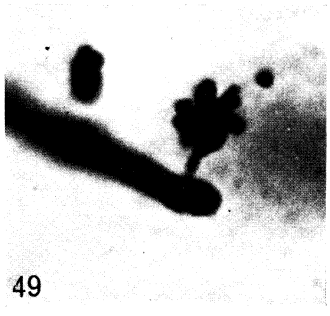
Figure 49. Knobbed extrinsic cell terminal seen end-on. *Sphinx*, Colonnier, × 1800.

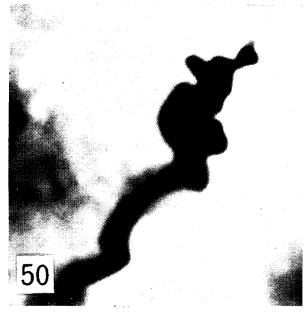
Figure 50. Knobbed extrinsic cell terminal in the calyx. *Triphaena*, Rapid, × 1800.

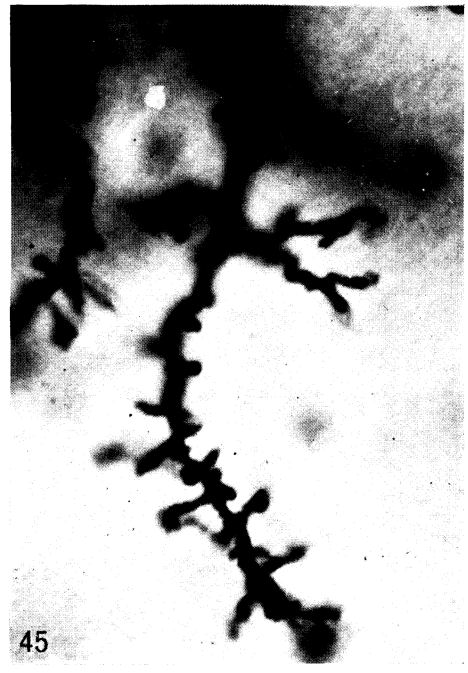
Figure 51. Bunched accessory cell in the calyx. *Sphinx*, Colonnier, × 600.



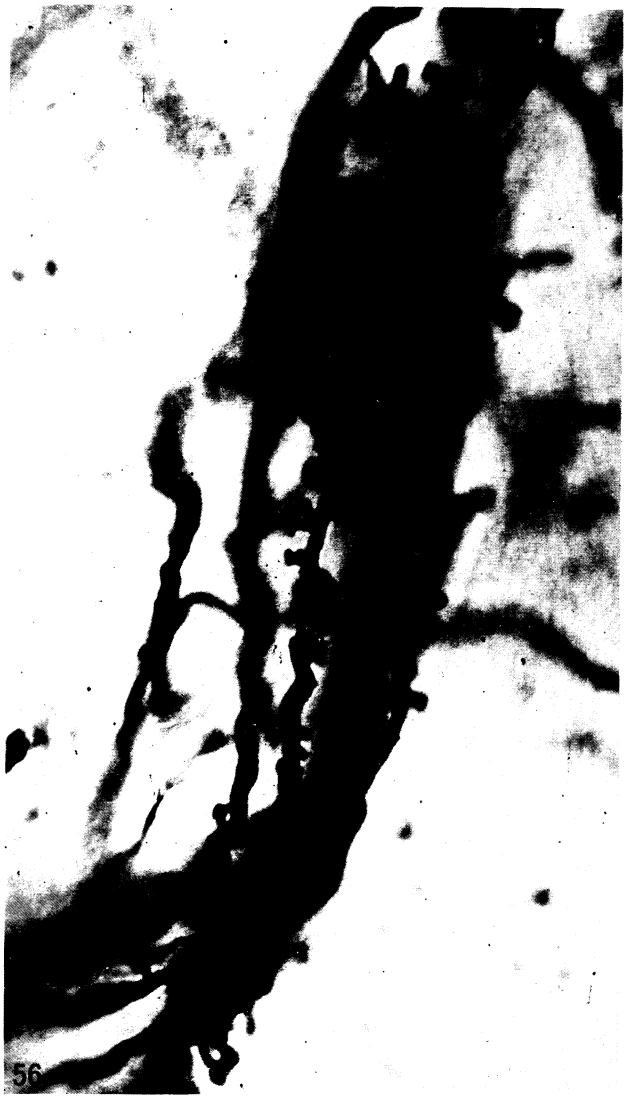








For legend see facing page.



For legend see facing page.

gives rise to a fibre which runs down the stalk to branch again in the roots. No processes from these cells pass out of the mushroom body complex. Accessory cells also branch only within the mushroom bodies; they link the calyx to the roots, or the calyces on both sides of the brain with each other, without using the stalk. The fibres which make up the Y-tract and lobe belong to accessory cells. Besides these intrinsic and accessory cells and their processes there are numerous extrinsic fibres, which have their cell bodies in various parts of the brain. Only one of their processes branches within the corpora pedunculata, while their other processes invade other areas. They thus form the means of input to, and output from, the mushroom bodies. For ease of description, it has been assumed that the terminals of extrinsic fibres in the calyx and stalk are presynaptic to intrinsic endings. In the roots, extrinsic terminals are considered to be postsynaptic elements. In other words, extrinsic fibres in the calyx represent inputs, while those in the roots represent outputs from the mushroom bodies. The physiological studies previously mentioned provide the only support for this view. It is stressed that this convention has been adopted merely to facilitate description of the fibres.

The calyx (figures 2; 38, 39, plate 31). Tracts enter the calyx at its base, where the stalk arises.

(*a*) *Antenno-glomerular tract* (*tractus olfactorio-globularis*). This arises in the centre of the antennal lobe, and leaves the lobe ventro-posteriorly. It runs posteriorly and dorsally, along the lateral edge of the central body. Beside the protocerebral bridge, the tract swings outwards to run across the base of the calyx above the stalk (figure 38). It is about 50 μm in diameter in *Sphinx*. No other antenno-calycal connexions have been found.

(*b*) *Lateral protocerebral tract.* Having given off branches which divide in the calyx, the fibres in the antenno-glomerular tract continue laterally (figures 38, 39, plate 31). After a short distance, they turn to run anteriorly and slightly ventrally, roughly parallel to the stalk but further laterally. The tract ends abruptly in the unspecialized neuropile of the lateral anterior protocerebrum, behind and below the optic tubercle. The connexions of this area are unknown, but they do not appear to include a contribution from the optic tubercle.

Description of plate 34

Figure 52. Branched accessory cell. This is a sagittal section through the central portion of the calyx (posterior at right). The fibre arises at the ventral edge of the calyx (below); after branching, it continues anteriorly, and veers sharply toward the mid-line, out of focus. Spiny intrinsic cell branches fill the section of the calyx. *Sphinx*, Colonnier, × 375.

Figure 53. Knobbed extrinsic fibres in the calyx. This is a horizontal section (posterior at top, centre at right). The antenno-glomerular tract is seen, out of focus, entering the calyx in the lower right corner of the picture. Branching fibres, which are swollen into knobs at intervals, arise from the tract. At the top right-hand edge of the picture are the 'ghosts' of two unimpregnated large cell bodies in the pars intercerebralis. *Sphinx*, Colonnier, × 600.

Figure 54. Blebbed extrinsic fibre (from accessory optic region) in the calyx. *Sphinx*, Colonnier, × 600.

Figure 55. Irregular branching extrinsic fibre entering the calyx at the lower centre of the picture (anterior margin of the calyx). Its branches in the calyx are blebbed in this preparation. *Sphinx*, Colonnier, × 600.

Figure 56. Spiny intrinsic fibres in the stalk, much enlarged to show their spines. *Sphinx*, Colonnier, × 1800.

Figure 57. Three intrinsic fibres in the stalk, with long and short side-branches. Horizontal section (posterior at top). The anterior limit of the calyx is at the top of the picture. *Sphinx*, Colonnier, × 600.

Figure 58. Single intrinsic fibre in the stalk. Horizontal section, posterior at top. The long branches occur at the junction of the calyx and the stalk. The outline of the anterior edge of the calyx may be seen as a faint line. *Sphinx*, Colonnier, × 600.

(*c*) *Medial protocerebral tract.* This tract enters the calyx above, and parallel to, the stalk. It can be traced horizontally forwards to an area of undifferentiated neuropile posterior and medial to the α lobe (figure 38). It is a 25 μm band of loosely packed fibres.

(*d*) *Tract from accessory optic area.* Fibre tracts from the optic lobe end in an area, not clearly defined in *Sphinx* or the other moths, in the dorsal posterior lateral protocerebrum. From here, fibres run across the back of the protocerebrum into the calyx, entering it tangentially. There is no definite tract in *Sphinx*, but in *Pieris* both the tract and the lobe from which it arises are clearly demarcated.

(*e*) *Suboesophageal connexion.* In Golgi-stained material, a large (4 μm) fibre, arising in the anterior part of the suboesophageal lobe, has been traced to the calyx. No corresponding tract has been seen in non-selective silver preparations; the fibres, of which there are probably few, must make their way individually to the calyx. (The fibre is not mentioned in the later cell-type descriptions, because its endings have never been satisfactorily stained. Nevertheless, it has been seen several times, in one case on a single section.)

(*f*) *Other protocerebral connexions.* Some fibres enter the calyx individually from the protocerebrum. These, and a possible commissural fibre, are described later (pp. 496, 493). None of these run in tracts, and there appear to be few of them.

The stalk. No tracts lead to the stalk, although a few fibres can be seen to enter it from the protocerebrum (see p. 500 for full description).

The roots. Numerous attempts to trace connexions between the roots and other areas have met with little success. The complex lobes are swathed in fibres; most of these bypass the roots without entering them. Of the fibres that do branch in the lobes, most do not form closely knit tracts; instead they leave the roots singly, or in loose bundles of few fibres. It has therefore proved almost impossible to trace connexions, although the direction in which extrinsic fibres run after leaving the roots could be ascertained.

α lobe. Most extrinsic fibres leave from the medial edge of the lobe, though some leave from its dorsal tip. Large-diameter fibres emerge from the medial and dorsal edge at the base of the lobe, at the junction of the lobe and the stalk. These fibres run directly medially and posteriorly into the protocerebral neuropile, and do not form a tract.

Y-lobe. Fibres from the Y-lobe usually arise from the medial side.

β lobe. Single fibres arise from the posterior edge of the β lobe. There is also a diffuse tract which leaves the posterior edge of the lobe, about halfway along it. The tract runs medially, upwards and backwards into the plexus in front of the central body. Here it is joined by other fibres running in the same direction. Many of these fibres run up through the plexus, and eventually reach the cell-body layer anterior to the lateral lobe. Corresponding fibres have been seen in Golgi preparations (Diii).

From the medial ends and sides of the β lobe, a number of fibres arise. They join the fibres in the medial tract (figure 43, plate 32) in front of the central body. These fibres all run into the suboesophageal lobe, where their destinations vary: some run straight down the ventral cord. It is impossible to distinguish the β lobe fibres from the others.

Other moths. Here, the situation is the same. In one *Automeris* preparation a possible antennal connexion with the α lobe was traced. It consisted of three fibres.

In contrast to the situation in the bee (Vowles 1955), extrinsic endings in the roots of the moth show no obvious orientation relative to the intrinsic fibres.

CELL-TYPE DESCRIPTIONS (Golgi-stained material)

Protocerebral fibres. Apart from the regions of 'structured' neuropile (mushroom bodies, protocerebral bridge, central complex and optic tubercle), the protocerebral lobes in Lepidoptera are composed of diffuse, 'unspecialized' neuropile. This is not divisible into distinct lobes. Fibres from a large number of other regions of the nervous system enter this neuropile, and branch therein; other protocerebral fibres branch only within the protocerebrum. The terminals of most protocerebral fibres ramify diffusely through a large volume. Exact location and measurement of the terminal field are often unobtainable. Furthermore, the connexions of these large-field fibres cannot be ascertained by anatomical methods.

Two 'typical' protocerebral fibres are shown in figure 3: one has blebbed endings, the other fine branching terminals. Both extend over at least 100 μm^3; both are only portions of cells.

The lateral 'lobes' occasionally mentioned in the descriptions are not well-defined lobes in Lepidoptera: the term merely refers to the areas of protocerebral neuropile lying roughly above and around the β lobes, extending as far laterally as the α lobes, and as far posteriorly as the centre of the central complex. These regions have numerous connexions with the central complex. In other insect groups they are often more clearly defined, forming distinct lobes.

The calyx

Intrinsic cells

Spiny intrinsic cell (figures 4, 5; 44, 45, plate 33). These are the most commonly stained, and most easily recognizable, cells in the protocerebrum. The cell body lies either within one of the calyx-cups, or in the cell-body layer posterior to the calyx. All the cell bodies found within the cups belong to cells of this type. Most are smooth and spherical, some 8 to 10 μm in diameter. The cell bodies lying posterior, or postero-lateral, to the calyx are frequently ovoid, and are larger—up to 20 μm across. The latter type sometimes appear to be covered in small warty protrusions. The presence of these may be an artefact, due to staining of intercellular material, or to cell shrinkage; or their absence may be due to defective impregnation; or there may be a real difference between warty and smooth cells. Kenyon (1896) noticed similar protrusions on these cell bodies in the bee, and was also undecided as to their nature.

The cell-body fibre, 1 to 2 μm in diameter, is smooth. It runs, often with several twists and turns, into the calyx-cup. Here it divides repeatedly, forming a dense network of branches throughout the wall of the calyx. Although the primary processes formed by this division are usually bare, the secondary and tertiary ones are covered in short (2 μm) spines, with a density of 7 to 17 spines per 25 μm fibre length (figure 5; 45, plate 33). Spine density varies within and between cells, and between individual preparations. A large number of counts of spine density was made, but statistical tests did not reveal the existence of more than one population in this respect. Furthermore, variations in spine density do not correlate with variations in other characteristics of the cells—size or pattern of branches in the calyx, or position or size of cell body. Evidence presented below (p. 498), however, suggests that the spiny intrinsic cells may be classified into varieties on the basis of other anatomical features.

The processes of a single cell extend through the thickness of the calyx wall, and for some distance around its circumference. The average spread is 120 to 150 μm round the circumference of the cup, 90 μm in depth, and 90 μm through the wall of the cup. There is again considerable variation, but the varieties or subtypes encountered form a continuum, rather than distinct

classes. One common variant is the 'umbrella' type (figure 4), whose processes (the umbrella) covered the whole outer surface of both cups, while the stalk fibre forms the handle. The cell body usually lies posterior to the calyx. At the opposite extreme is the bipartite type (figure 5), whose stalk fibre is a direct continuation of the cell-body fibre. From this fibre, two main secondary processes are given off at right angles, opposite each other. One branches in the outer wall of one cup, while the other branches in the central, common, wall. This variant is thus restricted to one cup, within which its cell body usually lies.

The field covered by the processes of these cells, in relation to the size of the calyx, is very large compared to that in the bee.

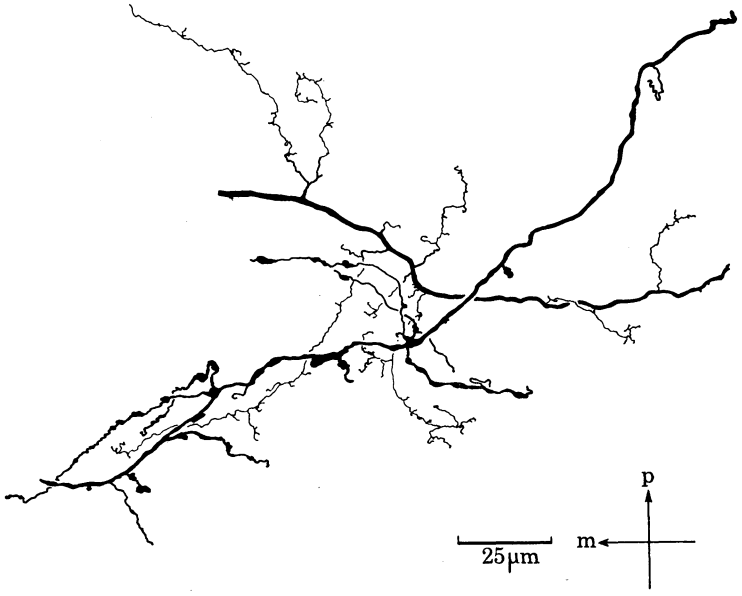


FIGURE 3. Two 'typical' protocerebral fibres in the dorsal protocerebrum of *Sphinx*. Rapid method.

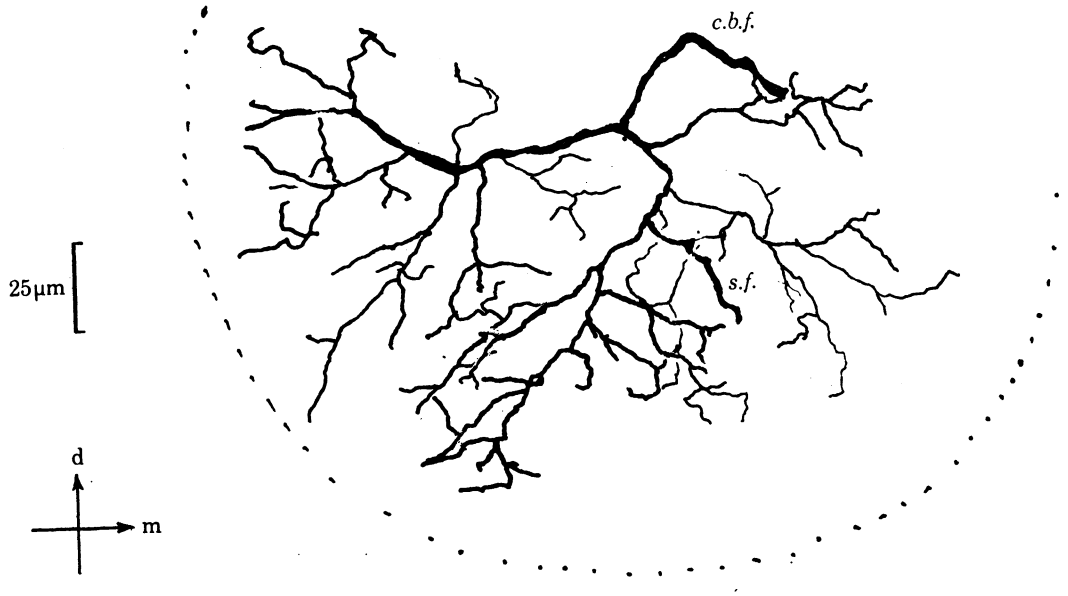


FIGURE 4. Spiny intrinsic cell in the calyx. *Sphinx*, Colonnier. Only the main branches are shown: the spines have been omitted. *c.b.f.*, cell body fibre; *s.f.*, stalk fibre.

From one of the primary processes, a branch (the stalk fibre) extends down the stalk, into the roots. There appears to be little regularity about the origin of this branch: it depends on the shape of the field covered by the processes. In some cases, the stalk fibre arises from one of the secondary branches; in others, it is a continuation of the cell-body fibre. The stalk fibres run down the inside of the cup wall, in the cup cavity. At the bottom of the cup, the fibres are gathered into two tracts, one for each cup. After running forwards for a little way,

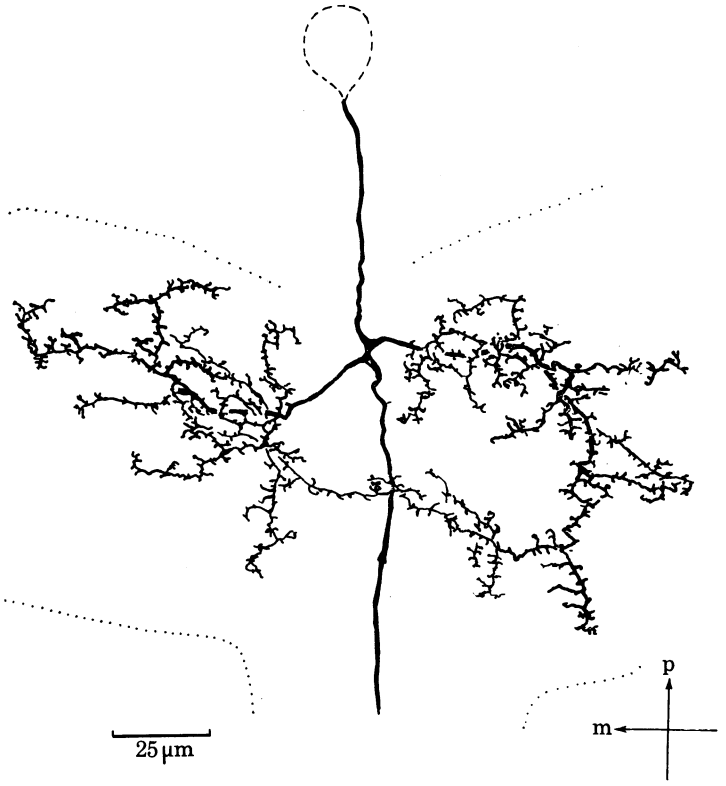


FIGURE 5. Spiny intrinsic cell in the calyx: 'bipartite' variant. *Triphaena*, Rapid. The cell body, which was on the next section, is shown in outline.

the two tracts join to form the stalk. The fibres are not divided small groups in the cup cavity, as they are in the bee (Kenyon 1896; Vowles 1955). Their continuations in the stalk and roots will be described later.

Bunched intrinsic cell (figures 6; 46, 47, plate 33). The cell bodies lie posterior to the calyx, and are similar to those of the spiny intrinsic cells in this location. The cell-body fibre is smooth, and 1 to 2 μm in diameter. It passes along the surface of the calyx before turning into the cup cavity. The fibre runs straight through the cup and out of its base, where it joins the stalk fibres of the spiny intrinsic cells and continues into the stalk and roots.

During its passage through the cup, the fibre gives off perpendicular secondary branches. These branches are up to 30 μm long; they infiltrate the wall of the cup. The field covered by these processes is thus restricted to a column whose cross-section is oval. At its widest, the column is about 60 μm in diameter.

Typically, the secondary branches terminate in an irregular, hollow, claw or bunch some 5 μm across. The bunch is formed by a group of processes arising from the division of the secondary branch. Occasionally, small branchlets may be present on the secondary processes,

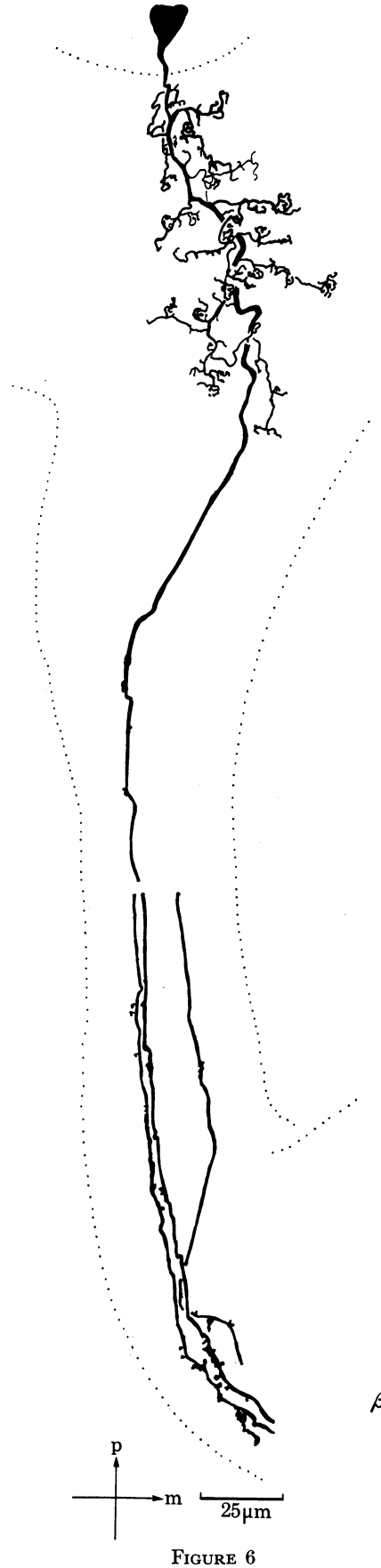


FIGURE 6

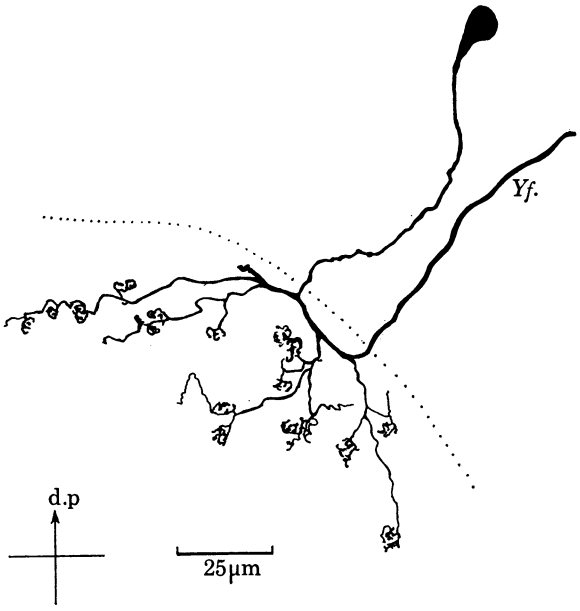


FIGURE 7

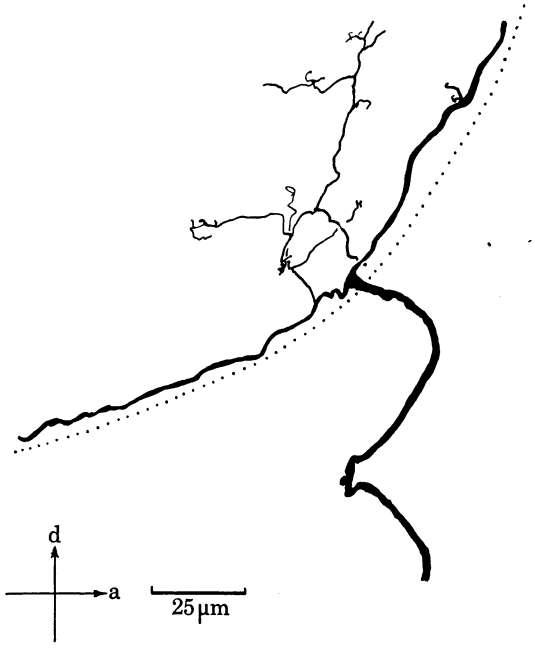


FIGURE 8

FIGURE 6. Bunched intrinsic cell in the calyx and stalk. *Triphaena*, Rapid. Three cells of this type were stained close together. One is drawn in the calyx and proximal stalk. All three cells continued in the next section: it is impossible to tell which process is that of the drawn cell. Note that all these processes run to the β lobe, and do not branch in the α lobe.

FIGURE 7. Bunched accessory cell in calyx. *Triphaena*, Rapid. *Yf.*, Y-tract fibre.

FIGURE 8. Branched accessory cell in the calyx. *Sphinx*, Colonnier. The central portion of the large fibre veers sharply towards the mid-line, and appears foreshortened in the drawing.

or may arise from the bunches. At the bottom of the cup, the fibre ceases to give off branches, and is smooth until it reaches the stalk.

It is evident that these cells branch over a much smaller area than the spiny intrinsic cells. Furthermore, if, by analogy with the spines of the Purkinje cells and the bunches of the granule cells in the vertebrate cerebellum (Eccles, Ito & Szentagothai 1964), both types of terminal are taken to represent synaptic specializations, then it is evident that, of the two types, the bunched intrinsic cells receive a much more restricted input.

These cells are impregnated in about one in every twenty brains stained by the Colonnier method, but are impregnated more often in rapid preparations.

Bunched accessory cell (figures 7; 48, 51, plate 33). The processes of these cells form the Y-tract. From the small cell body in the 'third group' of globuli cells, a 1 μm fibre runs downwards, forwards, and centrally, past the antero-lateral border of the calyx. Here it gives rise to a branch which enters the calyx, and divides repeatedly there, filling an irregularly shaped volume up to 60 μm^3. The branches thus formed terminate in bunches of the same size and general appearance as those of the bunched intrinsic cells, though the accessory cell bunches are tighter and more knotted than the open, clawlike intrinsic ones.

