GOLGI STUDIES ON INSECTS
PART I. THE OPTIC LOBES OF LEPIDOPTERA

By N. J. STRAUSFELD† and A. D. BLEST
Department of Zoology and Comparative Anatomy,
University College London

(*Communicated by G. P. Wells, F.R.S.—Received* 24 *January* 1969—*Revised* 26 *June* 1969)

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† Present address: Max-Planck Institut für biologische Kybernetik, 74 Tübingen, West Germany.

Vol. 258. B. 820. (Price £6; U.S. $15.60) 11 [Published 21 April 1970

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Variants of the Golgi–Colonnier (1964) selective silver procedure have been used to show up neurons in insect brains. Neural elements are particularly clearly impregnated in the optic lobes. Three classes of nerve cells can be distinguished; perpendicular (class I), tangential (class II) and amacrine cells (class III). There are many types of neurons in each class which together have a very wide variety of form. Their components are related to specific strata in the optic lobe regions. Short visual cells from the retina terminate in the lamina in discrete groups of endings (optic cartridges). Pairs of long visual fibres from ommatidia pass through the lamina and end in the medulla. Class I cells link these two regions in parallel with the long visual fibres and groups of these elements define columns in the medulla. These in turn give rise to small-field fibres that project to the lobula complex. Tangential processes intersect the parallel arrays of class I cells at characteristic levels. Some are complex in form and may invade up to three regions. Another type provides a direct link between the ipsi- and contralateral optic lobe. Amacrine cells are intrinsic to single lobe regions and have processes situated at the same levels as those of classes I and II cells. A fifth optic lobe region, the optic tubercle, is connected to the medulla and lobula and also receives a set of processes from the mid-brain. There are at least six separate types of small-field relays which could represent the retina mosaic arrangement in the lobula.

INTRODUCTION