The continuation of the cell-body fibre runs down the Y-tract into the Y-lobe. These are the only fibres seen in the tract. However, fibres in the tract are sometimes stained with no sign of the characteristic endings in the calyx. As the calyx branch comes off the cell-body fibre at a sharp angle, it is likely that the impregnation is at fault, but it remains possible that there are two cell types.

These cells are infrequently stained—about one in every twelve brains treated by the Colonnier method contains impregnated cells, and the rapid method stains them less often.

Branched accessory cell (figures 8; 52, plate 34). Although this cell type has been stained very infrequently, its appearance on each occasion was striking and characteristic. A smooth fibre, 1 to 2 μm in diameter, arises from the ventral posterior border of the calyx. The cell body has not been found, but is possibly here. The fibre runs forwards and upwards, closely following the margin of the calycal neuropile. Halfway up the calyx, approximately level with the stalk but medial to it, the fibre turns abruptly to run straight forwards. At the turn two or three processes arise. These turn and run just below the surface of the calycal neuropile, upwards, downwards, and laterally. Short (10 μm) side branches are sparsely distributed (not less than 15 μm apart) along the secondary processes, particularly towards their ends. The main fibre, after running forwards for a short distance, veers towards the mid-line, maintaining its position high in the protocerebrum. It has been traced as far as the mid-line; it is believed to be a fibre forming a commissure between the two calyces.

Extrinsic fibres

Knobbed extrinsic fibre (figures 9 to 11; 49, 50, 53, plates 33 and 34). The processes of these cells form the large antenno-glomerular tract. The cell bodies mostly lie at the margins of the antennal lobe, surrounding the neuropile. The cell-body fibre passes between the antennal glomeruli (the sensory antennal neuropile). In the centre of the lobe it branches several times, giving rise to large processes which invade several glomeruli (figure 9). At this point, the fibre is very large, reaching 5 to 6 μm in some cases. One branch, more slender than the others, passes out of the lobe at its ventral anterior margin into the antenno-glomerular tract. A few of the fibres in the tract arise directly from the antennal nerve, though this appears to be the exception

rather than the rule. At the point where the fibres run across the base of the calyx, they are 2 to 3 μm in diameter. From here, secondary fibres, 1 to 2 μm in diameter, arise at right angles to the primary ones. These fibres enter the calyx, where they divide once or twice, and ramify

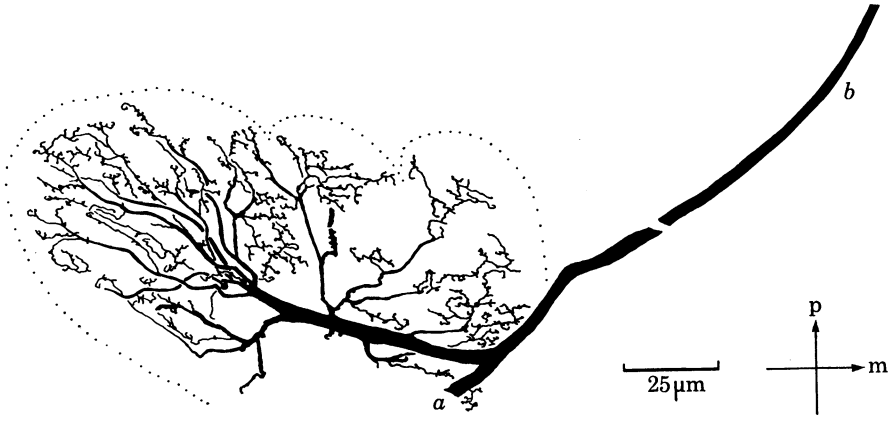


FIGURE 9. Antenno-glomerular tract fibre: terminal in the antennal lobe. This large terminal covers several antennal 'glomeruli'. Fibre *a* continues deeper into the antennal lobe, where it has branches similar in form and overall field size to those shown here. Fibre *b* runs in the antenno-glomerular tract to the calyx. The break in the fibre represents its transition to another section. *Triphaena*, Rapid.

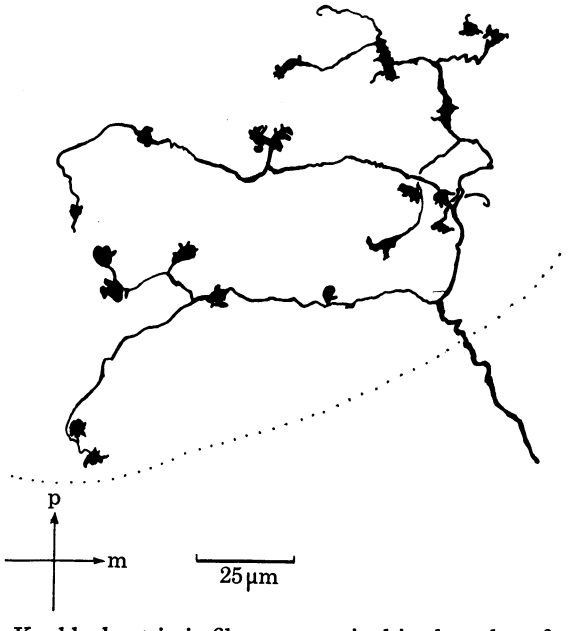


FIGURE 10. Knobbed extrinsic fibre, or terminal in the calyx of a fibre from the antenno-glomerular tract. *Triphaena*, Rapid.

over most of the calyx (figure 10). The processes arising from one primary fibre are not restricted to one calyx-cup. At irregular intervals (usually more than 15 μm), the fibres are swollen into large warty knobs, about 5 to 8 μm long and 2 to 3 μm wide. A few short (1 μm) spines are sometimes present on the knobs. The fibres almost always terminate in a knob, which may also mark the point of division of the fibres.

It is suggested, on the basis of comparative size, shape and form, that these antennal knobs in the calyx are the central core of a glomerulus as described in the electron microscope studies

of Trujillo-Cenoz & Melamed (1962). This point is further considered in the Discussion (p. 509).

Some of the secondary fibres, instead of plunging straight in to the calyx, run around its circumference giving off branches into the neuropile. These fibres usually terminate in the calyx. Most of the fibres from the antenno-glomerular tract, however, continue in the lateral protocerebral

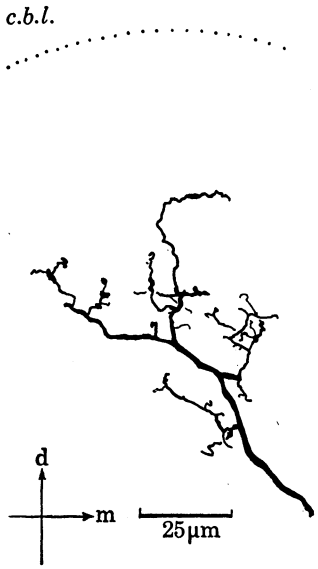


FIGURE 11. Antenno-glomerular tract fibre: terminal in the lateral protocerebrum. *Sphinx*, Colonnier.

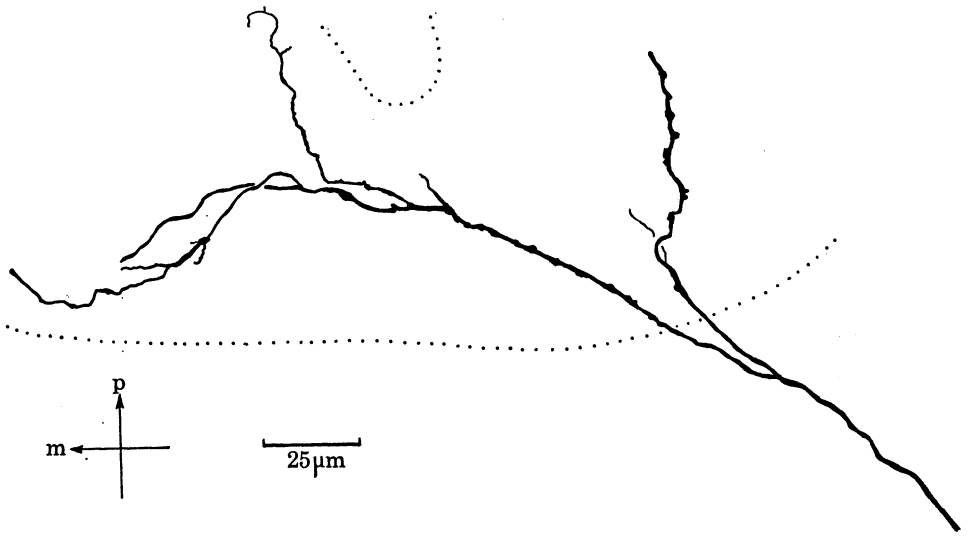


FIGURE 12. Blebbed extrinsic fibre in the calyx. Fibre *a* runs to the accessory optic area in the dorsal lateral protocerebrum. *Sphinx*, Colonnier.

tract. They sweep round in an arc, running forwards to an area behind and below the optic tubercle. Here they divide several times, giving rise to a network of fine, slightly blebbed, branches (figure 11). The area covered by these terminals is about 100 μm^3.

These fibres are stained in about 90 % of all preparations.

Blebbed extrinsic fibre (figures 12; 54, plate 34). This fibre has its origin in the dorsal accessory optic region. Within this area, its terminals are of the diffuse branching type, and cover the

whole region. A 1 μm fibre runs to the base of the calyx. Here, the fibre is swollen into smooth blebs at regular intervals (5 to 8 μm). It turns into the cup, usually via one of the two tracts which join to form the stalk. It divides three or four times; the branches diffusely cover most of one calyx-cup. These branches are blebbed at regular intervals; the diameter of the blebs is about twice that of the fibre at any particular point. Fibre diameters vary according to the number of divisions which have taken place. The terminal branches are extremely fine—less than $\frac{1}{4}$ μm in diameter.

It is presumed that these blebs represent *en passant* synapses (compare, for example, with the blebs on the parallel fibres in the vertebrate cerebellum: Eccles *et al.* 1964). Due to the

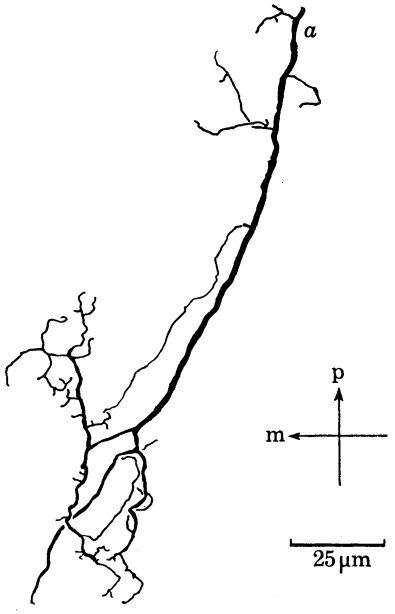


FIGURE 13. Protocerebral terminal of a medial protocerebral tract fibre. Fibre *a* runs to the calyx in the medial protocerebral tract. *Sphinx*, Colonnier.

vagaries of the stain, many fibres appear blebbed at times. This artefactual blebbing is usually not as regular, nor as rounded, as that which characterises these fibres.

The fibres are stained in about 20 % of all preparations.

Branching extrinsic fibres (figures 13, 14; 55, plate 34). There are several varieties of these fibres. One arises from a $\frac{1}{2}$ μm fibre in the medial protocerebral tract. Entering the calyx via one of the two hemi-stalks, it divides several times; the field covered by the fine, branching endings is restricted to part of one calyx-cup. There are no specialized terminals, although the branches may appear slightly blebbed at times (probably artefactually). At the anterior end of the medial protocerebral tract the fibres divide in a second series of endings (figure 13). These are diffuse, slightly blebbed branches typical of the protocerebrum, covering a volume of about 80 μm^3 posterior to the α lobe.

Other branching fibres do not run in any tract, but enter the calyx individually from the protocerebrum. This small group of fibres forms a heterogeneous assortment. But all the fibres have one character in common: they have diffuse, extensive, unspecialized endings in the dorsal protocerebrum. Examination of non-selective silver preparations suggests that there are few of these fibres—possibly no more than half a dozen. They are infrequently stained. They seldom look similar; three variants are shown.

The first type of ending (figure 14*a*) arises from a small fibre with diffuse protocerebral branches. The fibre branches repeatedly within the calyx. The branches thus formed are

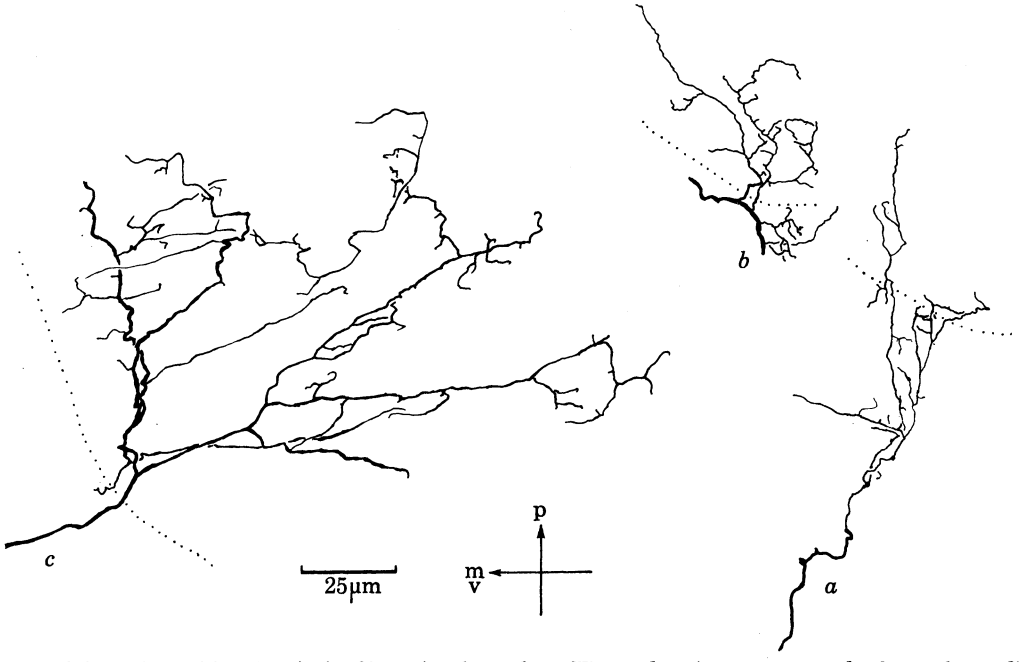


FIGURE 14. Three branching extrinsic fibres in the calyx. These drawings were made from three different preparations. *Sphinx*, Colonnier. *a*, *b* and *c* as in text.

extremely fine and smooth, and ramify over 30 μm^3. The second (figure 14*b*) has extensive branches in the protocerebrum, and a 2 μm main fibre. One of the protocerebral branches enters the calyx, there to divide again. The fields of both these fibres cover only the basal portion of the calyx, near the origin of the stalk. A third variant (figure 14*c*) ramifies over the whole calyx.

Other Lepidoptera. Cells of the preceding types have been found in all the moths, and there is no evidence to suggest that they differ in any way from *Sphinx*. In *Pieris*, all cell types except bunched accessory cells have been identified. *Pieris* preparations tend to have the mushroom bodies either very heavily stained or totally unstained; thus, bunches which were thought to belong to bunched intrinsic cells might have been those of cells homologous to bunched accessory cells.

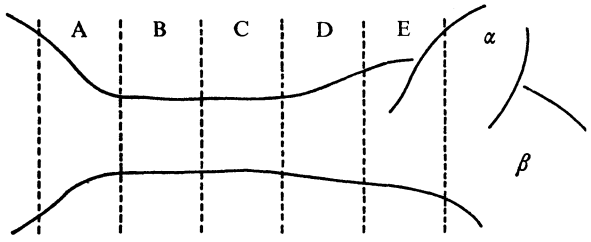
The stalk

Intrinsic cells (figures 15, 16; 56 to 58, plate 34).

The stalk is composed almost entirely of the processes (1 to 2 μm in diameter) of the intrinsic cells, running from the calyx to the roots. The most striking characteristic of the Hymenopteran stalk is the strictly parallel course of these descending fibres. In contrast, those in Lepidoptera weave across each other's paths, especially at the top of the stalk. The fibres give off fine perpendicular side branches during their course down the stalk. The distribution of side branches varies, but, typically, the branches at the base of the calyx and the origin of the stalk are long (up to 30 μm) and may bifurcate once or twice themselves (figure 58, plate 34); those in the stalk itself are usually shorter, taking the form of 2 to 4 μm spines; those arising where the stalk runs into the roots are again longer.

Intrinsic fibres in the stalk can be divided into a number of different varieties, according to three characteristics: the exact form and pattern of branches on the fibre; the position of the fibre in the stalk (central, lateral, etc.; precise localization is difficult to measure in Golgi preparations); and the subsequent branching pattern in the roots. This classification is given in table 2.

TABLE 2. A CLASSIFICATION OF THE VARIETIES OF INTRINSIC FIBRE IN THE STALK AND ROOTS



	appearance of branches in A	B	C	D	E	position in stalk	destination in roots	figure
1	4 μm	*very* few, to 8 μm			few, to 20 μm	medial margin	α (only)	15
2	4 μm	4 μm	4 μm	4 μm	—	medial	α+β	15, 19
3	20 μm	to 10 μm	to 15 μm	none/ few 4 μm	to 20 μm	central	α+β	
4	4 μm	many 4 μm	few 4 μm	—	to 30 μm	central	β. Ends in E are beginning of β ends: long branches	15
5	—	4 μm	4 μm	—	—	central/ ventral	β. Short (10 to 15 μm) ends in β	6
6	4 μm	4 μm	10 to 16 μm	4 μm	—	lateral	β or β+γ (running through)	15
7	—	—	—	—	to 15 μm	lateral	to β through γ (stain variant of 6?)	—
8	to 20 μm	to 15 μm	to 8 μm	few 4 μm	—	lateral	α+β+γ (branch)	20

Probably, not all the variants have been seen. It seems likely that the different variants have different branching patterns in the calyx too. That these could not be found may be due to the large size of the fields occupied by the branching processes of the intrinsic cells, in comparison to the small size of the calyx.

All the bunched intrinsic fibres which have been traced into the stalk have been of type 5. However, since they are infrequently stained it is difficult to be certain that all bunched intrinsic fibres are of this type, or that all type 5 fibres arise from bunched intrinsic cells. The same reservations apply to the branching pattern of this fibre in the roots.

Fibres in the stalk often do not stain singly, but rather as groups or bundles of a few fibres each. The fibres of one bundle lie close together, are all of the same type (as detailed in table 2), and all have the same branching pattern in the roots. Fibres of type 5 have not been seen to form a bundle—possibly because they are rarely stained. Golgi preparations do not allow measurements of the numbers of fibres in a bundle but, from non-selective silver preparations, the number of bundles in the stalk can be estimated at twenty. Thus, either some varieties of stalk fibre have not been stained, or, more probably, there is more than one bundle of each fibre type.

FIGURE 15. Examples of five variants of stalk fibre arising from spiny intrinsic cells in the calyx. These drawings were made from three different preparations. Numbers of variants as detailed in the text and in table 2. (1) Note that this is finer than the other variants, and has no β lobe branch. The α lobe branch of this and variant 3 run perpendicular to the plane of section, and appear foreshortened. (2) This fibre continues into the roots, branching in both the α lobe and the β lobe (shown in figure 19). (3) Note branches to both α and β lobes. (4) Note long branches at the distal end of the stalk (approximately region E in table 2, continuing into the β lobe). There is no α lobe branch. (6) Again, there is no α lobe branch. Note lack of branches in region E, and spines in the proximal part of the β lobe.

Although the fibres in one bundle may cross each other, and the bundles themselves intertwine, there appears to be no interdigitation, or exchange, of the fibres from different bundles (figure 16).

The spines and branches of the intrinsic fibres in the stalk almost certainly represent synaptic specializations. The very small number of extrinsic fibres (see below), and the very large number of intrinsic fibre spines, indicate that the majority of these synapses involve interactions between different intrinsic cells, not between intrinsic and extrinsic cells. Furthermore, the

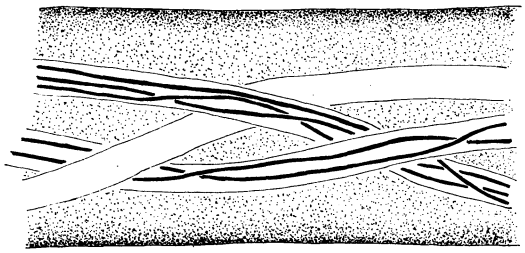


FIGURE 16. Diagram to show three bundles and a few of their fibres in the stalk.

formation of bundles suggests that, as far as their short spines are concerned, most fibres interact only with the other fibres in the same bundle. Some fibres bear spines on one side only. Generally, these appear to be the fibres on the outside of a bundle, with the spines projecting into its centre.

The crossing of the fibres at the top of the stalk may represent the fibres sorting themselves out into their correct bundles. Fibres from both cups can, and usually do, participate in the formation of one bundle.

Extrinsic fibres

There are no tracts leading to the stalk, and extrinsic fibres are very infrequently stained; Holmes preparations show that there are very few of them. In Golgi preparations, two types have been seen: fibres whose terminals run perpendicular to the stalk, and those with endings lying parallel to the stalk fibres. The former type of ending (figure 17) arises from a 1 μm fibre which branches high in the dorsal protocerebrum, above the stalk. Its cell body probably lies in the cell-body layer posterior and dorsal to the optic tubercle. From the protocerebral branches, one very fine branch runs down to enter the stalk, where it divides once or twice; the terminals are inextensive and unspecialized. The second type (figure 18) also arises from a fibre which branches in the dorsal protocerebrum. The main fibre runs above, and parallel to, the stalk. Posteriorly, it turns into the stalk, where it divides repeatedly. The fine branches cover a strip 15 μm wide and at least 60 μm long, with the long axis lying antero-posteriorly.

The small number of extrinsic fibres in the stalk suggests that most synaptic interactions which take place here are between intrinsic fibres.

Other Lepidoptera. The other moths appear almost identical to *Sphinx*; some of the main variants of intrinsic fibres were identified. In *Pieris*, however, no long side branches on the intrinsic cell fibres were seen, although the spines are present. Bundles are present. Not enough preparations were made (the stalk tends to stain very heavily) to classify variants of intrinsic fibres.

Only one extrinsic fibre was stained in the other Lepidoptera: this was a parallel ending in *Triphaena*.

The roots

Intrinsic fibres

Emerging from the stalk, the intrinsic fibres spread out and divide in the roots. Their main branching pattern varies according to type, being correlated with the form of the branches in the stalk, as shown in table 2.

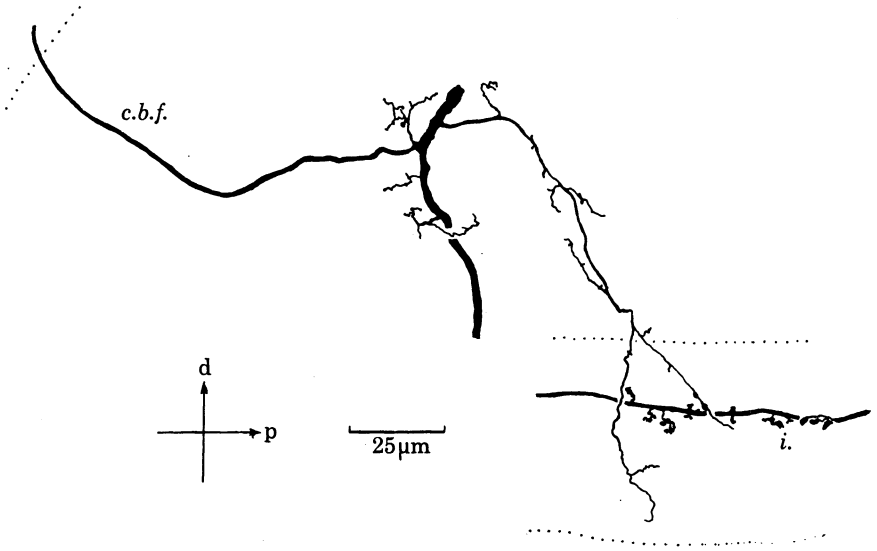


FIGURE 17. 'Perpendicular' extrinsic terminal in the stalk. *c.b.f.*, cell body fibre; *i.*, intrinsic fibre in stalk. *Sphinx*, Colonnier.

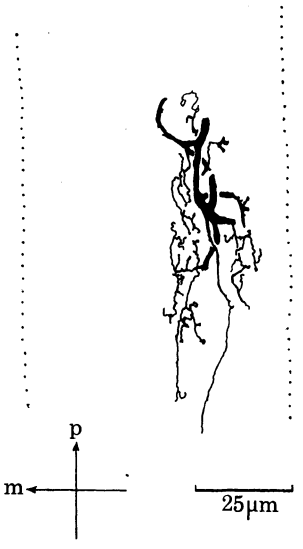


FIGURE 18. 'Parallel' extrinsic terminal in the stalk. *Sphinx*, Colonnier.

Within the α, β and γ lobes, the fibres all run in the same direction, though they may swing across each other's paths (figure 19). They run to the tips of the lobes, where they end, tapering into branches. Side branches are given off perpendicularly to the main fibres

(figures 59, 60, plate 35). These side branches are usually 15 to 30 μm long, and may bifurcate once or twice; they are often slightly blebbed, particularly at their ends (figures 61, 62, plate 35). Some portions of the main fibres are devoid of branches. For instance, type 4 fibres are smooth where they pass the base of the α lobe, although they bear branches above and below this region. All the fibres within the β lobe show this uneven distribution of branches. Furthermore, the fine branches differ in length and pattern of division themselves, according to the region. Thus regions within the main lobes differ in two respects as regards intrinsic fibres: firstly,

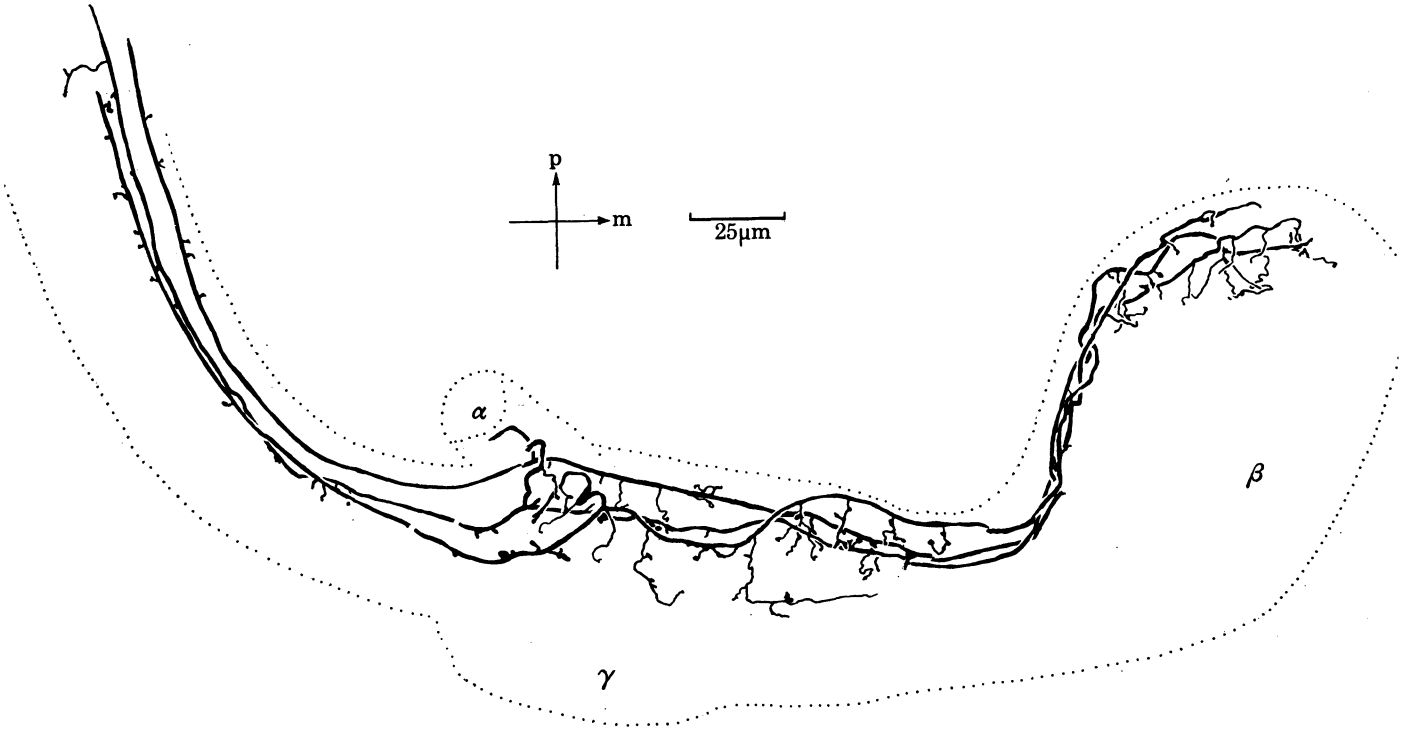


FIGURE 19. Three intrinsic fibres (variant 2) in the roots. The α lobe branches run perpendicular to the plane of section, and appear foreshortened. There are no γ lobe branches. Note the regional distribution of side branches within the β lobe. *Sphinx*, Colonnier.

different fibre types invest them; secondly, the fine branches of these fibres differ. The regions are so small that no attempt to classify them has been made.

The fibres of type 1 (figure 15) are narrower than the other types, and they branch only within the α lobe. Here their side branches are very fine and sparse. Types 2 and 3 (figures 19, 15) both divide at the base of the stalk. One 1 μm process invades the α lobe, to branch more densely than type 1; the other runs along the β lobe. In the region of the γ lobe, their side branches are up to 50 μm long; but no major process from this type of fibre invades the area. Elsewhere in the roots, their branches are usually 25 μm or so long. Types 4 (figure 15) and 5 (figure 6) invest only the β lobe. Both are 1 μm fibres, but their side branches differ: type 5 has short, stubby branches while type 4 has longer, subdivided ones. All bunched intrinsic fibres which have been traced to the roots have been of type 5. Types 6 (figure 15) and 7 run through the γ lobe before swinging back into the β lobe, where they run to the tip of the lobe. Type 8 fibres appear to be the only ones to have a major (1 μm) process investing each of the three lobes (figure 20). All the processes have side branches.

The classification of fibres is probably incomplete. It is further complicated by the possibility that some of the processes may not have been impregnated on some occasions. However, the basic pattern emerges clearly: intrinsic fibres differ sharply in their patterns of division in the roots.

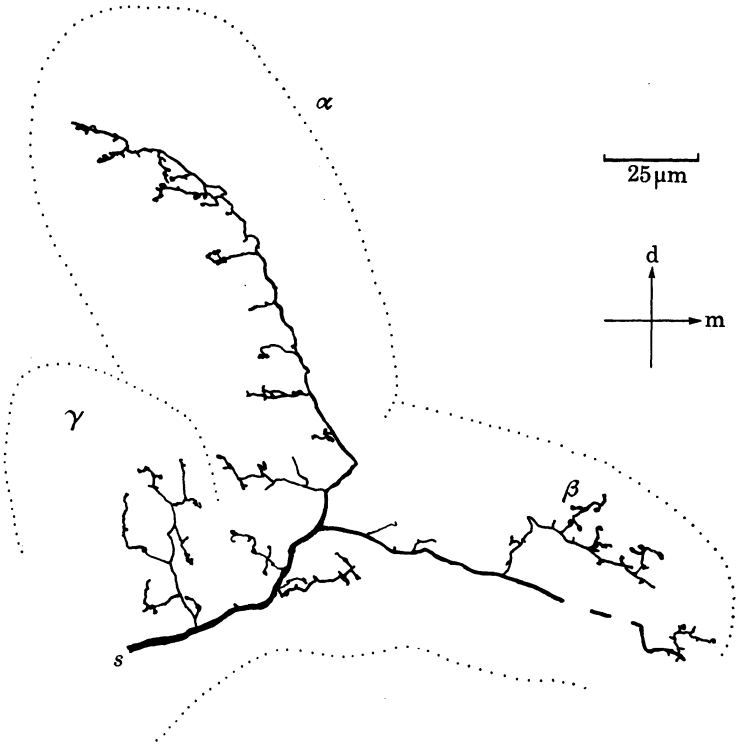


FIGURE 20. Intrinsic fibre (variant 8) in the roots. This fibre branches in the α, β and γ lobes. *Sphinx*, Colonnier.

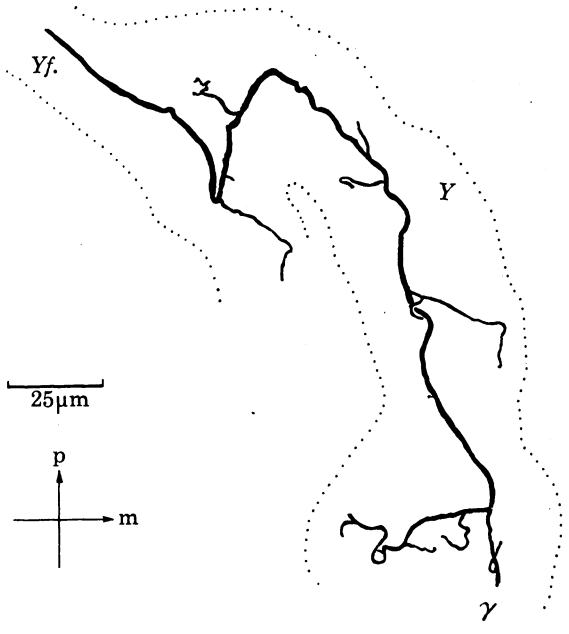


FIGURE 21. Bunched accessory fibre branching in the Y-lobe, and extending into the γ lobe. *Sphinx*, Colonnier. *Yf.*, Y-tract fibre.

The Y-lobe complex

This is composed of the processes of bunched accessory fibres and those of extrinsic fibres; no intrinsic fibres invade any part of it. From the Y-tract, bunched accessory fibres run into the dorsal part of the Y-lobe. Here they divide once or twice (figures 21; 63, plate 35). A loose network of fibres, criss-crossing each other with no apparent pattern, is formed. There are no terminal specializations, and no fine branches. The lateral, ventral limb of the Y is formed by branches arising from this tangle of fibres. These branches are finer ($\frac{1}{2}$ μm), and run downwards to the ventral margin of the roots. Here they form a tangle similar to that in the dorsal part of the lobe. Although this part of the lobe is entirely surrounded by intrinsic fibres, it has no connexion with them. The anterior portion of the 'Y' is simply an extension of the dorsal tangle of fibres. These continue into the γ lobe, where they mingle with the intrinsic fibres.

Extrinsic fibres

Because of the small size and contorted nature of the roots, it is difficult to trace fibres emerging from them for any distance. It has not, therefore, been possible to classify extrinsic fibres in terms of their origins. They have been grouped according to the position and type of their terminals. Many have the same diffuse branching pattern of terminal, and differ clearly only in their location. No extrinsic fibre branches in more than one lobe; indeed, most of them cover only a portion of a lobe. These probably correspond, in most cases, to the regions or sublobes previously mentioned (pp. 484, 502).

Extrinsic fibres are infrequently stained, but almost all the types listed below have been seen several times in good preparations.

Description of plate 35

Figure 59. A number of intrinsic fibres in the α lobe. The whole lobe is shown in frontal oblique section (dorsal at top). *Sphinx*, Colonnier, × 375.

Figure 60. A number of intrinsic fibres in the β lobe of *Triphaena*. The whole lobe is shown in frontal oblique section (anterior at top, mid-line at left edge of picture). The small size of the lobe (cf. figures 61, 62) is due to the small size of *Triphaena's* brain. The large fibres in the lower left of the picture are part of the central commissure below the central body. Rapid, × 375.

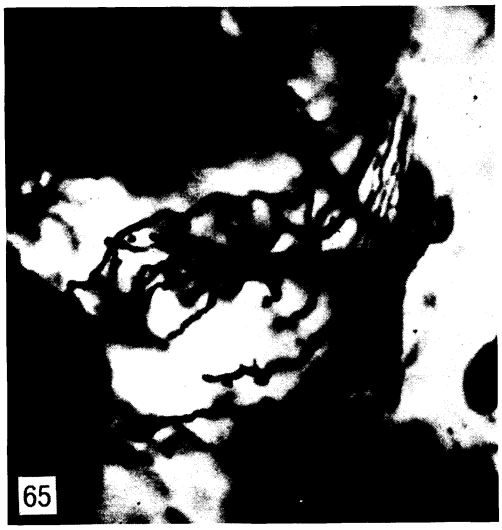
Figures 61, 62. Single intrinsic fibre in the β lobe of *Sphinx*, at two planes of focus. The outline of the lobe is seen clearly in figure 61. Oblique section, mid-line at left. Colonnier, × 375.

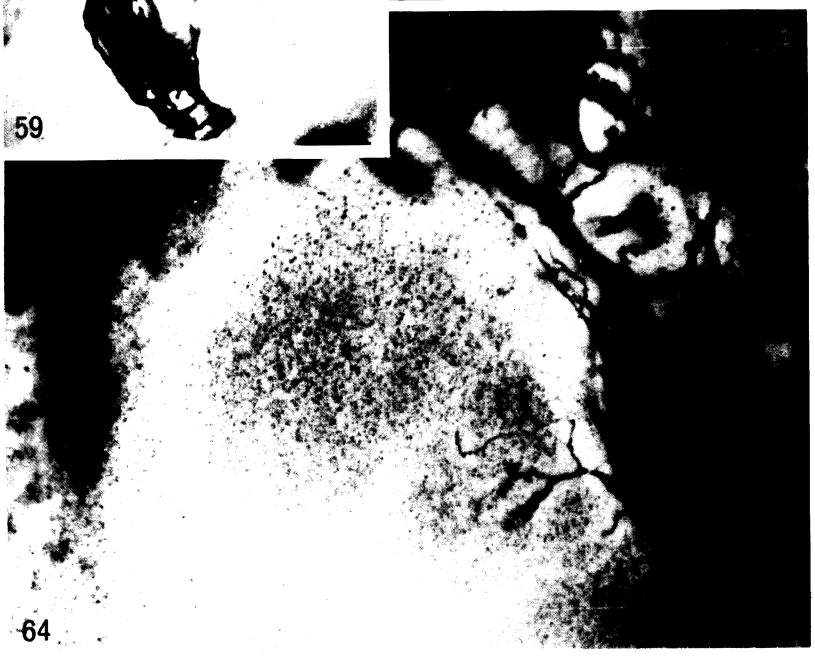
Figure 63. Bunched accessory cell fibres ramifying in the dorsal part of the Y-lobe (above) and running ventrally into the ventral posterior limb of the lobe (below). Frontal section: mid-line towards right. The outline of the bulbous base of the stalk can be seen at the left. *Sphinx*, Colonnier, × 600.

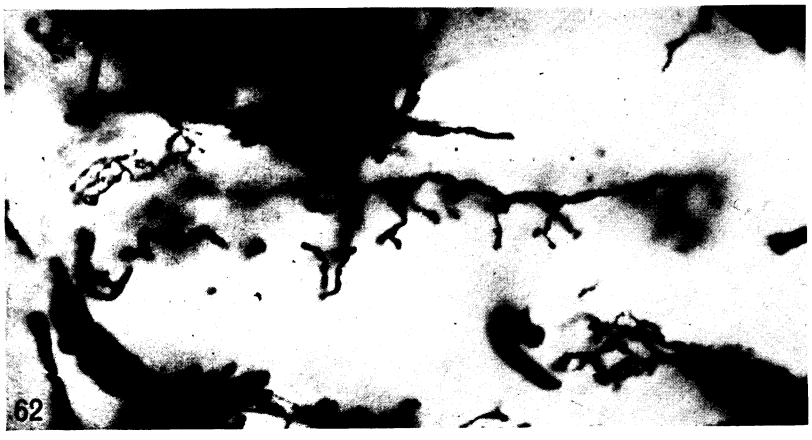
Figure 64. Extrinsic fibre entering the base of the stalk from the protocerebral lobes (heavily stained, at left). The outlines of the different lobes of the 'roots' can be seen. On the right are unimpregnated cell bodies lying between the 'roots' and the antennal lobe. Horizontal section (anterior top right, centre top left). *Sphinx*, Colonnier, × 600.

Figure 65. Three types of intrinsic fibre in the 'roots' of *Pieris*. The base of the stalk and part of the β lobe are shown in horizontal section (posterior at top, mid-line at left). The first type of fibre (see p. 507) has long side branches and lies in the left centre of the picture. The second, with short branches, is in the lower centre. Three fibres of each of these types can be distinguished. At the right (i.e. lateral edge of the base of the stalk) are several very fine fibres which have no side-branches, although some of them bifurcate. Colonnier, × 375.

Figure 66. Tracheae stained by the Colonnier method. *Pieris*, × 600.







For legend see facing page.

(A) *Endings in the α lobe*

(i) A 1 μm fibre, which has a few branches in the lateral dorsal protocerebrum, divides two or three times in the dorsal part of the α lobe. The branches are very fine, and spread over the whole of the lobe in a diffuse network (figure 22).

(ii) A fibre enters the basal part of the α lobe from the medial side. It immediately breaks up and divides repeatedly, giving rise to fine, close-packed branches. These terminals appear dense and bushy (figure 23). The field covered is restricted to about 16 μm^3.

(iii) Some endings, with connexions in the dorsal protocerebrum, are intermediate between these two types. They are very variable in appearance, and may represent a series of a single-fibre connexions.

(B) *Endings at the base of the stalk and the origin of the roots*

(i) A 1 to 2 μm fibre leaves from the very base of the α lobe. It can be traced a short distance medially and posteriorly. Its terminals are fine, and may appear blebbed (probably artefactual). They are sparse and diffuse, and cover the whole of the base of the α lobe.

(ii) A large ending covers the entire base of the stalk. It arises from a thick (3 to 5 μm) fibre which has been traced out of the roots, under the Y-lobe, and followed a little way upwards and medially from there. Reduced silver preparations indicate that there is probably only a single such fibre. The terminal is composed of fine, very closely packed branches (figure 24), which form a series of open bunches about 2 μm in diameter. This fibre must contact a very large number of processes in the area—possibly all the intrinsic fibres, before they divide in the roots.

(iii) A 1 μm fibre runs in a small tract between the base of the roots and an area of protocerebral neuropile medial to the anterior optic tract and the optic tubercle. (The tract could not be traced in Holmes preparations. The area is anterior and lateral to the protocerebral terminals of the lateral protocerebral tract.) At the base of the α lobe the fibre divides once or twice, giving rise to a few fine branches which cover a small field.

(iv) A very similar ending arises from a 1 μm fibre which has been traced towards the mid-line from the base of the stalk. It is possible that both this and the previous fibre arise from the same terminal (one of them, probably the former, may be a cell-body fibre) and that only one of the two has been stained in one section.

(v) A 2 μm fibre leaves the base of the roots medially, and branches a few times in the dorsal protocerebrum, above the roots. Within the roots it divides two or three times; the field covered by these branches is 30 μm × 20 μm × 20 μm. The terminal branches are more numerous than those of (iii) or (iv) above (figure 64, plate 35).

(C) *Endings in the dorsal portion of the Y-lobe*

(i) A 2 to 3 μm fibre, of the typical protocerebral type, has branches in the protocerebrum medial and posterior to the Y-lobe. One small branch arises from the Y-lobe, where it divides several times, forming a tree of fine branches which covers all the dorsal portion of the lobe (figure 25).

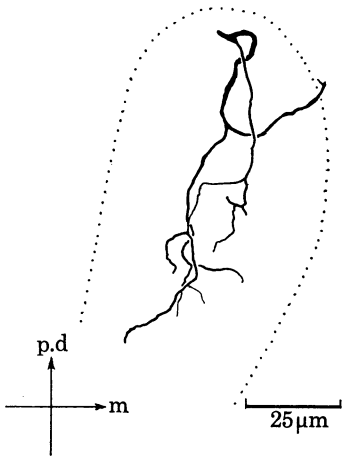


FIGURE 22

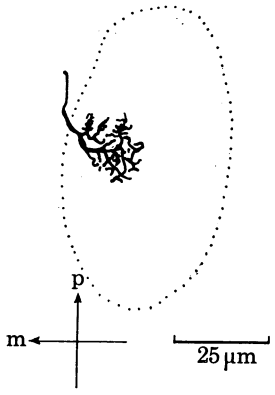


FIGURE 23

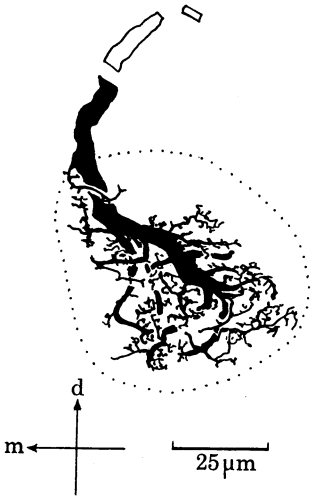


FIGURE 24

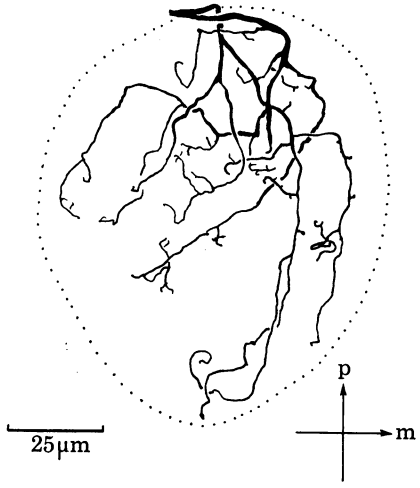


FIGURE 25

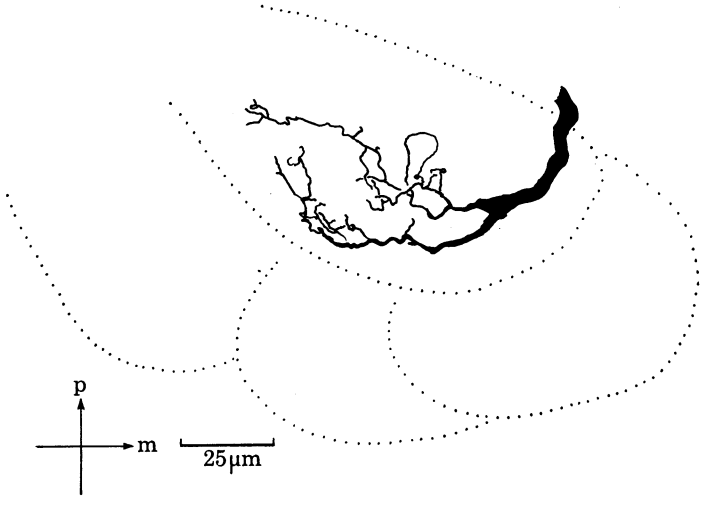


FIGURE 26

FIGURE 22. Extrinsic terminal Ai in the α lobe. *Sphinx*, Colonnier.

FIGURE 23. Extrinsic terminal Aii in the α lobe. *Sphinx*, Colonnier.

FIGURE 24. Extrinsic terminal Bii in the base of the stalk. The foreshortened continuation of the fibre in the next two sections is shown in outline. *Sphinx*, Colonnier.

FIGURE 25. Extrinsic terminal Ci ramifying through the whole of the Y-lobe. *Sphinx*, Colonnier.

FIGURE 26. Extrinsic terminal Di in the Y-lobe/γ lobe. *Sphinx*, Colonnier.

(D) *Endings in the base of the Y-lobe/γ lobe*

(i) A diffuse network of fine branches in this area is formed by the terminal of a 2 μm fibre. The fibre (figure 26) runs over the top part of the β lobe to the mid-line area.

(E) *Endings in the β lobe*

(i) A diffusely branching terminal is found in the dorsal β lobe. The endings (figure 27) are fine and unorientated, covering 35 μm^3. They arise from a 1 to 2 μm fibre which emerges from the top of the lobe, runs downwards and medially across it, and is lost in the lateral lobe.

(ii) In the protocerebral neuropile above the stalk a 1 μm fibre runs antero-laterally. Above the base of the roots, one or two branches are given off. These dip down into the β lobe. Within the lobe, they bifurcate again. These branches run parallel to the intrinsic fibres (figure 28). Each bears short (8 μm), fine perpendicular side branches. The terminals are restricted to the anterior dorsal part of the lobe. Orientated fibres of this type are apparently very common in Hymenoptera (Vowles 1955), but they are very infrequently seen in Lepidoptera.

(iii) In the medial half of the β lobe there are clumpy, bushy endings (figure 29), which are formed by the repeated branching of a large fibre over a limited area. The terminal field tends to be ovoid, about 50 μm × 30 μm^2, with the long axis lying at right angles to the intrinsic fibres. More than one clump of endings may arise from a single fibre. Some of these terminals come from a cell-body fibre which leaves the lobe at its posterior edge and runs upwards and forwards, round the β lobe, into the cell-body layer anterior and dorsal to the lobe. Others give rise to a 2 to 4 μm fibre which emerges from the posterior mid-line edge of the β lobe. It runs posteriorly before turning sharply downwards in front of the central body. These two fibres are probably branches of the same cell.

(iv) A single isolated specimen of a fibre orientated at right angles to the intrinsic fibres has been found. It is shown in figure 30.

Thus, most extrinsic fibres do not have terminal fields which are orientated with respect to the intrinsic fibres, but instead are of the diffusely branching type. Their field sizes imply that they contact relatively few intrinsic fibres. In contrast, other extrinsic cells, such as B(ii) and E(iii), possibly contact every fibre in the area in which they divide.

Other species. The other moths showed essentially similar structures in the roots, with no obvious differences from *Sphinx*.

In *Pieris*, most of the intrinsic fibres in the roots were of the same general appearance as those in *Sphinx*. Three types could be distinguished (figure 65, plate 35): the first was very like the fibres in *Sphinx*, with side branches up to 20 μm long. Secondly, there were similar fibres with short (8 μm) side branches. These are like the bunched intrinsic fibres in *Sphinx*; but they could not be traced back to the calyx. Thirdly, on the lateral edge of the stalk is a group of fibres which are more slender than most stalk fibres, and are smooth throughout their passage down the stalk. Their terminals in the roots are very reminiscent of those of the bunched accessory fibres in the Y-lobe in *Sphinx*. It seems possible that the Y-tract and lobe are incorporated into the stalk and root system in *Pieris*.

Very few extrinsic fibres were seen in *Pieris*; they all had diffuse, branching terminals.

Glia. Glia were not intensively studied, but it was noted that all the glia stained by the

Golgi rapid method were of much the same appearance. The small cell body (usually not more than 10 μm long) is often ovoid or indented, and lies outside the area in which its processes divide—for instance, in the cell-body layer; around the central body, corpora pedunculata, or optic tubercle; or beside one of the large tracts leading through the protocerebral neuropile. A process arises from the cell body and enters the relevant area of neuropile; infrequently, there are two or three such processes. Within the neuropile, the processes divide to give a very dense arborescence which may invade as much as 120 μm^3. Figure 31 shows a typical glial cell. The drawing is slightly diagrammatic, for it is impossible to draw the small dense processes accurately.

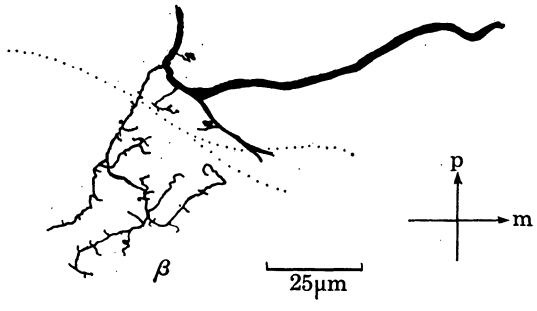


FIGURE 27

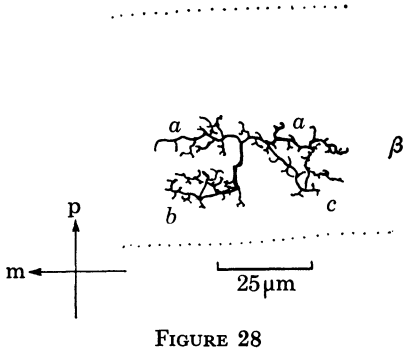


FIGURE 28

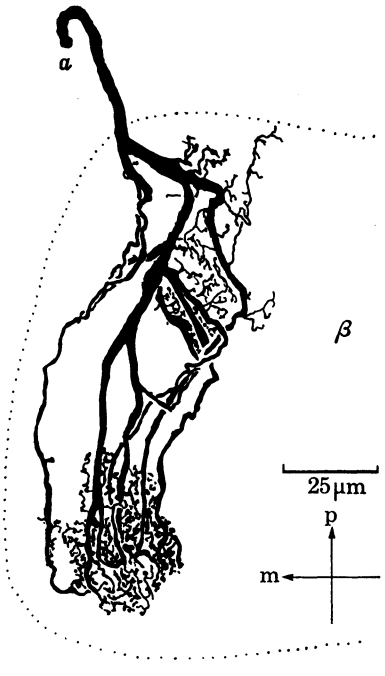


FIGURE 29

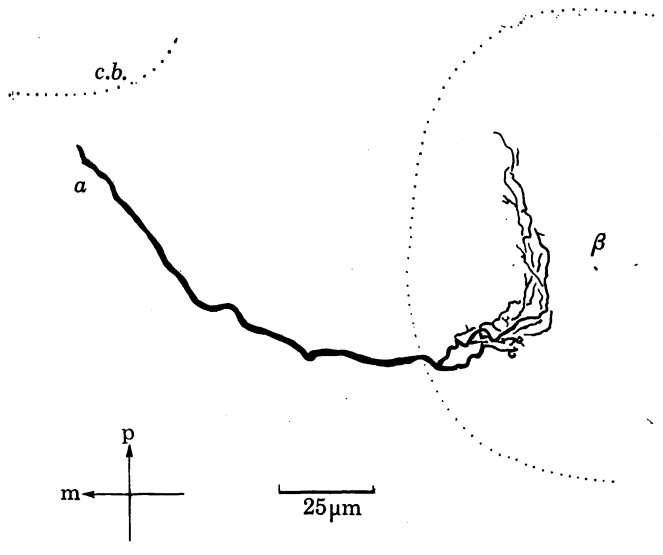


FIGURE 30

FIGURE 27. Extrinsic terminal Ei in the proximal dorsal part of the β lobe. *Sphinx*, Colonnier.

FIGURE 28. A portion of extrinsic terminal Eii in the central part of the β lobe. The three processes, *a*, *b* and *c*, lie at different depths in the lobe, but the branches linking them appear very foreshortened in the drawing. In the plane perpendicular to that of the section the terminal has a field 70 μm in depth. *Sphinx*, Colonnier.

FIGURE 29. Extrinsic terminal Eiii in the distal part of the β lobe. Fibre *a* runs ventrally in front of the central body. *Sphinx*, Colonnier.

FIGURE 30. Extrinsic terminal Eiv in the distal part of the β lobe. Fibre *a* runs dorsally in front of the central body (*c.b.*). *Sphinx*, Colonnier.

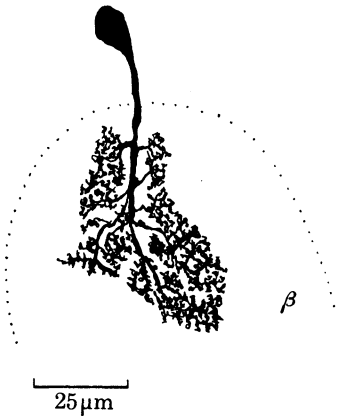


FIGURE 31. Glial cell in the β lobe. *Sphinx*, Rapid.

DISCUSSION

The calyx

It has been assumed that extrinsic endings in the calyx are presynaptic to intrinsic terminals. There is physiological evidence that the antennal and optic connexions represent inputs (Maynard 1967; Vowles 1964); but there is no clue as to whether the protocerebral and suboesophageal contributions represent inputs or outputs, nor is the nature of the information they carry known. The diffuse form of the extrinsic terminals, and the large field sizes of most of the intrinsic cells, indicate that there is little or no spatial separation of inputs in the moth.

The antenno-glomerular tract provides a relatively massive input of fibres; most of these are second-order, though some arise directly from the antennal tract. Whether these fibres originate from chemoreceptors or from mechanoreceptors is unknown. The magnitude of this input readily explains the correlation between the size of the antennal lobe and that of the calyx (Hanstrom 1928).

The forms of the various types of ending in the calyx throw some light on the structure of the 'glomeruli' of the calycal neuropile. In light microscope sections (except, of course, Golgi preparations), these glomeruli consist of knots or whorls of fibres, and are about 5 to 10 μm in diameter. Electron microscope studies (Trujillo-Cenoz & Melamed 1962) have revealed that each is composed of a single large presynaptic terminal, surrounded by many smaller fibres. The size and shape of the presynaptic ending correspond exactly to those of the antennal knobs described here. Moreover, the size and shape of the bunched intrinsic and bunched accessory endings suggest that they fit round the antennal terminals (figures 47 to 50, plate 33). Glomeruli which have been studied in various locations in vertebrate nervous systems have proved to be composed of more than two types of fibre, interacting in a complex manner (Szentagothai 1956). This may be the case here. It is possible that the calycal glomeruli are not all composed of the same elements, but that different types exist. There are evidently other modes of synaptic interaction in the calyx, but they have not yet been described in electron microscope studies.

The visual input is probably less than a quarter as large as the antennal input, and is carried by interneurons which may be at least fifth order in the visual pathway. Visual information reaching the calyx has thus passed through many more synapses than antennal

information. The synaptic connexions of these fibres within the calyx cannot be ascertained in Lepidoptera.

In Hymenoptera, there is some morphological evidence suggesting that visual and antennal inputs are processed separately in the calyx. Howse & Williams (1969) have pointed out that the composition of the calycal neuropile is not the same in all insects. In some (e.g. termites) it is strikingly glomerular; in others it is not. In most Hymenoptera only the basal part of the calyx is glomerular: the rims of the cups are non-glomerular. On the basis of comparative studies, Howse & Williams suggest that glomerular neuropile is mainly concerned with the integration of olfactory stimuli, while non-glomerular neuropile has other functions—for instance, integration of visual information. The hypothesis presented above supports the view that glomerular neuropile integrates antennal inputs.

Goll (1967) has shown that there are several intrinsic cell types in the calyx of *Formica*. All have fields which are very small relative to the size of the calyx. The different types are restricted to different regions of the calyx (figure 33). Their fibres in the stalk and roots are also located according to type (figure 34). If the suggestions of Howse & Williams are correct, this indicates that certain types of intrinsic cell only have a visual input, while others only have an antennal input. Furthermore, these different cell types are spatially separated in the stalk and roots. However, these conclusions are only tentative at present, for there is no detailed information concerning the extrinsic terminal field distributions in the calyx.

Glomeruli are visible, though not very conspicuous, throughout the calyx in Lepidoptera. Apparently, both types of neuropile are mixed together in this calyx (this is probably true of most other insect groups).

The large fields of most calycal elements make it impossible to tell whether different subgroups of intrinsic cells make contact with more than one type of extrinsic cell.

The small region at the base of the calyx, where the stalk arises, appears to be functionally distinct from the remainder of the calyx, for it is invested by two types of element in addition to those found in other parts of the calyx. These are, first, the small-field branching extrinsic fibres, and secondly the long sidebranches arising from the stalk fibres of certain intrinsic cells. This region does not correspond to either of Howse & Williams's types of calycal neuropile: it is a third type.

FIGURE 32. Summary diagram of cell types and their terminals in the mushroom bodies of *Sphinx* and other Lepidoptera. Left: intrinsic and accessory cells. Right: some extrinsic cells.

Lobes and tracts:

α	α lobe	*cup*	calyx-cup
a.g.t.	antenno-glomerular tract	γ	γ lobe
a.l.	antennal lobe	*l.p.t.*	lateral protocerebral tract
β	β lobe	*s.*	stalk
b.s.	base of stalk	*Yl.*	Y-lobe
c.	calyx	*Yt.*	Y-tract

Cells and terminals:

b.a.	bunched accessory cell	*s.i.*	spiny intrinsic cell
b.i.	bunched intrinsic cell		

Extrinsic terminals:

bl.	blebbed extrinsic fibre	*pa.*	parallel extrinsic terminal
br.	branching extrinsic fibre	*p.*	perpendicular extrinsic terminal
k.	knobbed extrinsic fibre		

Ai, Aii, Bii, Biii, Ci, Eiii = extrinsic terminals in the roots, as detailed in text.

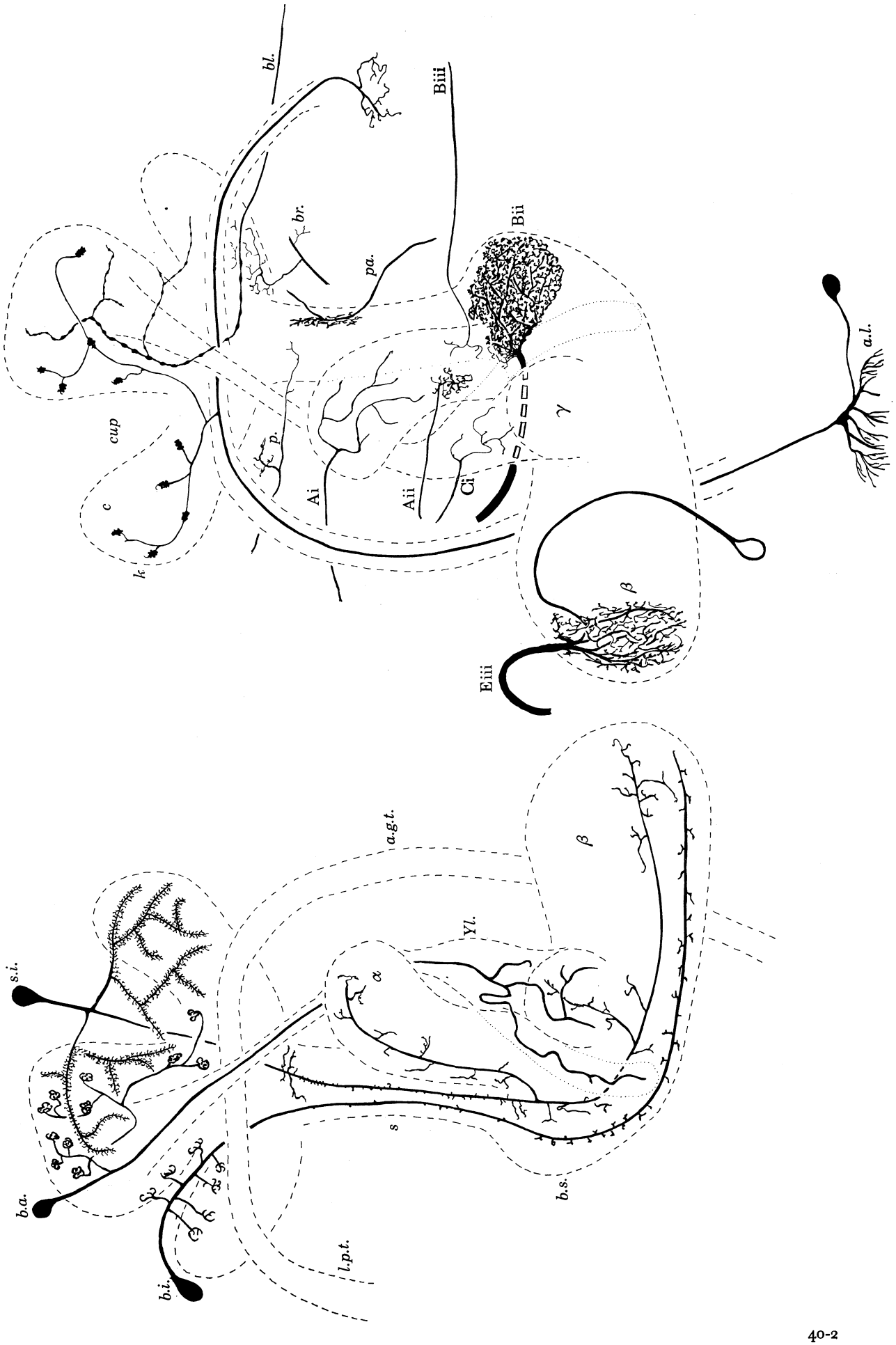


FIGURE 32. For legend see facing page.

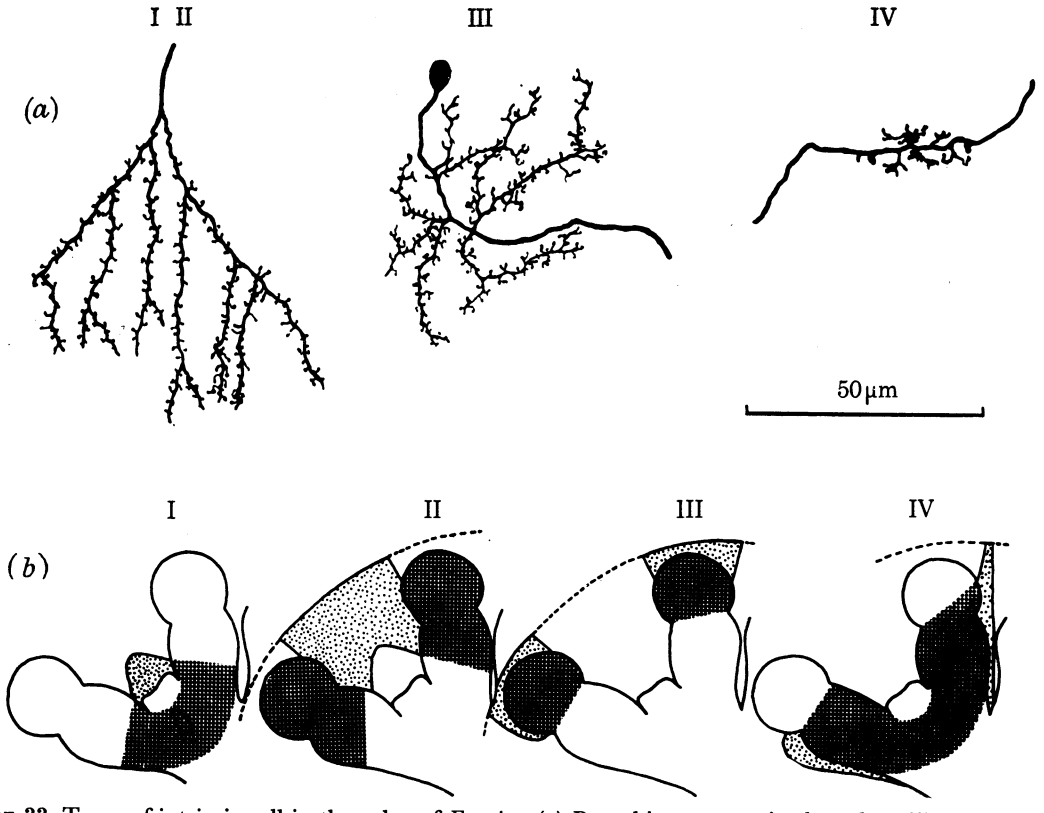


FIGURE 33. Types of intrinsic cell in the calyx of *Formica*. (*a*) Branching pattern in the calyx. (*b*) Distribution of cell bodies (stippled) and branching processes (cross-hatched). Figures 33 and 34 reproduced from Goll (1967).

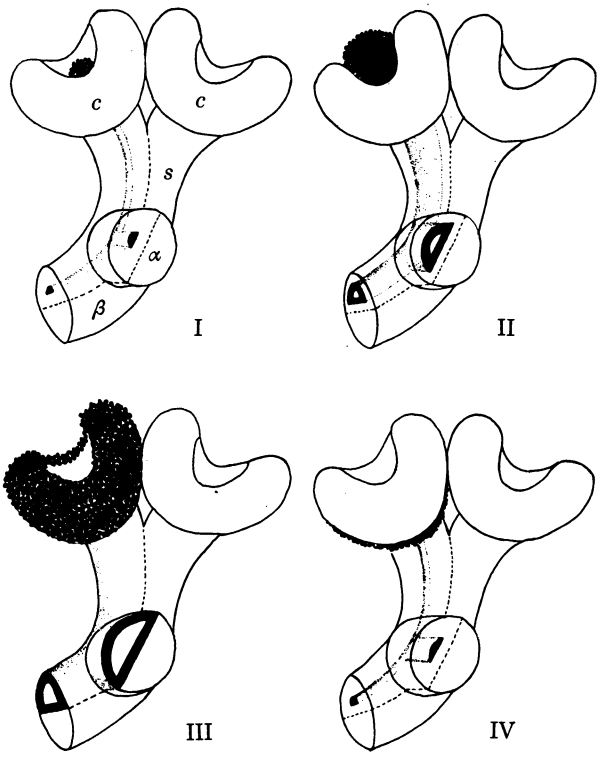


FIGURE 34. Distribution of intrinsic cell bodies and their processes in the stalk, α lobe and β lobe of *Formica*.

The stalk

It has been suggested that, in the stalk, intrinsic fibres run together in groups or bundles. The stalk fibres bear short side branches, so disposed that synaptic interactions may occur between fibres of the same bundle. It is possible that contacts between members of different bundles may also occur. Electron microscope evidence supports the hypothesis that fibres are assorted into bundles: Maynard (1967), and Mancini & Frontali (1967) noted that the stalk fibres are divided into discrete groups by glial envelopes. Possible sites of synaptic contact in the stalk have not been described however.†

Postsynaptic interactions between intrinsic cells were also suggested by the physiological studies of Maynard (1967). He stimulated the antennal nerve of the cockroach, and recorded a single large 'spike' in the stalk of the mushroom body. He concluded that many small intrinsic fibres had been activated in synchrony.

In Hymenoptera, the parallel course of the stalk fibres, and the spatial localization of different fibre types, suggest that bundles, if present, would be composed of fibres which had similar inputs in the calyx. The situation in Lepidoptera is uncertain.

The paucity of extrinsic endings in the stalk emphasizes that though this area is an important site of synaptic interaction between intrinsic cells, it is of little significance in the interplay of the mushroom bodies with other areas.

The 'roots'

The lobes and their sublobes receive combinations of branches from different varieties of intrinsic cell, and in this sense are functionally diverse. Most extrinsic terminals in the roots ramify through only one sublobe, within which their branches cover a large area. It is reasonable to assume that they make contact with more than one type of intrinsic fibre. Thus, even if the various inputs to the calyx are processed separately by the intrinsic cells, they are probably integrated in the roots.

The connexions of the roots are obscure. The Golgi studies of Goll (1967) and myself do not support Vowles's (1955, 1964) claim that the α lobe has connexions with sensory centres, and the β lobe with motor centres. Huber (1962, 1965, 1967), investigating the control of singing and locomotor behaviour in Orthoptera, found that the individual motor acts which make up these behaviour patterns were controlled by thoracic ganglia, while the central body coordinated them into the appropriate pattern. The selection of this pattern as opposed to any other, appeared to be the function of the mushroom body. Fibre pathways from the mushroom bodies to the central body would therefore be expected. Since they could find no such connexions, Howse & Williams (1969) rejected Huber's hypothesis. The results presented here tend to support this conclusion, but it is possible that, although direct connexions do not exist, the two regions are connected by way of a relay in the protocerebral lobes or the lateral lobes. Further theorizing as to the function of the corpora pedunculata is precluded by the lack of physiological and comparative anatomical data.

The reason for the separation of the accessory terminals in the Y-lobe is far from clear. This

† (*Note added in proof*, 6 *August* 1970.) Schurmann (*Z. Zellforsch.* **103**, 365–381, 1970) has recently described synaptic structures in the stalk of the cricket mushroom body. It is not possible to discuss this interesting paper fully here: suffice it to say that Schurmann has found synapses between intrinsic fibres in the stalk, and that in many cases synaptic vesicles were found on both sides of the synaptic membrane. Golgi preparations of the cricket reveal that the stalk fibres bear blebs and spines similar to the short spines described here.

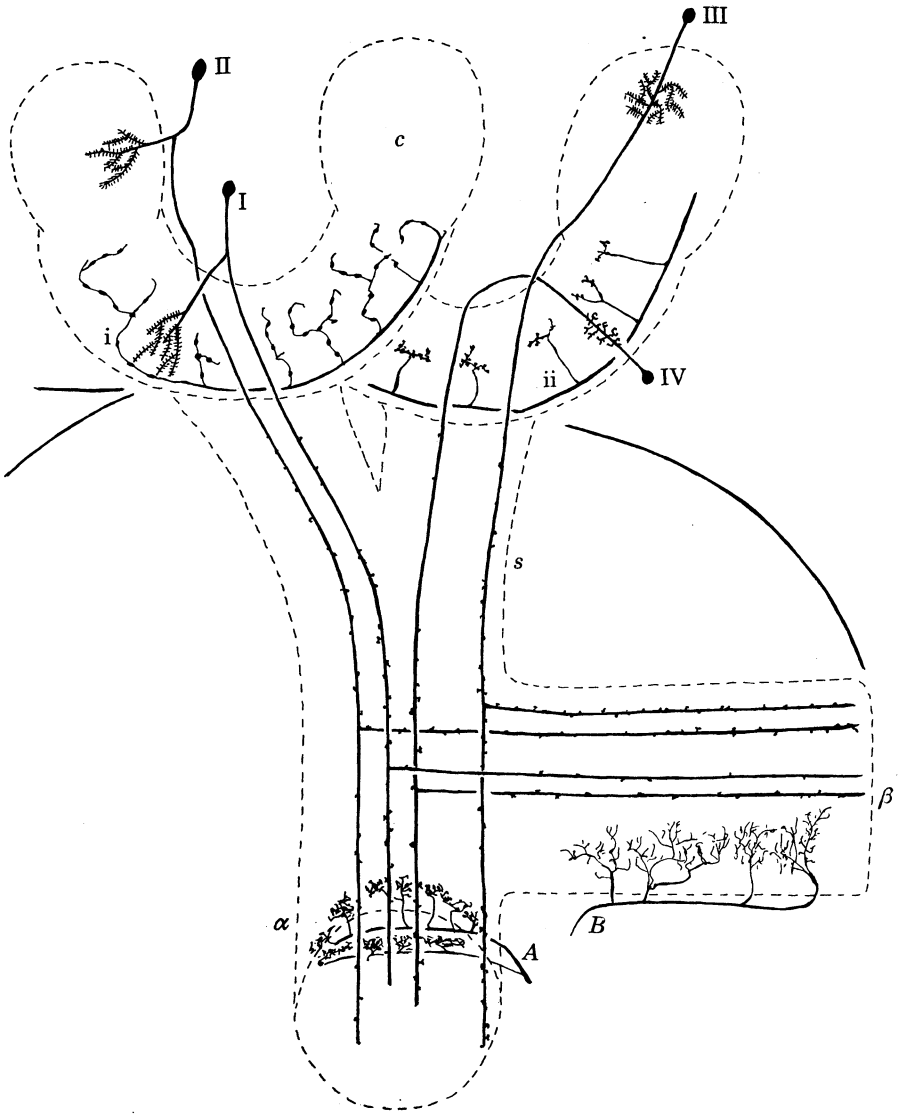


FIGURE 35. Summary diagram of some cell types and their terminals in the mushroom bodies of Hymenoptera. After Kenyon (1896) and Goll (1967). α, α lobe; β, β lobe; *c.*, calyx; *s.*, stalk. I, II, III, IV, types of intrinsic cell as detailed by Goll (1967). i, ii, two extrinsic fibres in the calyx. *A*, extrinsic fibre in α lobe, with field perpendicular to the intrinsic fibres. *B*, extrinsic fibre in β lobe, with field parallel to the intrinsic fibres.

TABLE 3. A SUMMARY OF THE DIFFERENCES BETWEEN THE CORPORA PEDUNCULATA OF LEPIDOPTERA AND THOSE OF HYMENOPTERA

	Lepidoptera	Hymenoptera
calyx	spiny intrinsic cells with large, overlapping fields	similar cells with small, discrete fields
	no evidence of spatial organization	spatial organization strikingly evident
stalk	fibres not parallel	fibres strictly parallel
	branches up to 30 μm long	branches very short—spines or blebs
roots	subdivision of main lobes	lobes appear homogeneous
	extrinsic fields localized but unspecialized (not orientated)	extrinsic fields very large, but specialized
Y-tract and Y-lobe	present in moths	not present; no reported evidence of homologue
	? homologue in butterflies	

lobe system, and its associated tract, may represent a Lepidopteran peculiarity. Only comparative studies can elucidate this problem. It is not known whether the accessory fibres interact with the intrinsic fibres in the Y-lobe, nor whether extrinsic fibres in this area synapse with both types of fibre. The possibility that the accessory fibres form a feedback link carrying impulses from the roots to the calyx has been considered. However, the position of the cell body and the form of the terminals of these fibres indicate that the direction of passage of impulses in the Y-tract is probably the same as in the stalk.

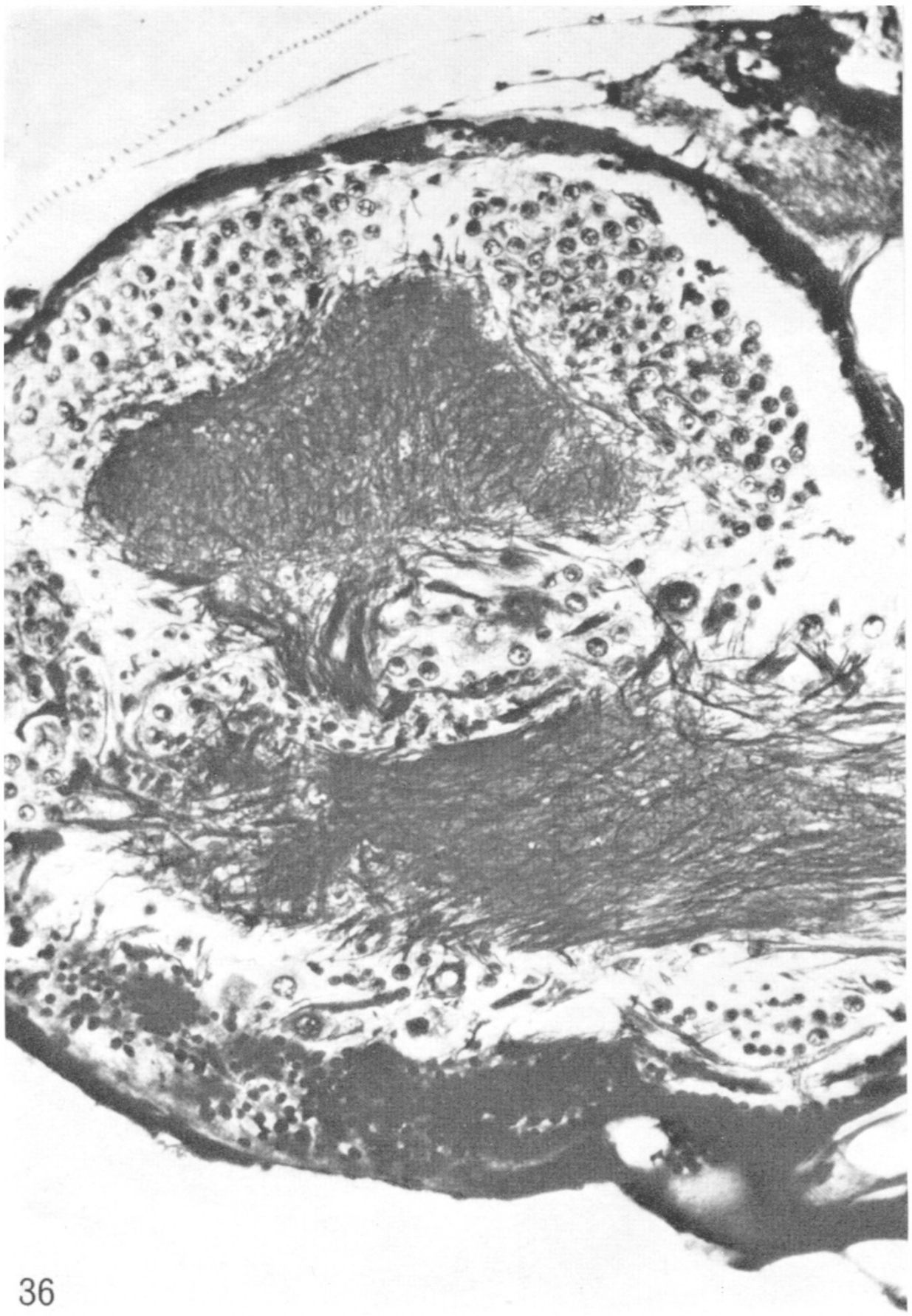
There are several striking differences between the organization of the corpora pedunculata of Lepidoptera and that in Hymenoptera. These differences are summarized in table 3 and in figures 32 and 35. While the functional implications of these differences are not entirely clear, it is apparent that the precise, well-defined patterning of the neurons—which makes the Hymenopteran corpora pedunculata so remarkable in Golgi preparations—is lacking in Lepidoptera.

I am deeply grateful to Dr Thomas Collett for his unfailing advice and constant encouragement. I would also like to thank Dr D. Blest and Dr N. Strausfield for the loan of some material, and for criticizing the manuscript. Finally, I thank Professor J. Z. Young, F.R.S., for his help in reading and commenting on the manuscript. The work was supported by a research grant to Dr Thomas Collett from the Science Research Council.

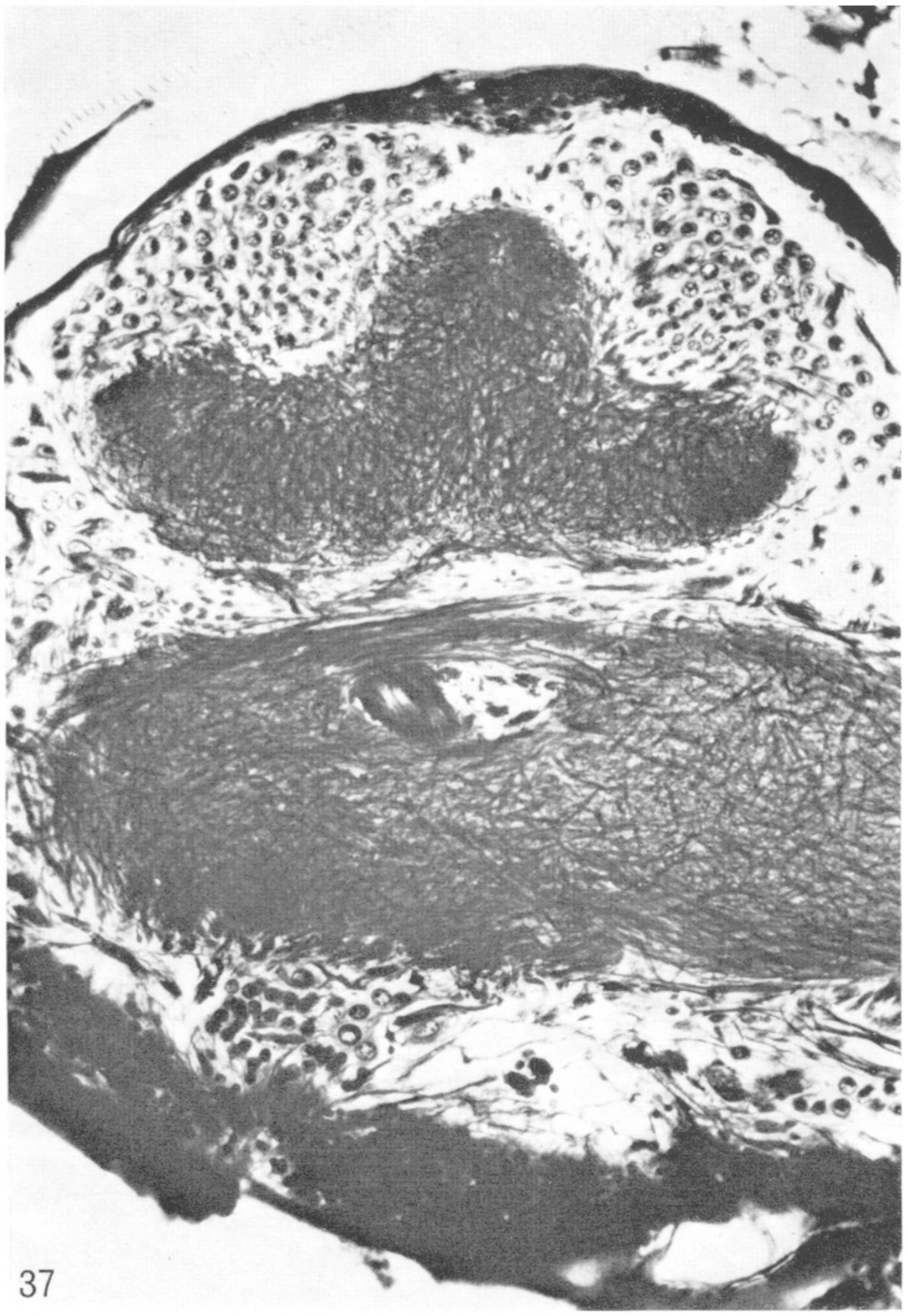
References

Blest, A. D. 1961 *Q. Jl. microsc. Sci.* **102**, 413–417.
Boycott, B. B. & Dowling, J. E. 1969 *Phil. Trans. Roy. Soc. Lond.* B **255**, 109–184.
Bretschneider, F. 1921 *Jena. Z. Naturw.* **57**, 423.
Bretschneider, F. 1924 *Jena. Z. Naturw.* **60**, 563–578.
Cajal, S. Ramon y 1911 *Histologie du Systeme Nerveux de l'Homme et des Vertebres.* Paris: Maloine.
Cajal, S. Ramon y & Sanchez, D. 1915 *Trab. Lab. Invest. biol. Univ. Madr.* **13**, 1–164.
Colonnier, M. 1964 *J. Anat.* **98**, 327–344.
Dujardin, F. 1850 *Ann. Sci. Nat. Zool.* (3), **14**,
Eccles, J. C., Ito, M. & Szentagothai, J. 1964 *The cerebellum as a neuronal machine.* Berlin: Springer-Verlag.
Ehnbom, K. 1948 *Opusc. ent.* Suppl. VIII.
Flogel, J. H. L. 1878 *Z. wiss. Zool.* **30**, (Suppl.), 556–592.
Goll, W. 1967 *Z. Morph. Ökol. Tiere* **59**, 143–210.
Hanstrom, B. 1928 *Vergleichende Anatomie der Nervensystems der wirbellosen Tiere unter Berucksichtigung seiner Funktion.* Berlin: Springer.
Holmes, W. 1947 In *Recent advances in clinical pathology* (ed. S. C. Dyke), p. 402. London: Churchill.
Horridge, G. A. 1965 In *Structure and Function in the nervous system of invertebrates* (by T. H. Bullock and G. A. Horridge). San Francisco: W. H. Freeman and Co.
Howse, P. E. & Williams, J. L. D. 1969 *Proc. VI Congr. IUSSI, Bern*, pp. 59–64.
Huber, F. 1962 In *Acoustic behaviour of animals* (ed. R. G. Busnel), pp. 440–488. Amsterdam: Elsevier.
Huber, F. 1965 In *The physiology of the insect nervous system* (ed. J. E. Treherne and J. W. L. Beament), pp. 233–246. London: Academic Press.
Huber, F. 1967 In *Invertebrate nervous systems* (ed. C. A. G. Wiersma). University of Chicago Press.
Kenyon, F. C. 1896 *J. comp. Neurol.* **6**, 133–210.
Mancini, G. & Frontali, N. 1967 *Z. Zellforsch.* **83**, 334–343.
Maynard, D. M. 1962 *Am. Zool.* **2**, 79–96.
Maynard, D. M. 1967 In *Invertebrate nervous systems* (ed. C. A. G. Wiersma), pp. 231–258. University of Chicago Press.
Melamed, J. & Trujillo-Cenoz, O. 1967 *J. ultrastruct. Res.* **21**, 313–334.
Pflugfelder, O. 1936 *Zoologica* **83**, 1–102.
Rowell, C. H. F. 1964 *J. exp. Biol.* **41**, 559–572.
Sanchez, D. 1933 *Trab. Lab. Invest. biol. Univ. Madr.* **28**, 149–185.
Strausfeld, N. J. 1970 *Phil. Trans. Roy. Soc. Lond.* B **258**, 135.
Strausfeld, N. J. & Blest, A. D. 1970 *Phil. Trans. Roy. Soc. Lond.* B **258**, 81.

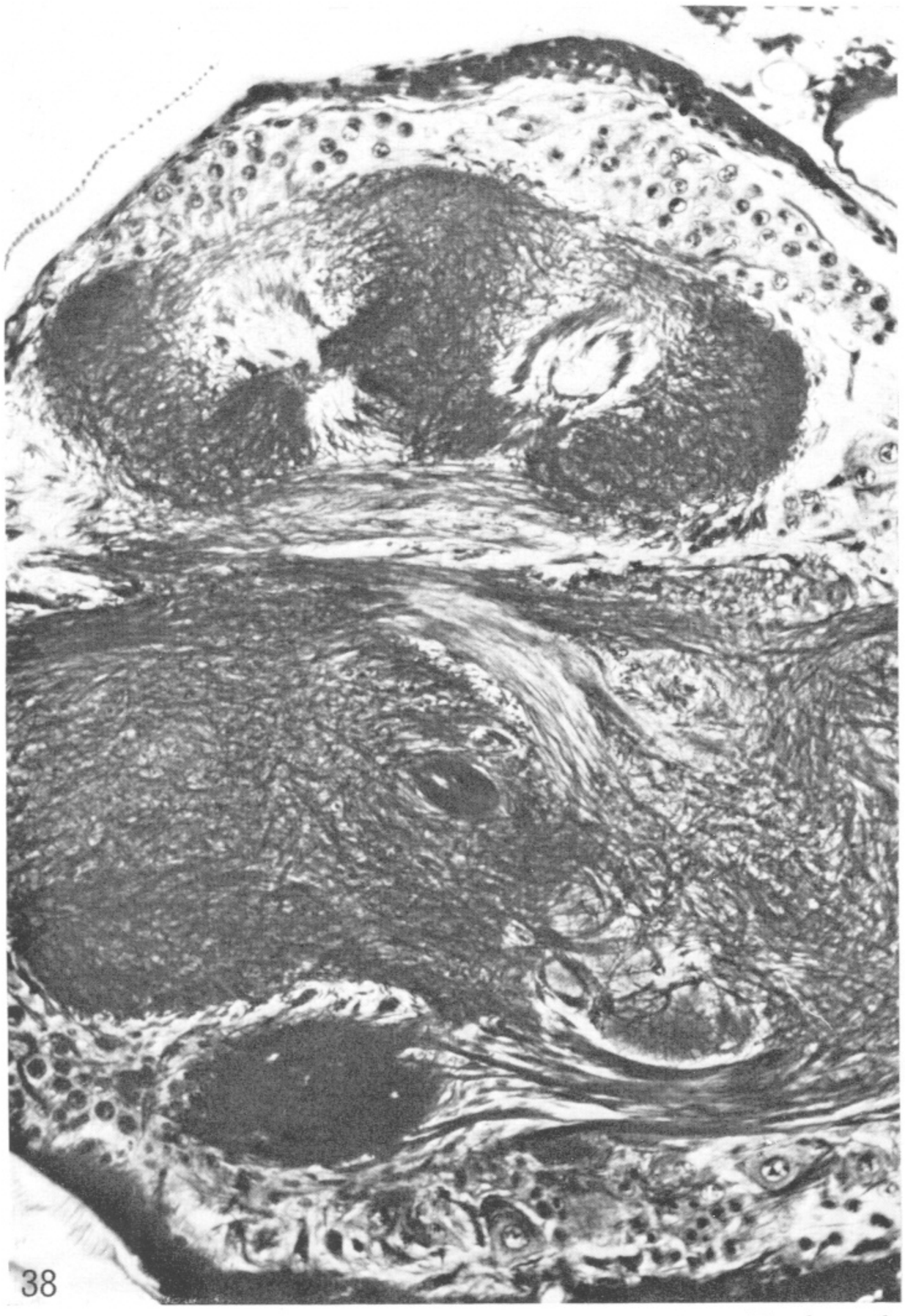
Szentagothai, J. 1956 In *Aus der Werkstatt der Anatomen* (ed. W. Bargmann), pp. 147–167. Stuttgart: Georg Thieme.
Trujillo-Cenoz, O. & Melamed, J. 1962 *J. Ultrastruct. Res.* **7**, 389–398.
Trujillo-Cenoz, O. & Melamed, J. 1963 *Z. Zellforsch.* **59**, 71–77.
Voskresenskaya, A. K. 1957 *Dokl. Akad. Nauk SSSR* **112**, 166–169.
Vowles, D. M. 1954 *Br. J. Anim. Behav.* **1**, 2, 116.
Vowles, D. M. 1955 *Q. Jl microsc. Sci.* **96**, 239–255.
Vowles, D. M. 1964 In *Neural theory and modeling* (ed. R. F. Reiss), pp. 377–399. California: Stanford University Press.
Zawarzin, A. 1913 *Z. wiss. Zool.* **108**, 175–257.



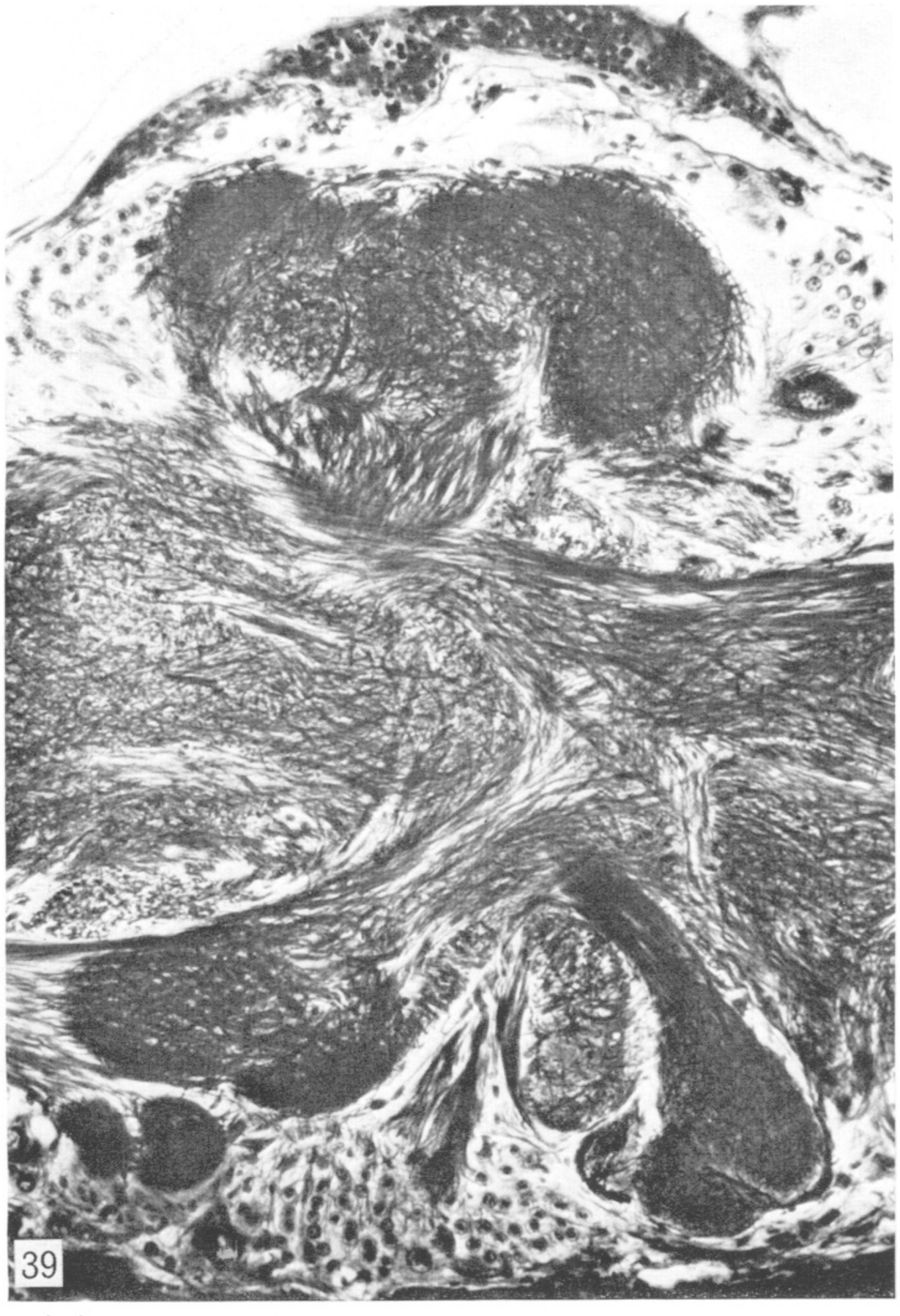
36



37

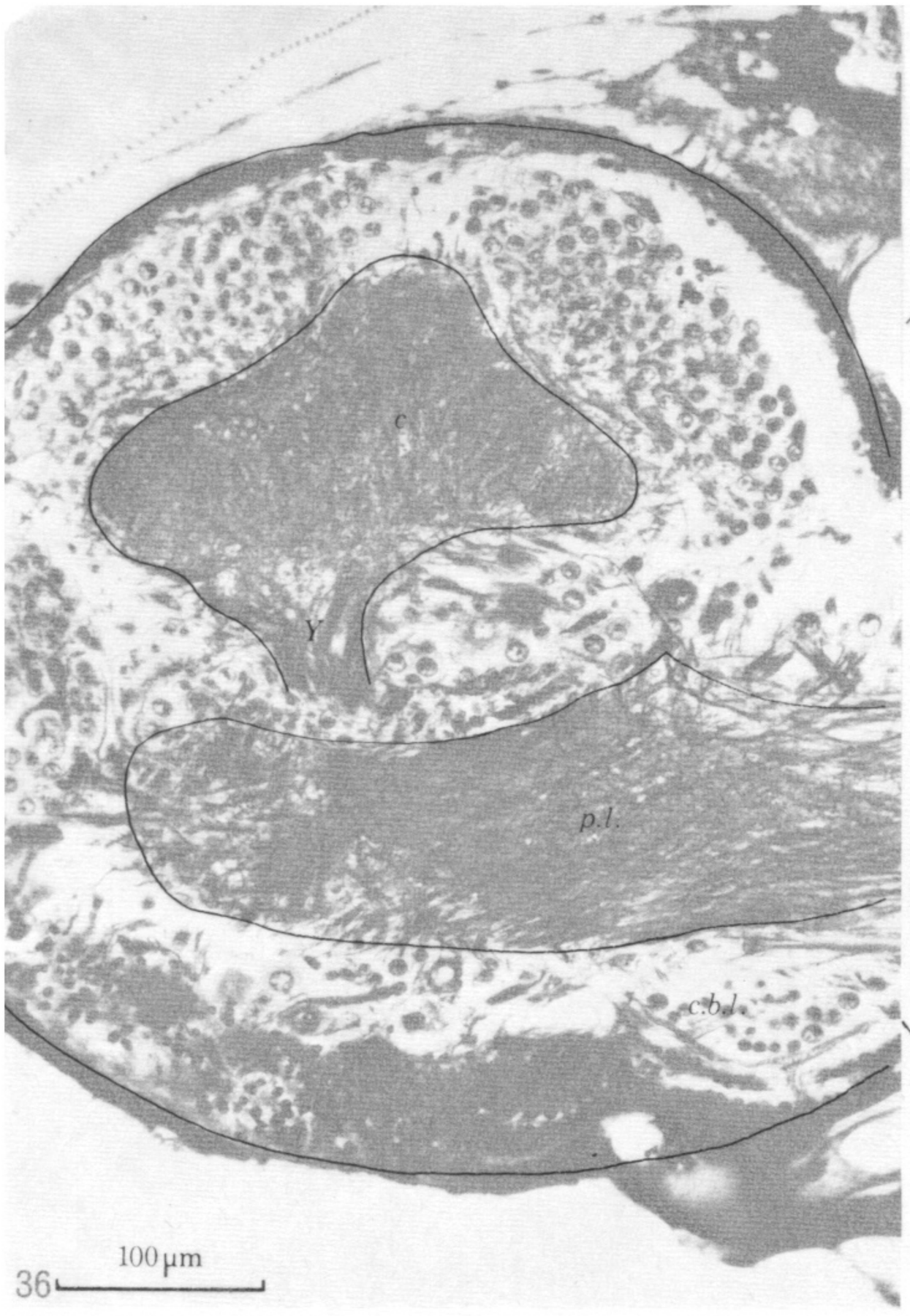


38

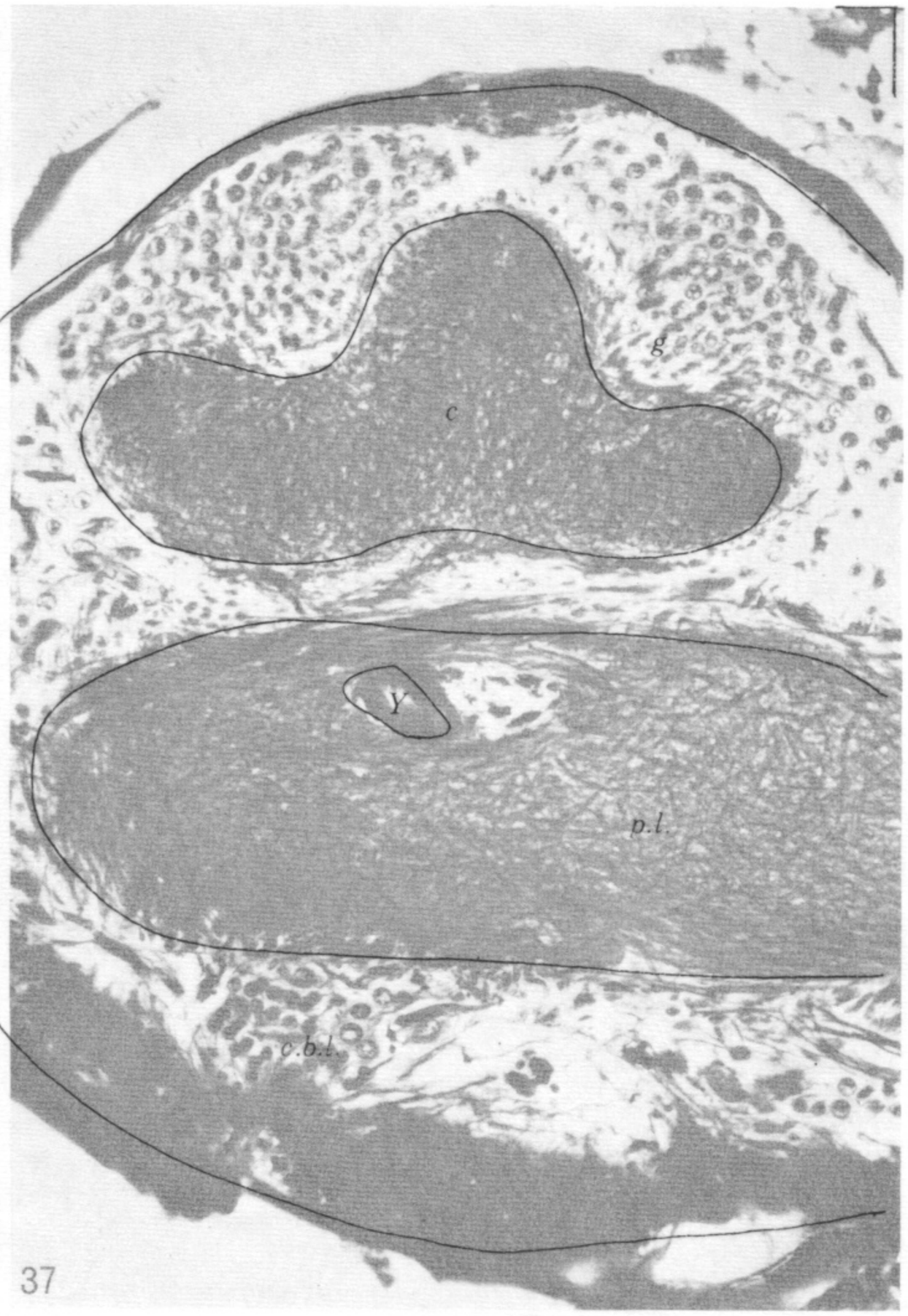


39

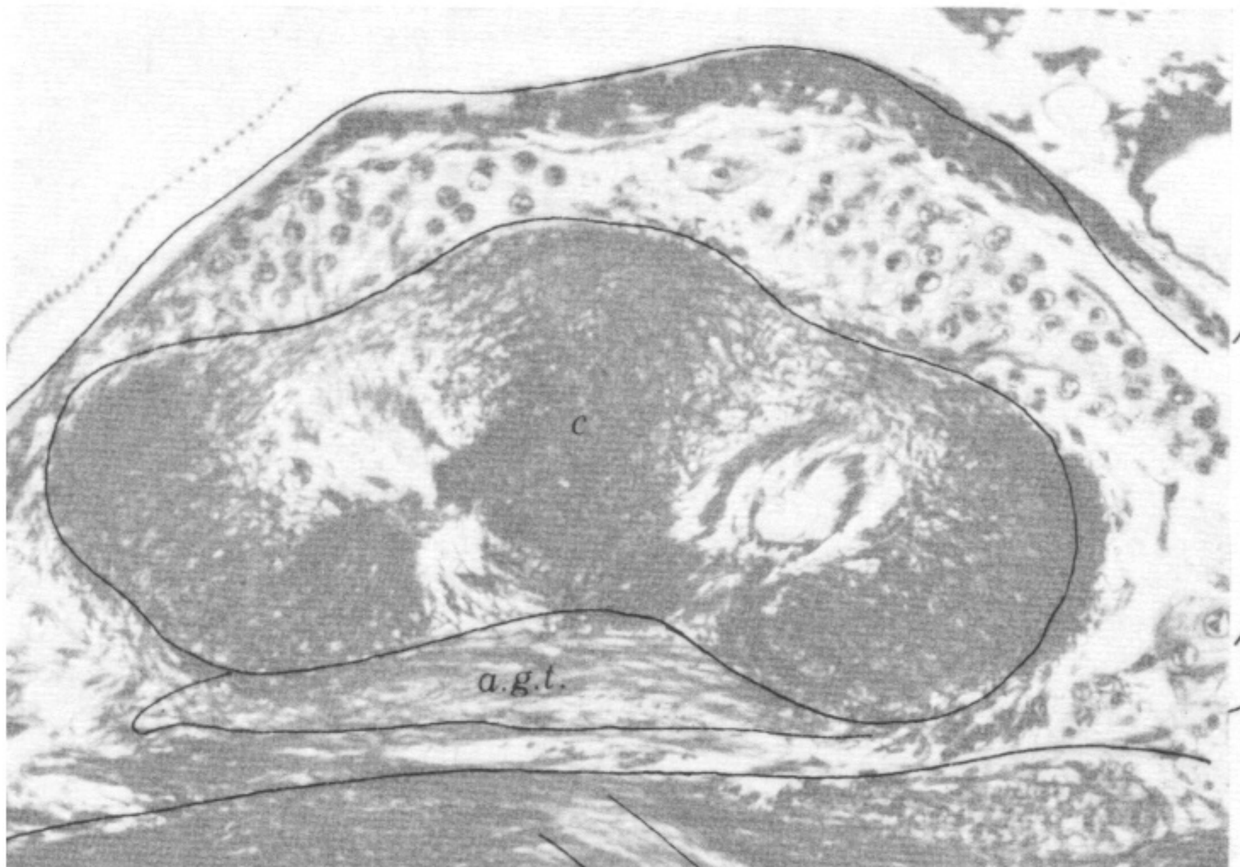
For legend see facing page.



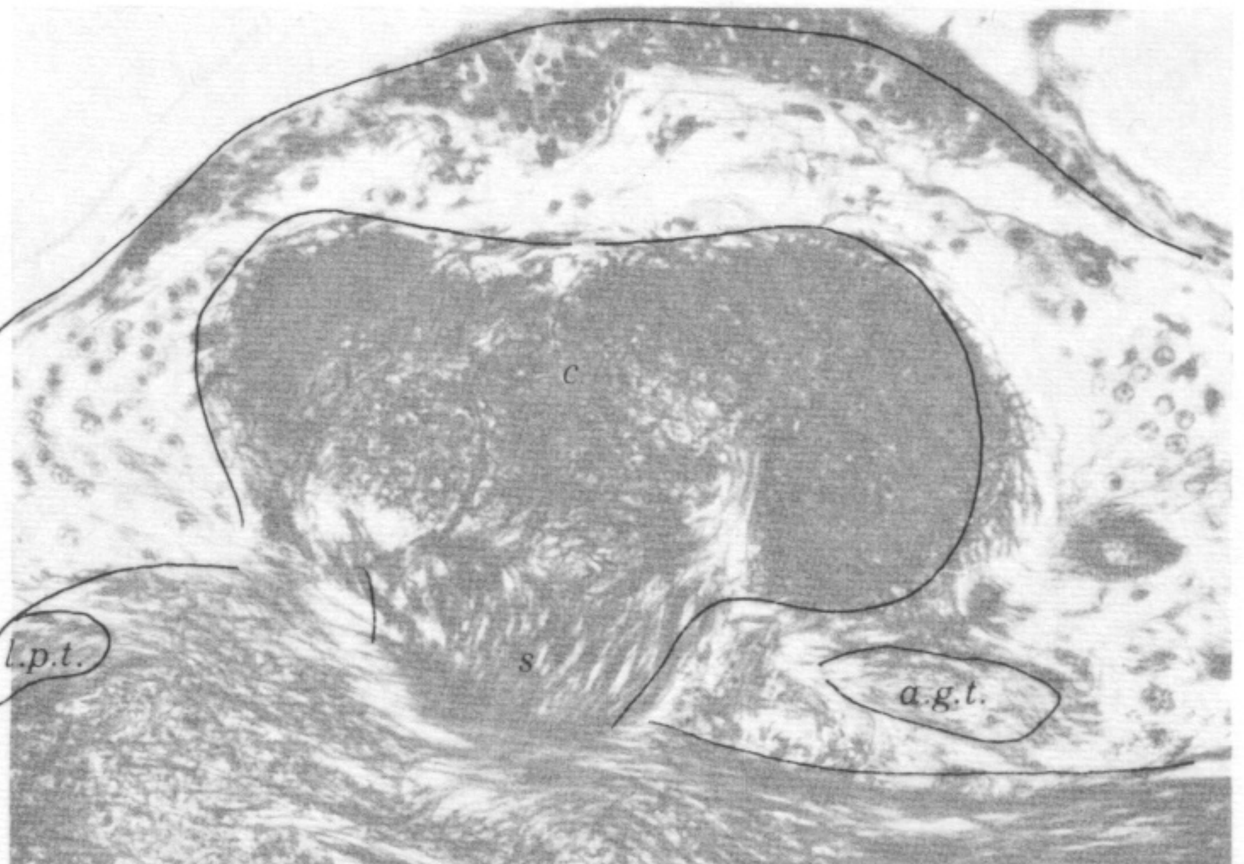
36



37

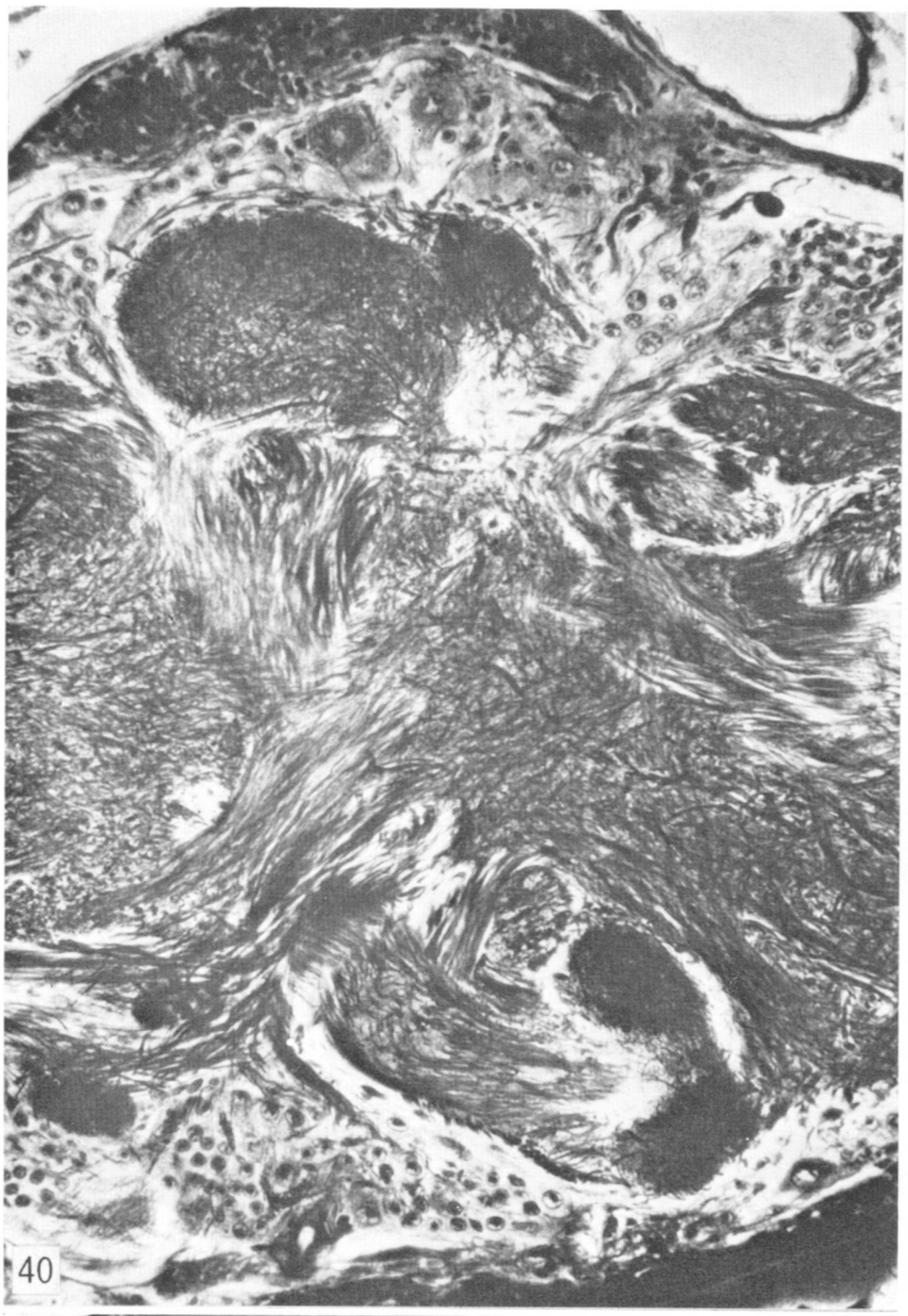


38



39

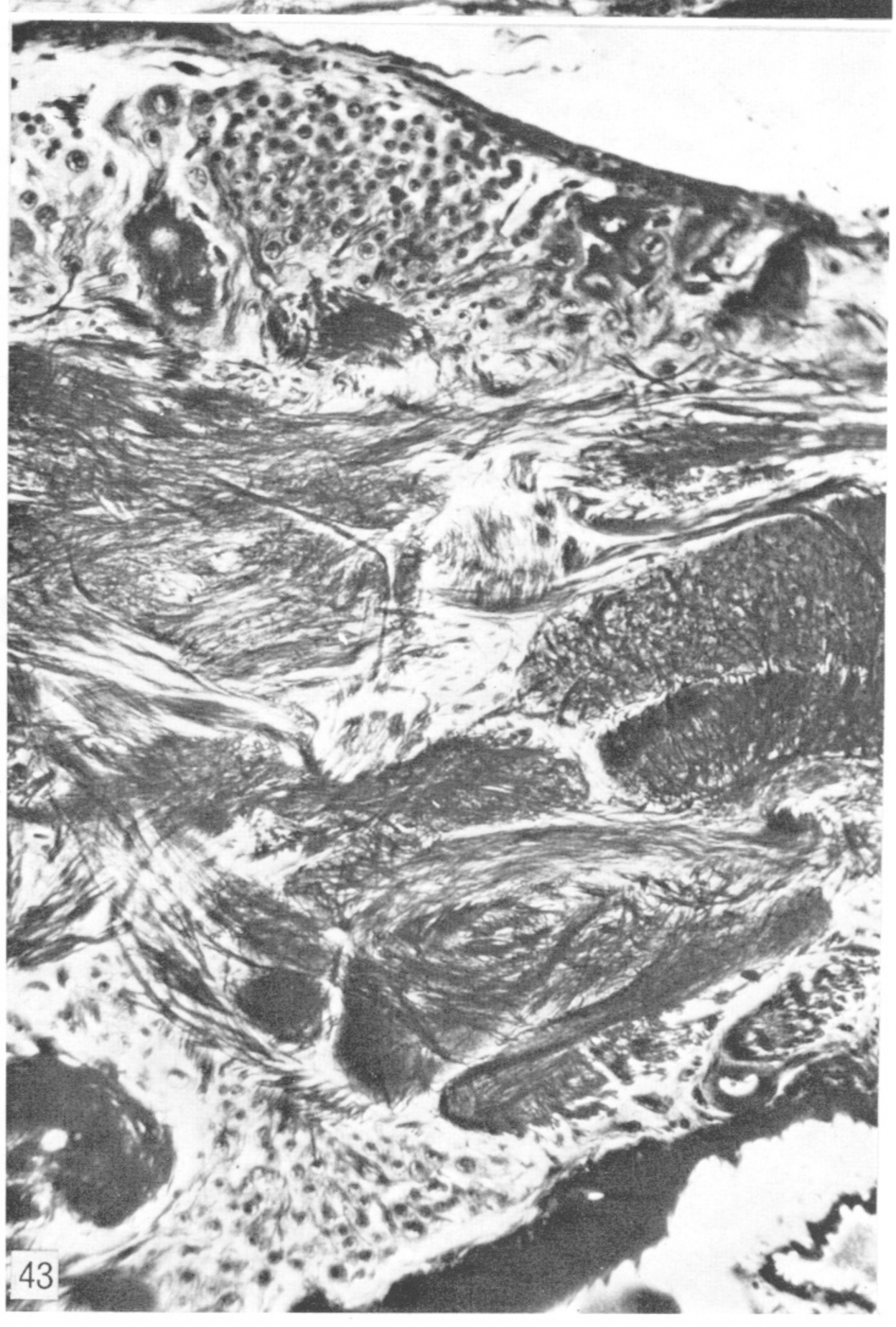
For legend see facing page.



40

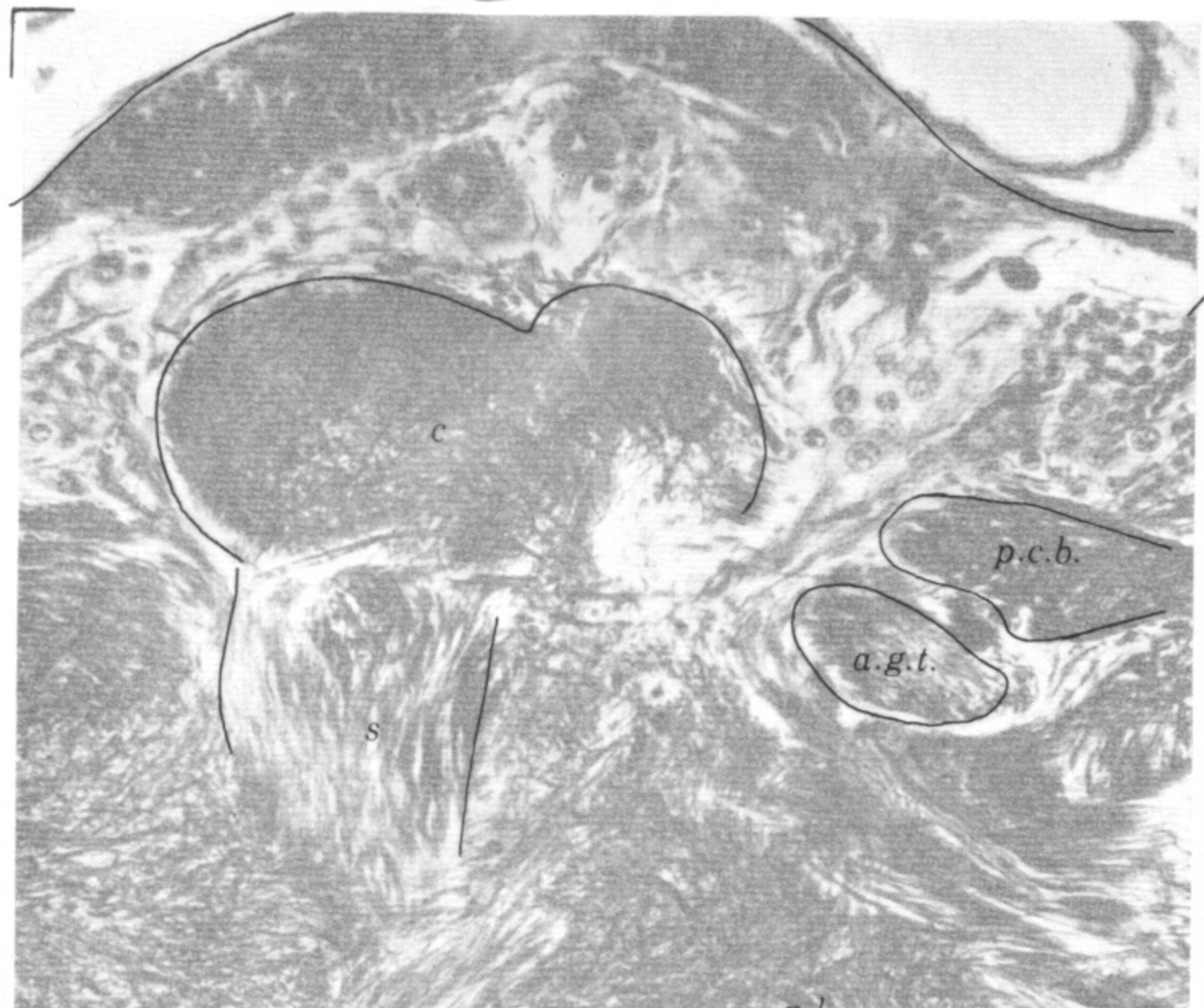
41

42

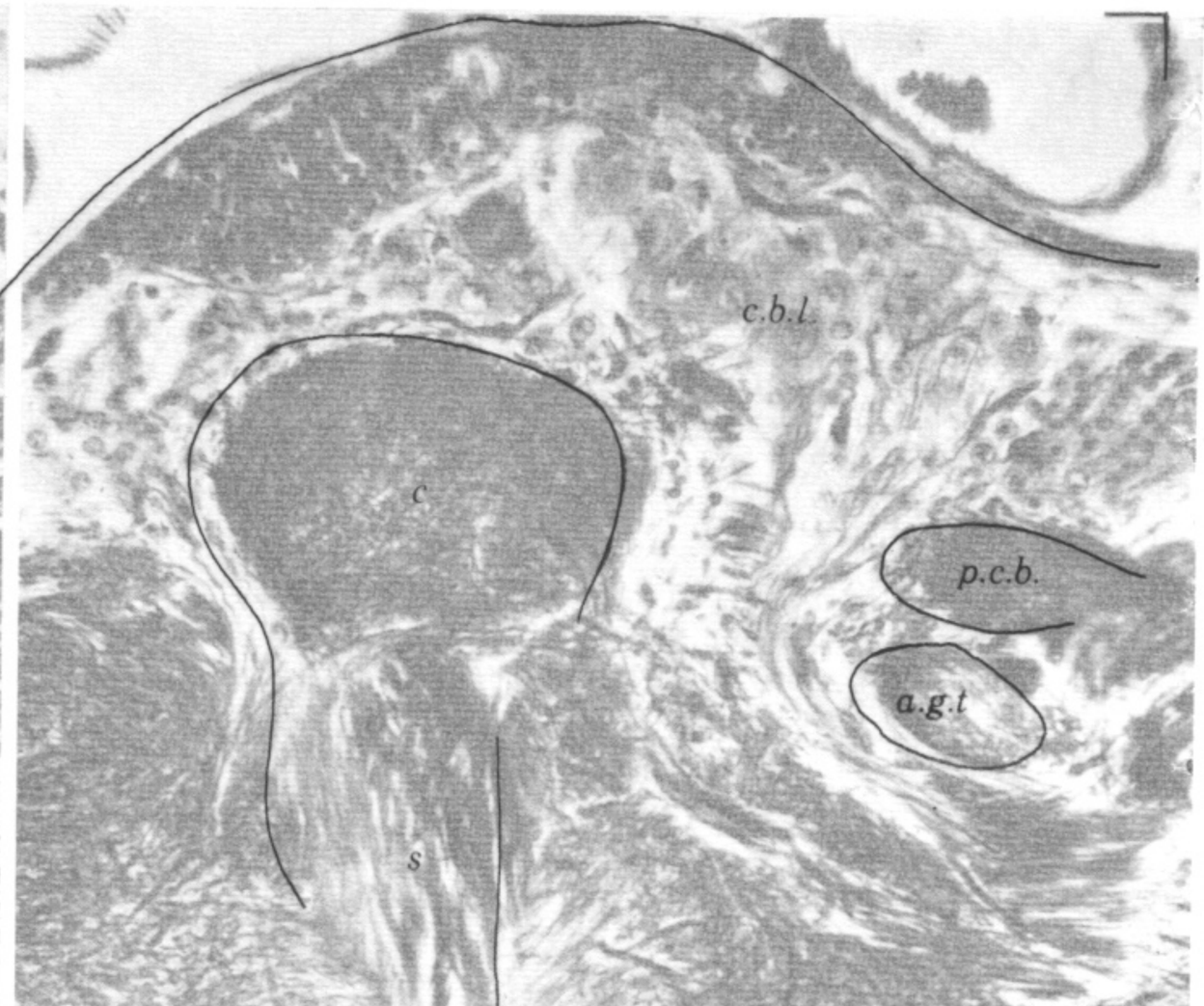


43

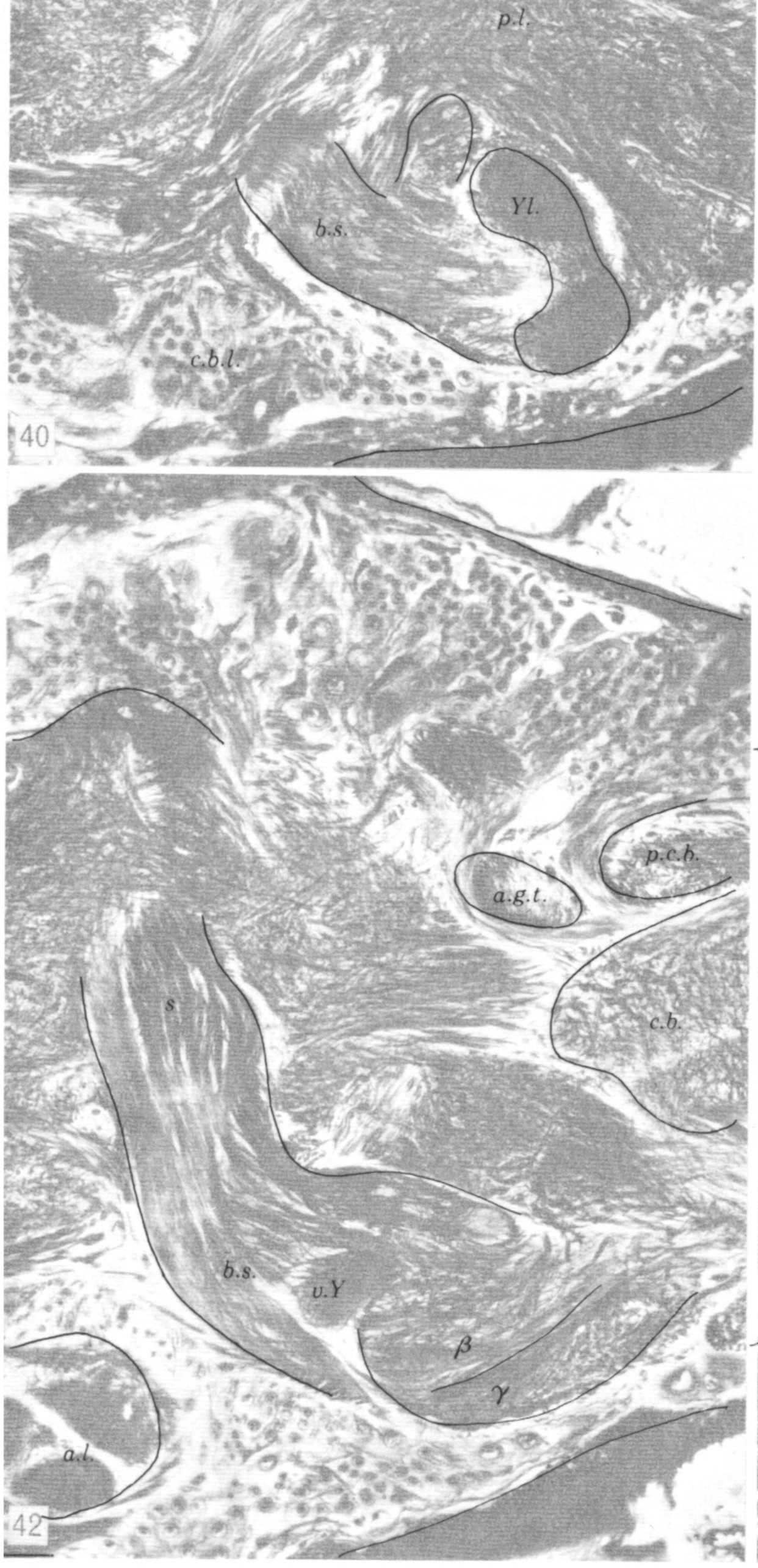
For legend see previous page



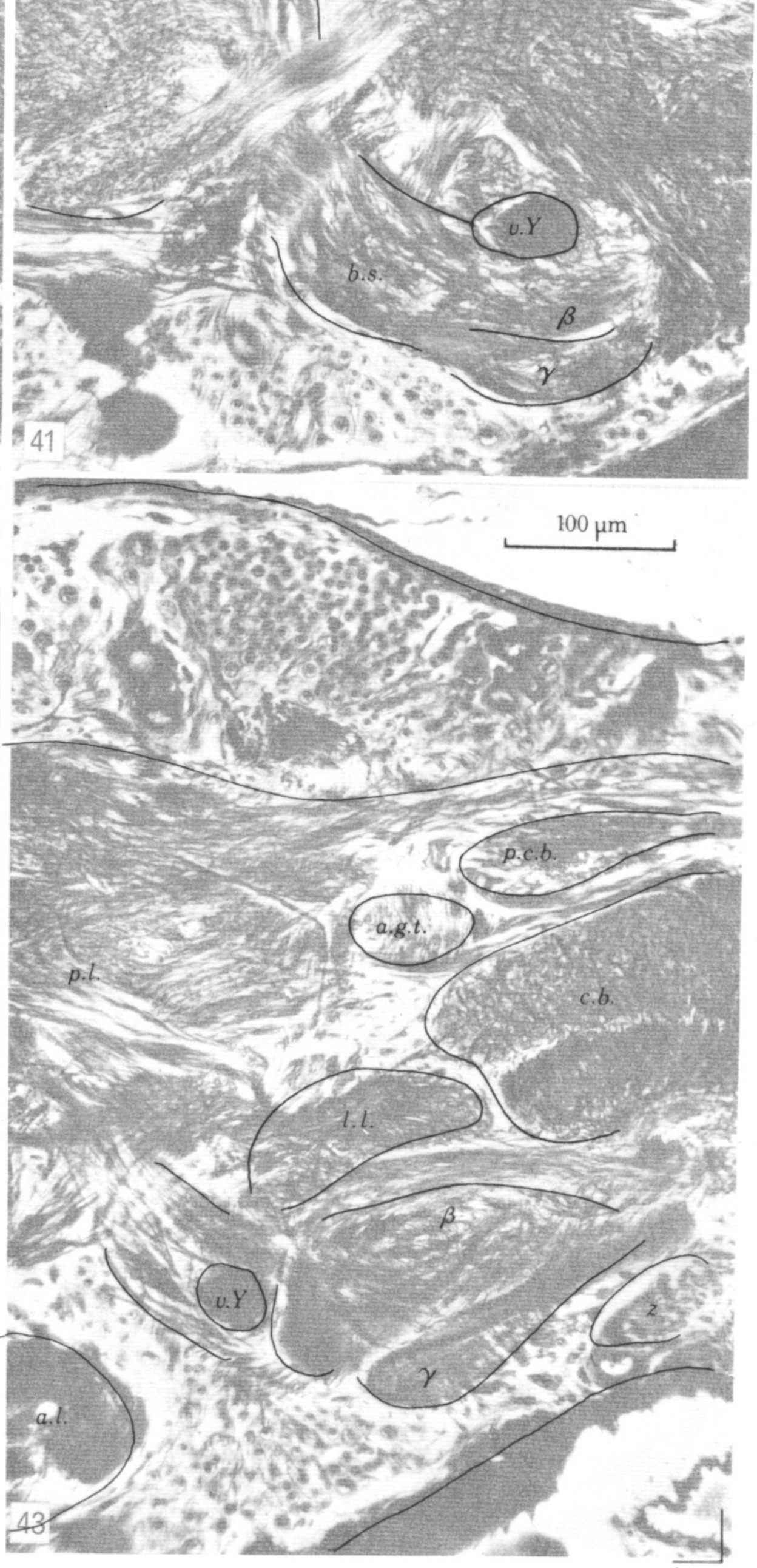
40



41

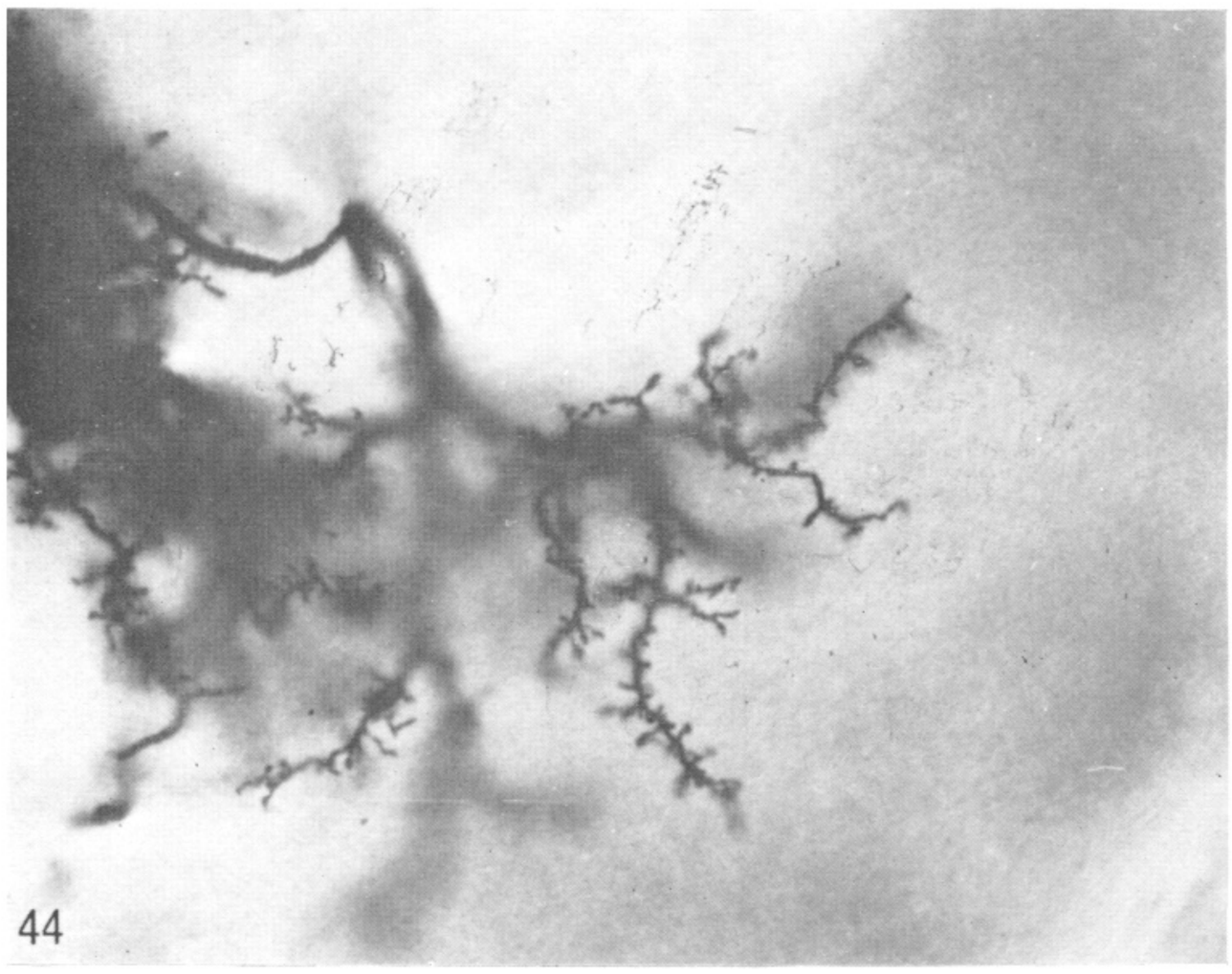


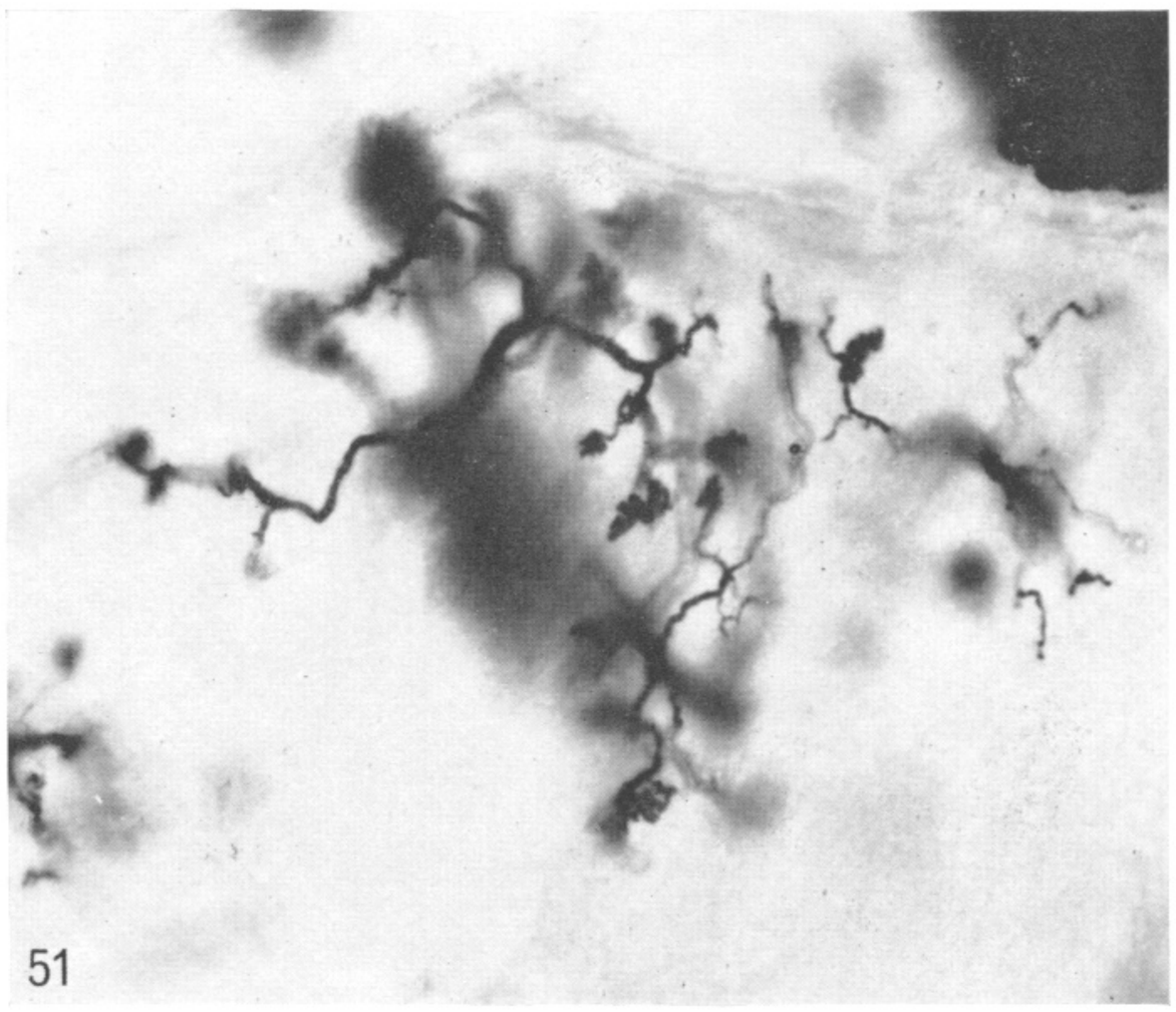
42

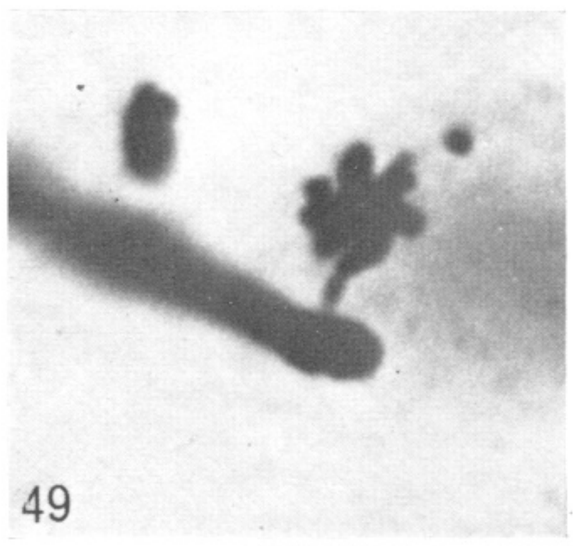


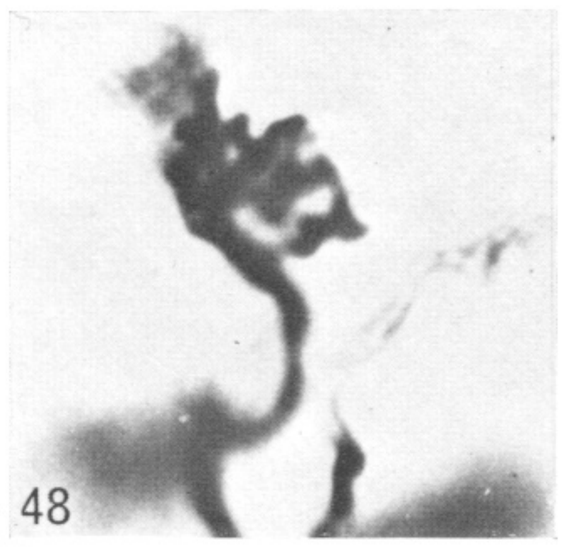
43

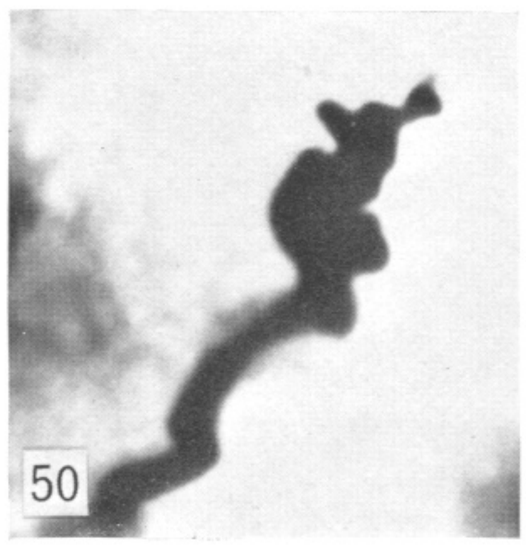
For legend see previous page.

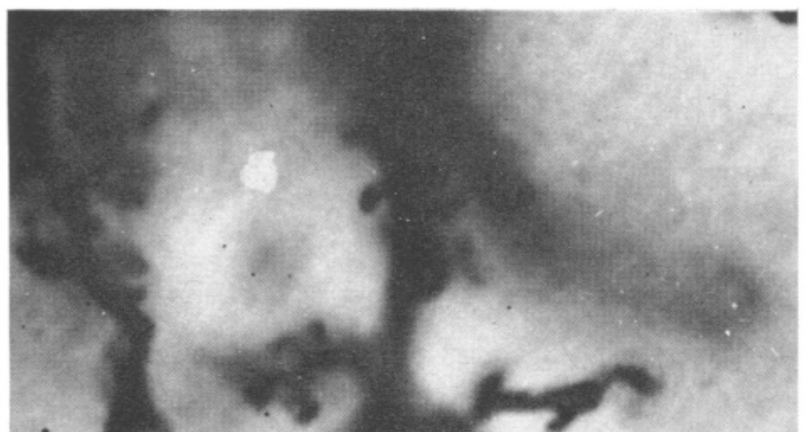


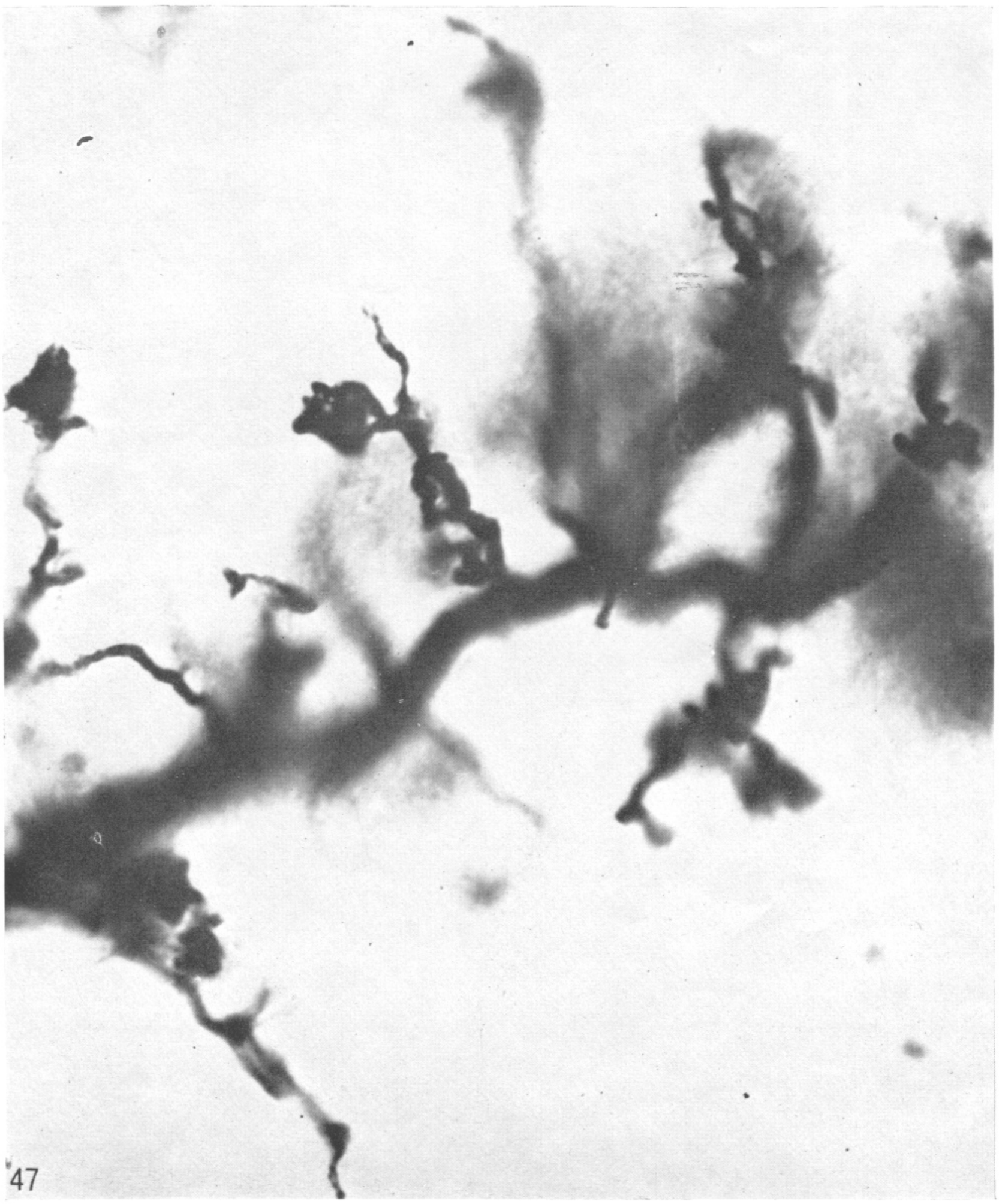


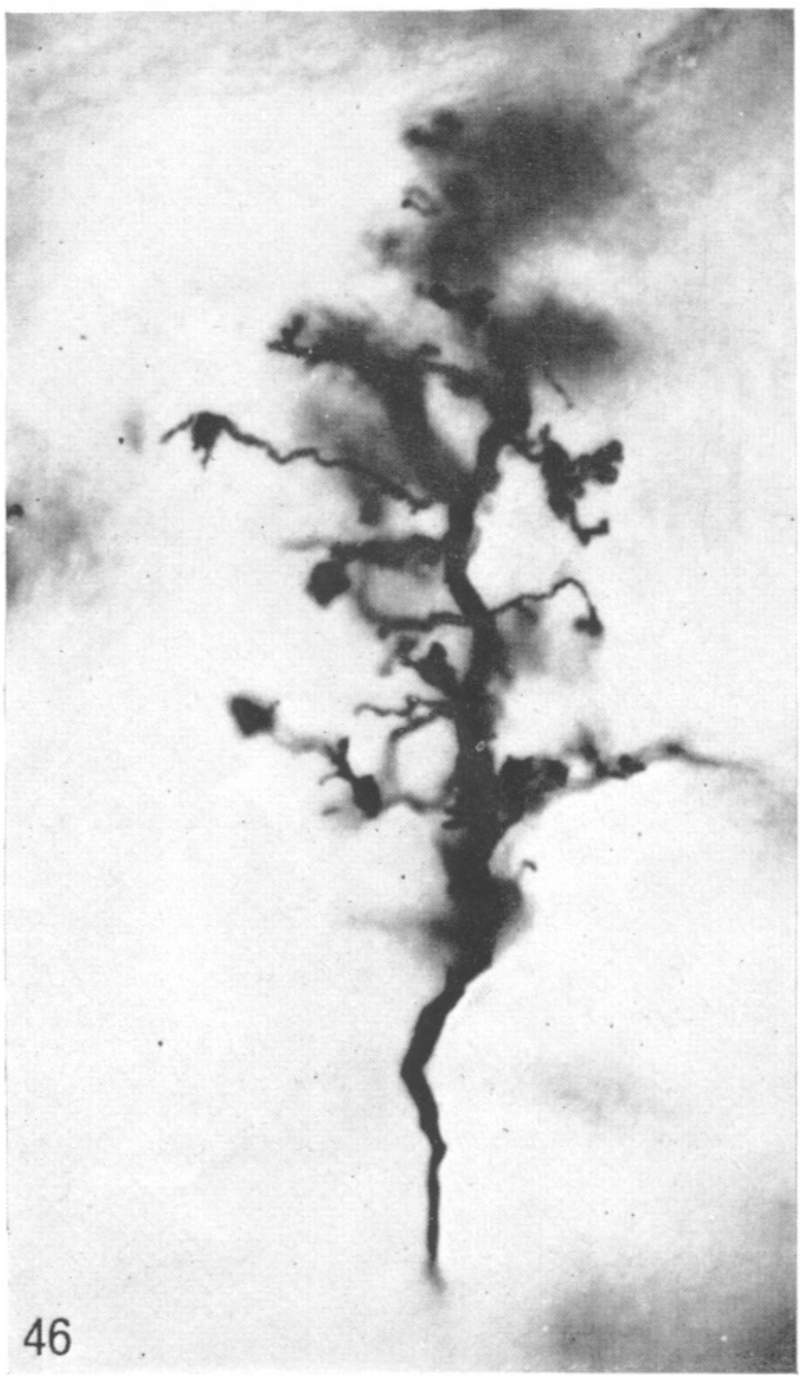




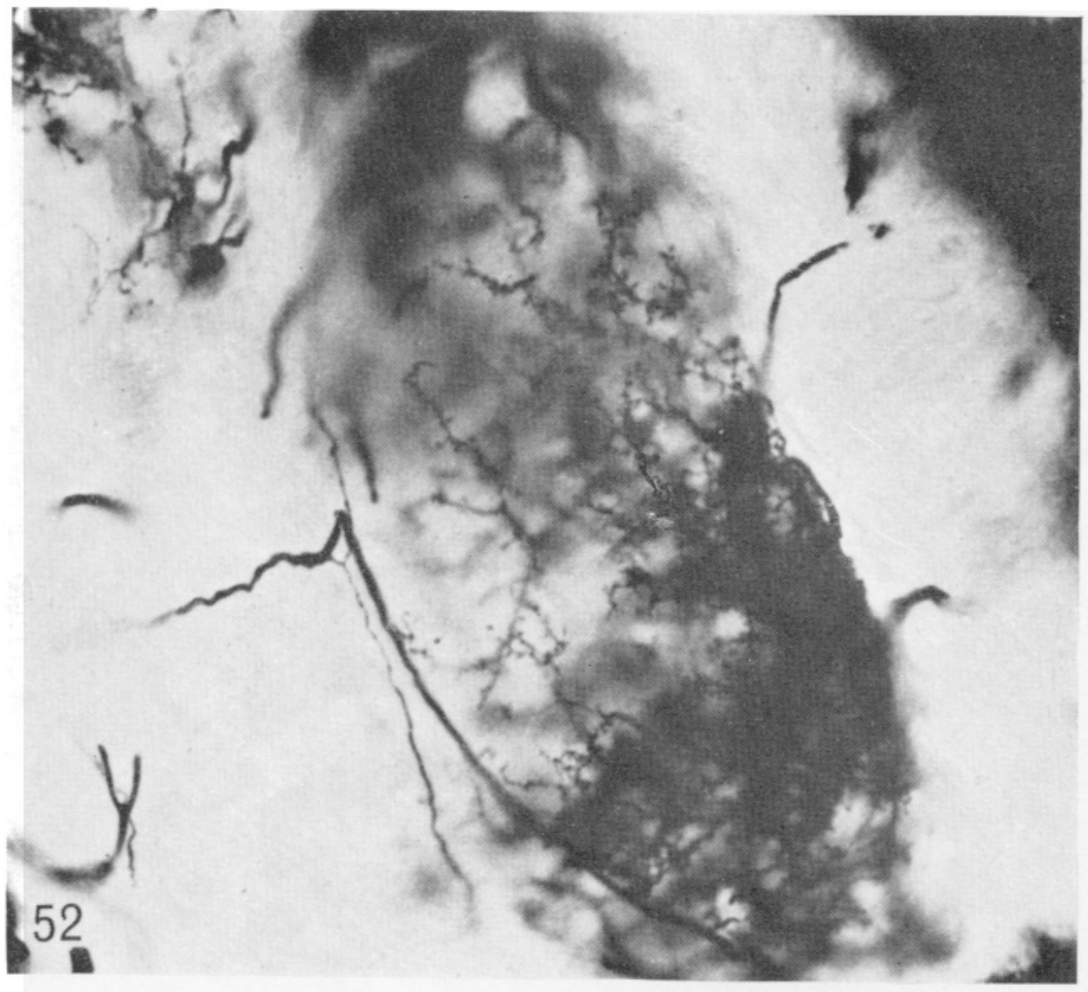




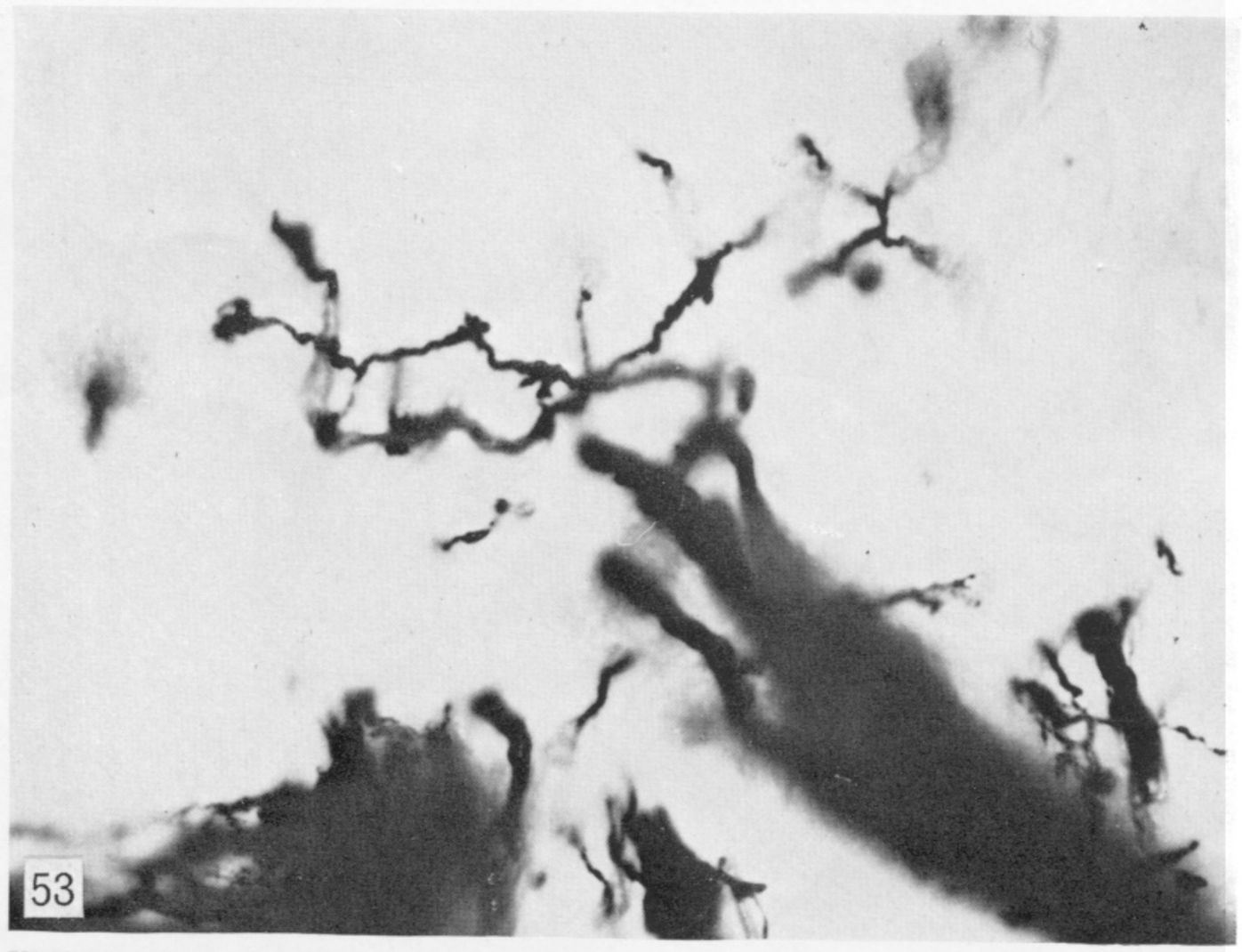




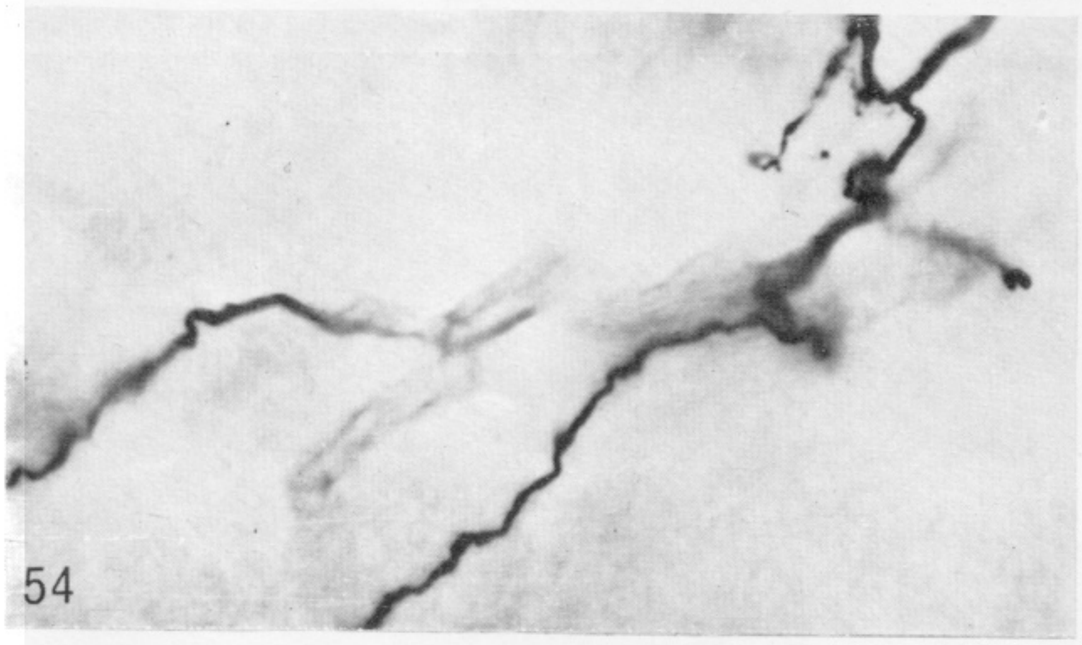
For legend see facing page.



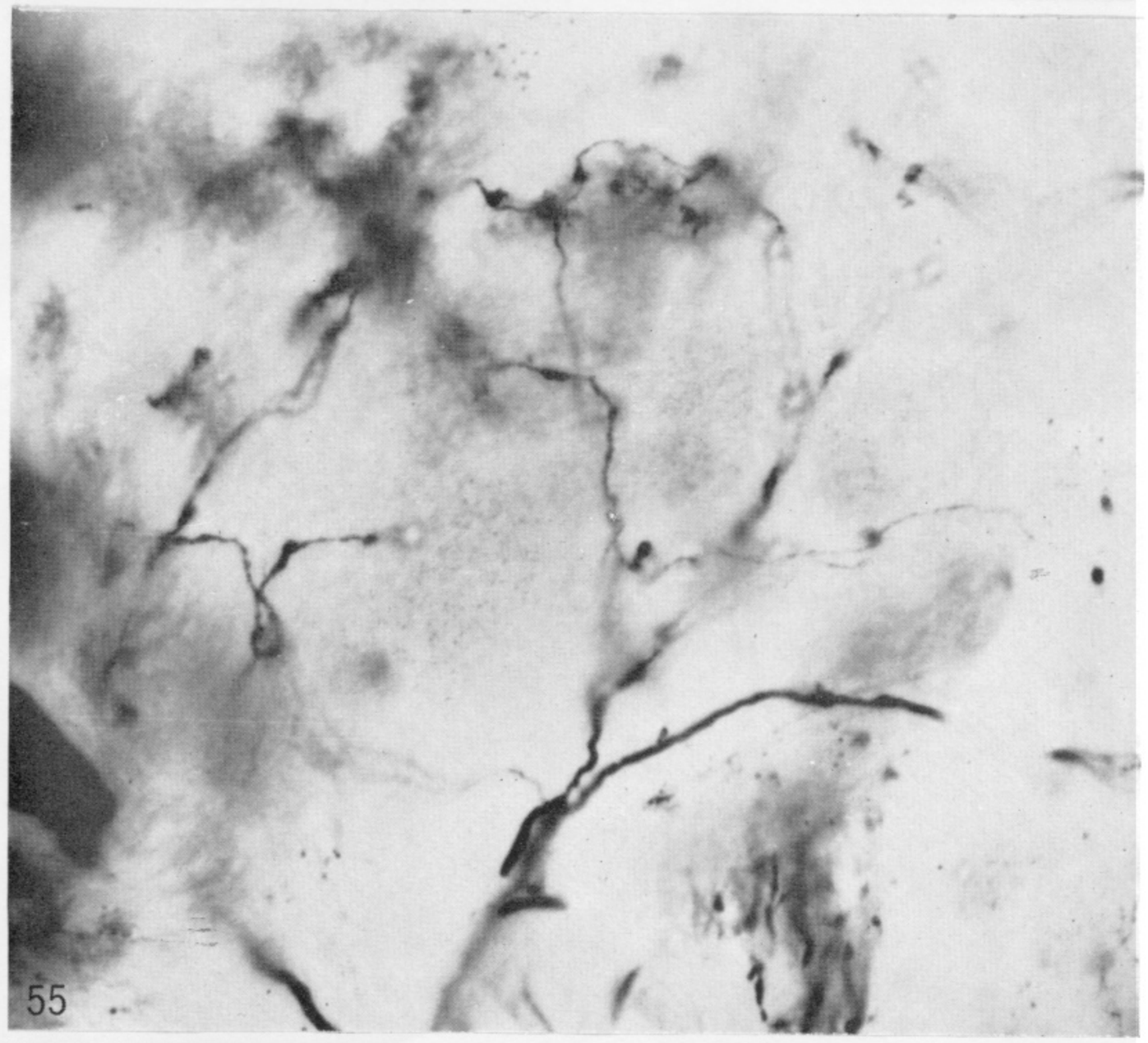
52



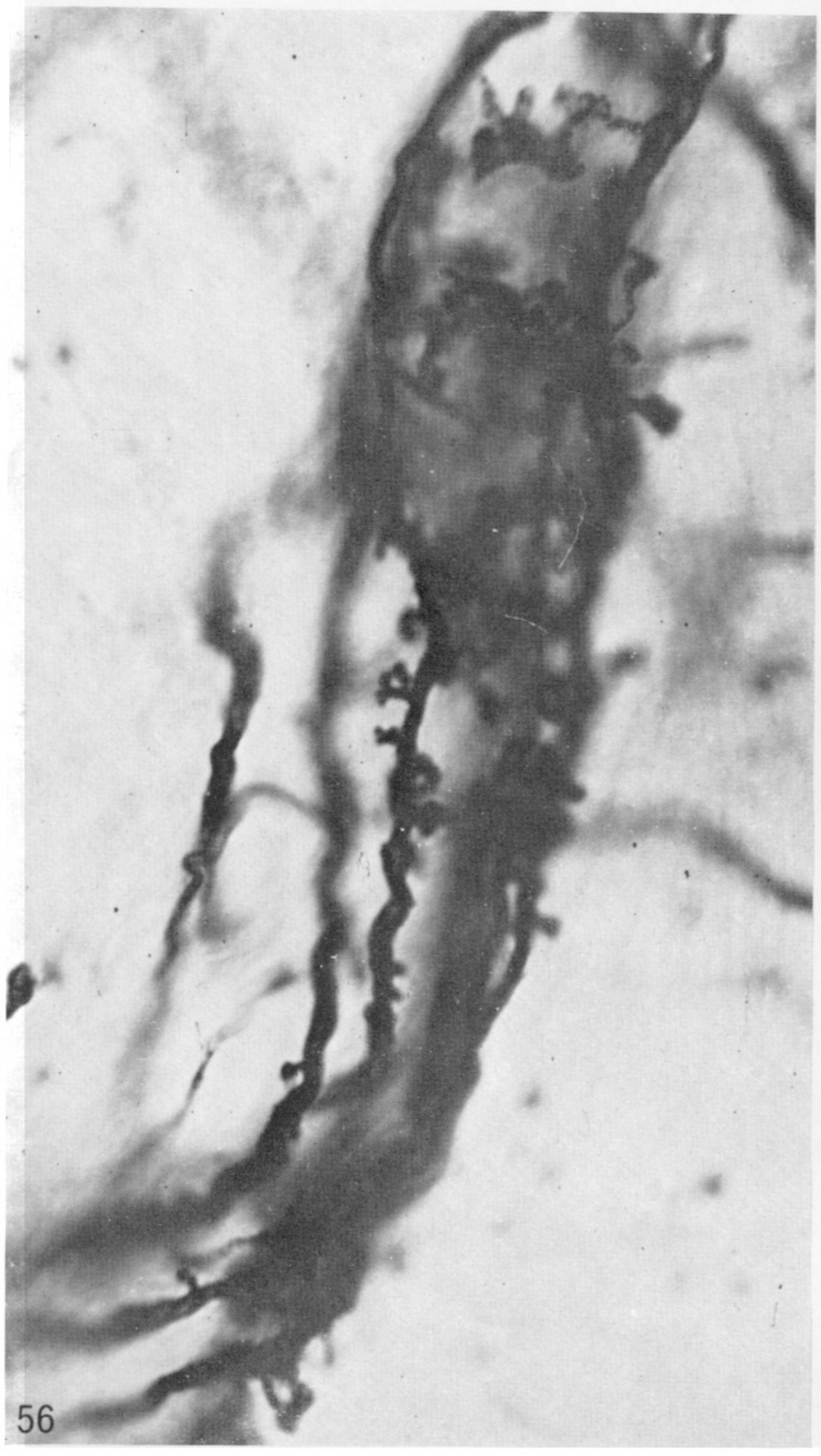
53



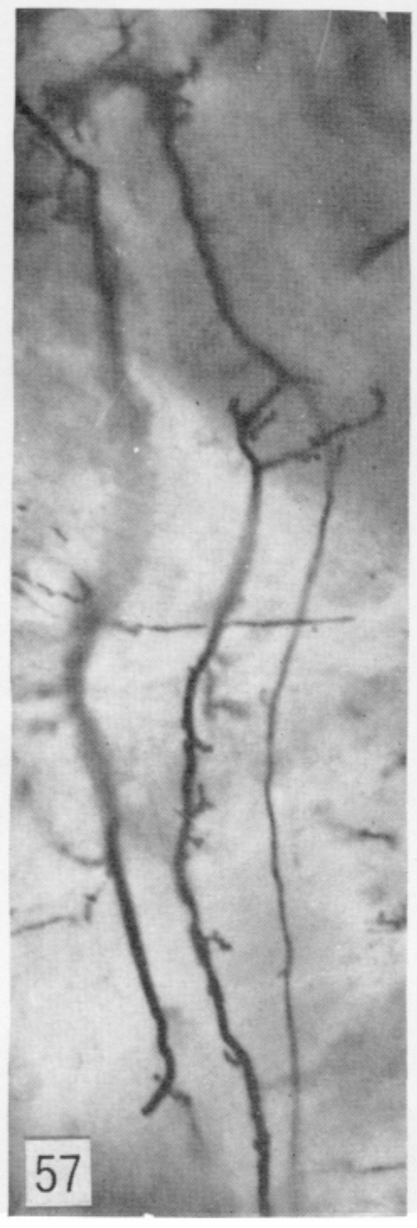
54



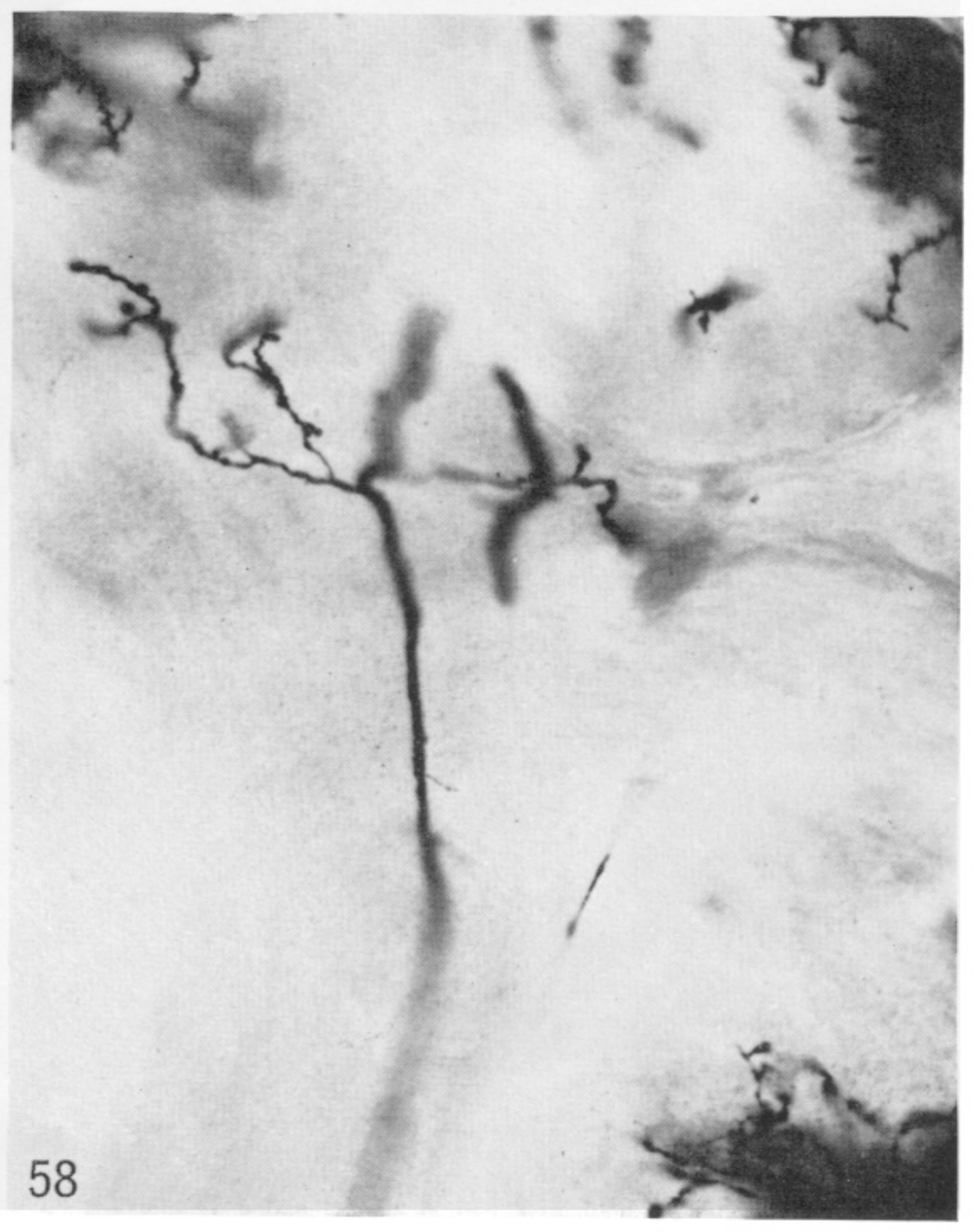
55



56

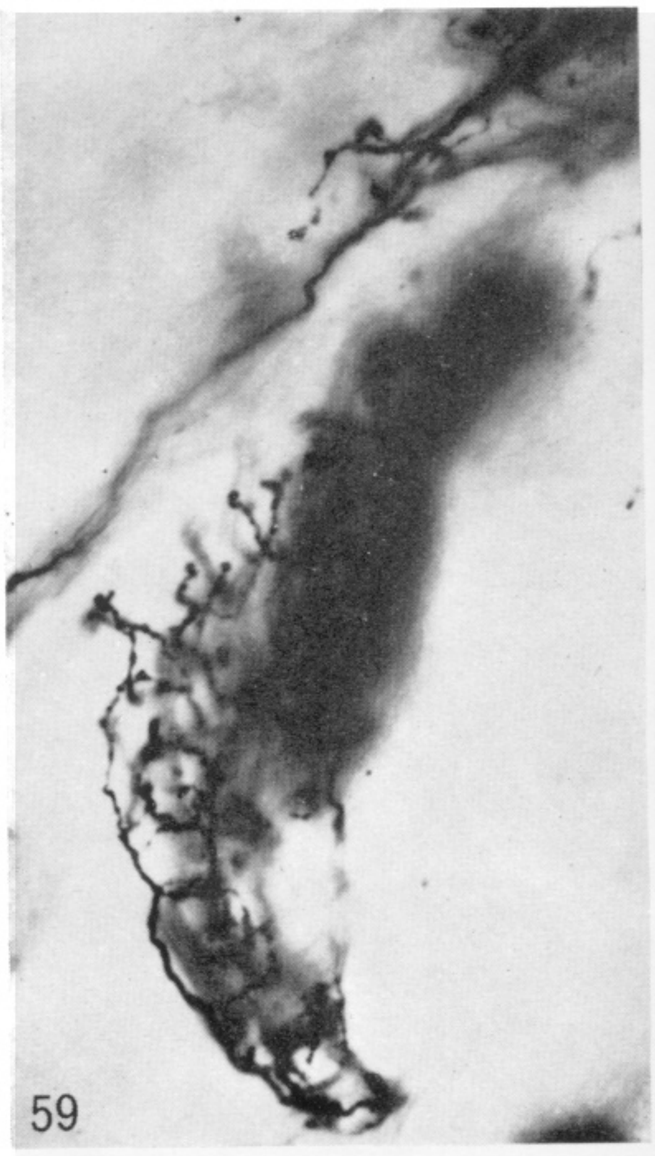


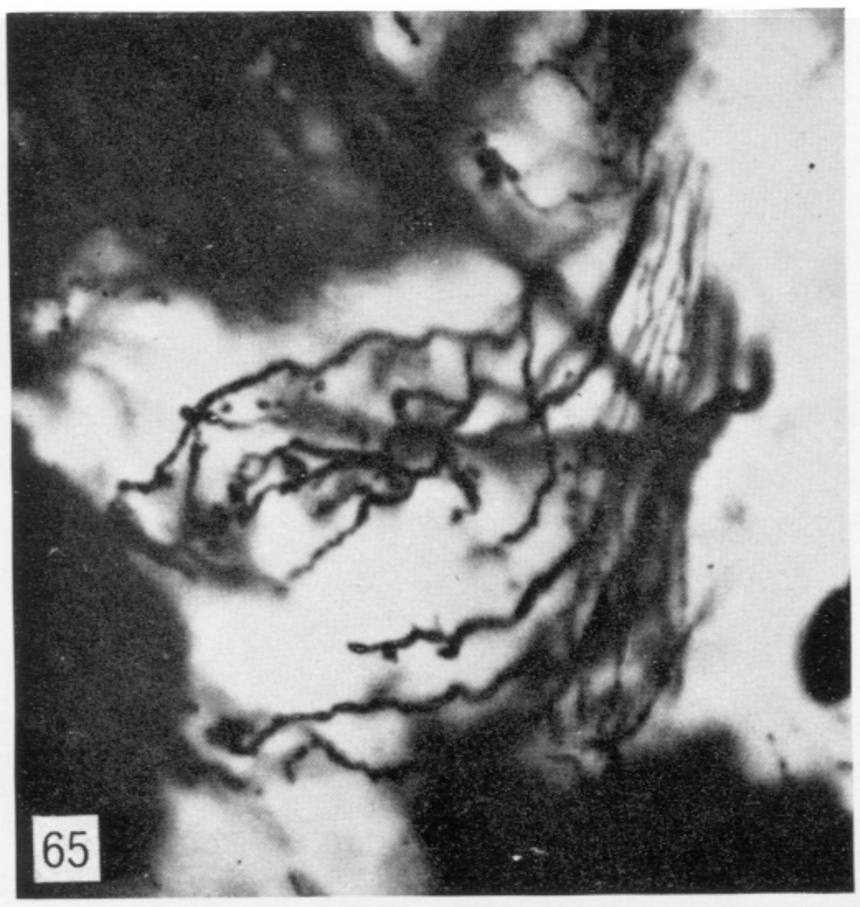
57



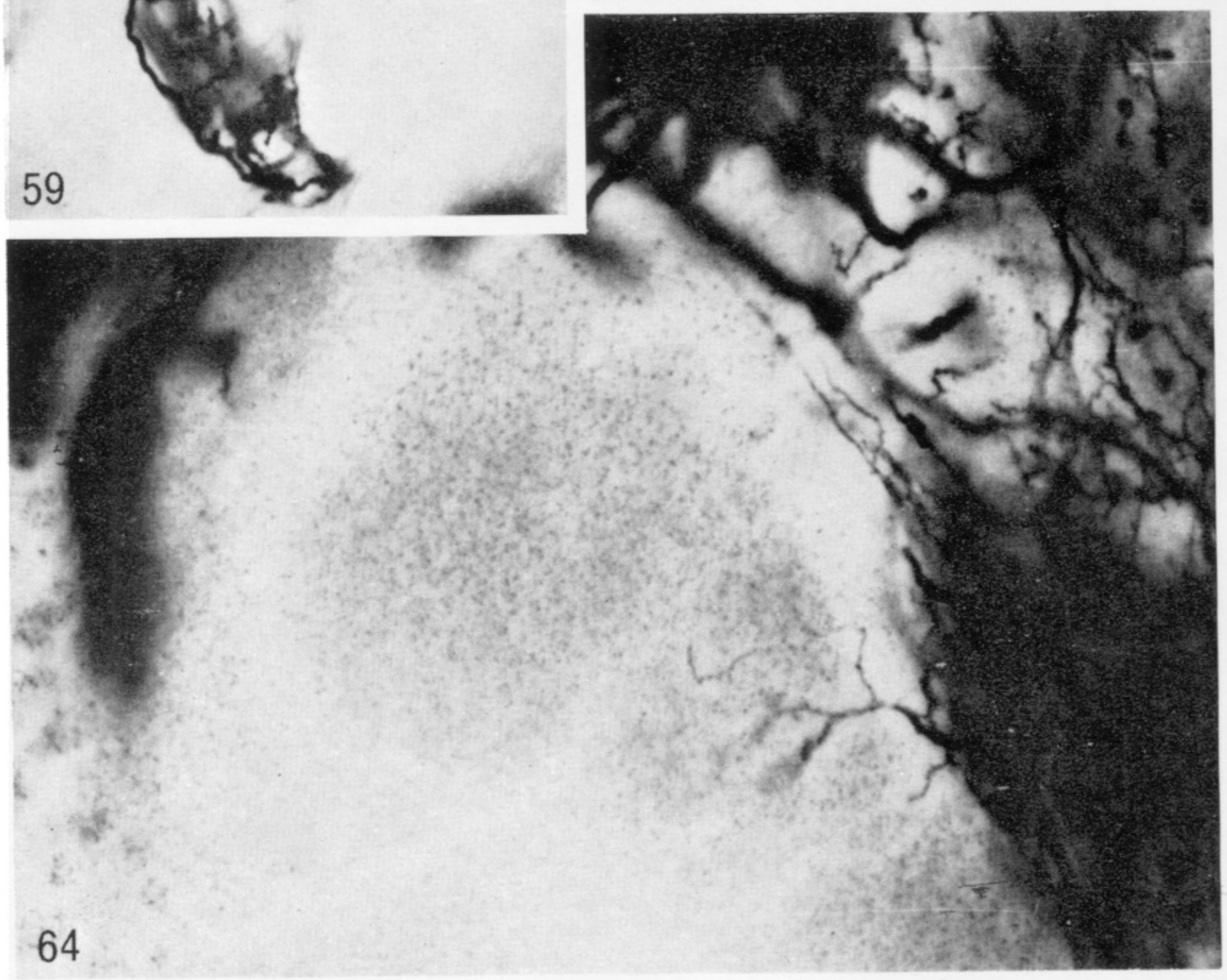
58

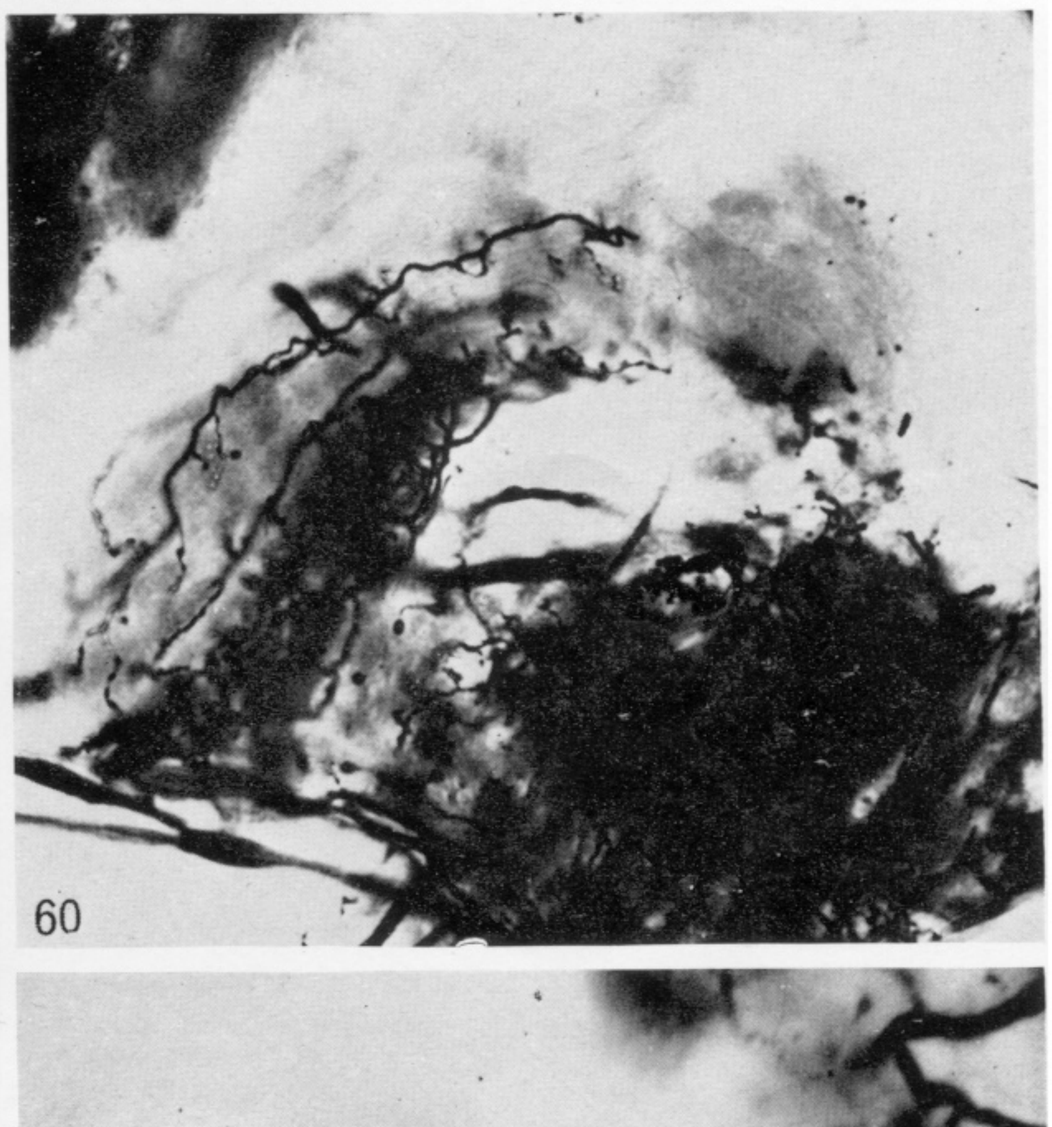
For legend see facing page.


59

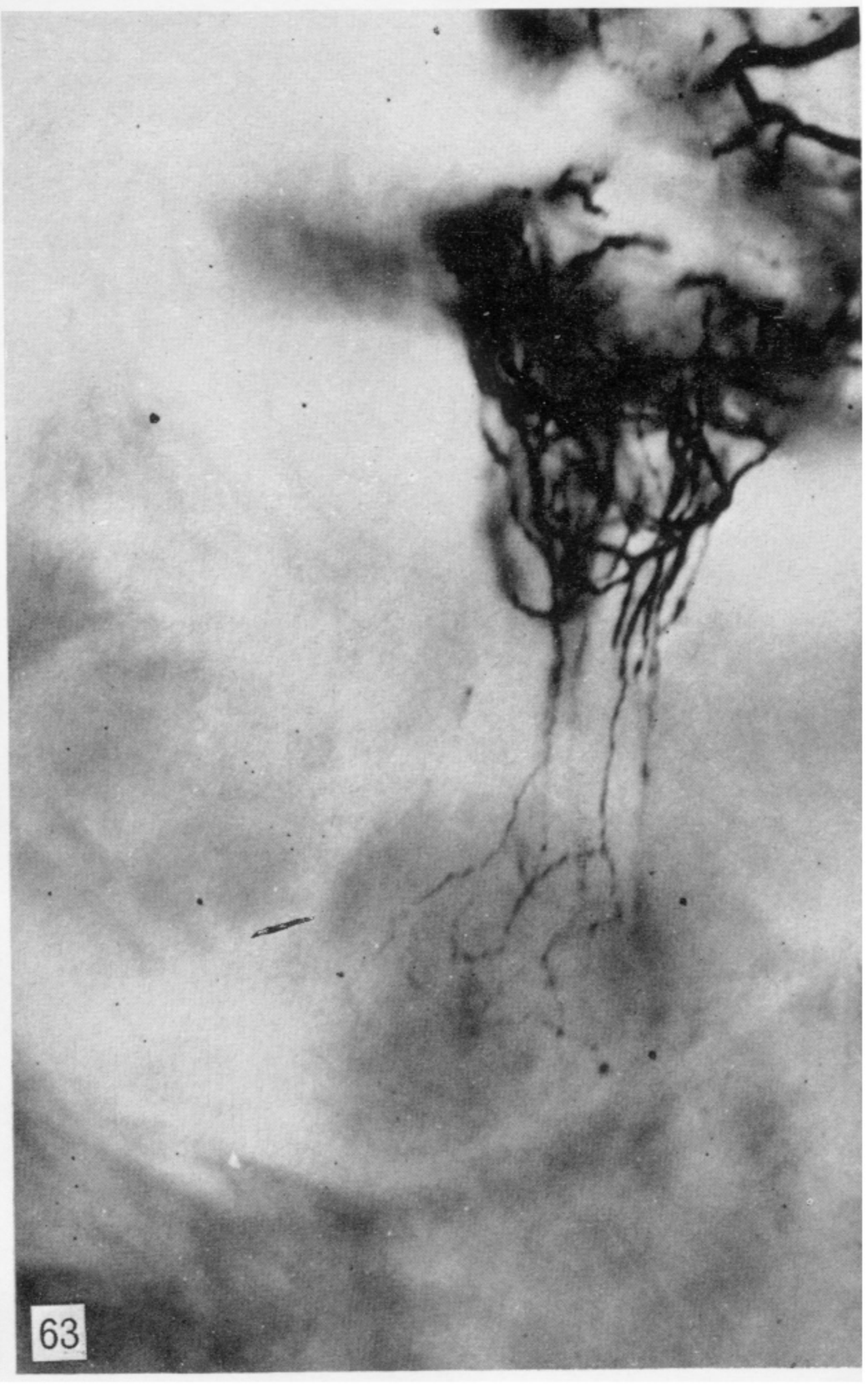

65

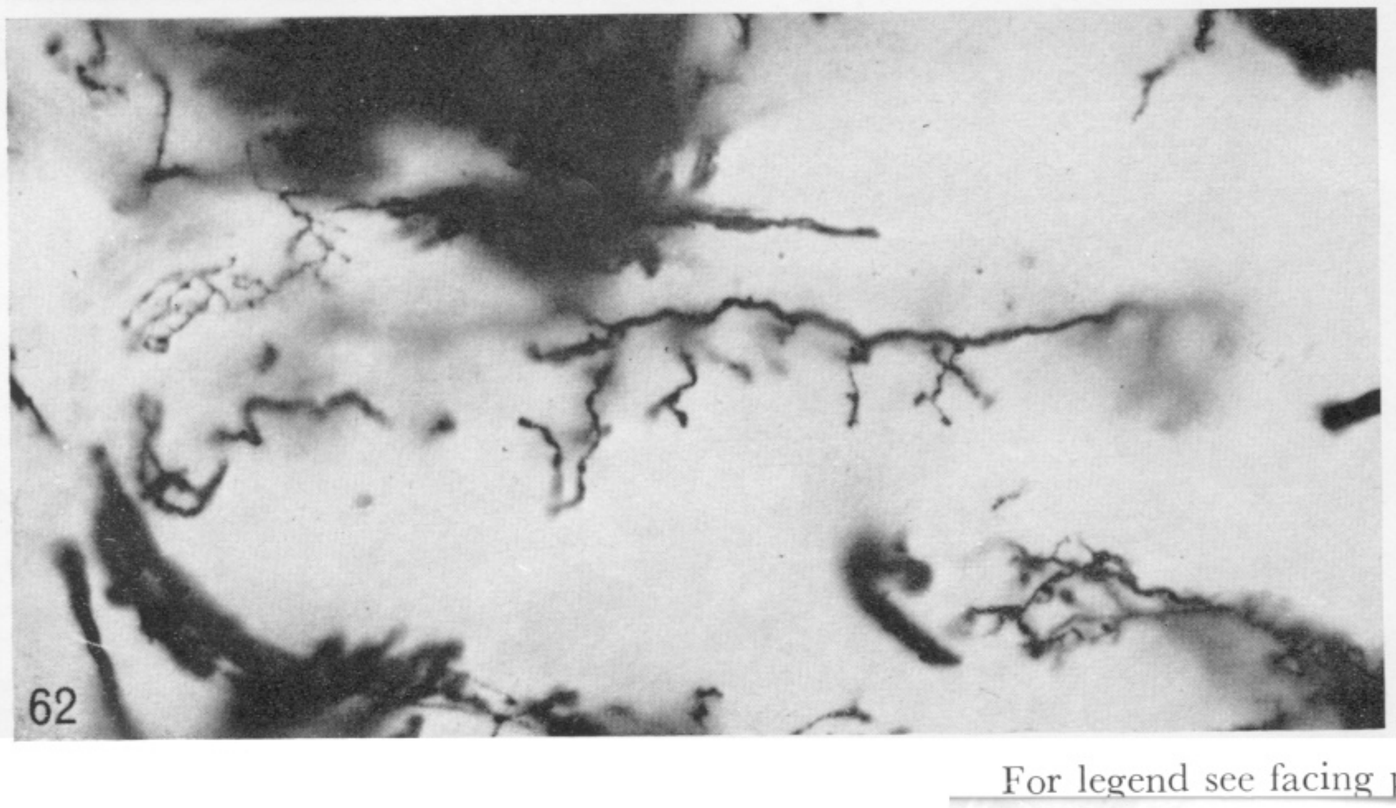

66


64


60


61


63


62

For legend see facing page.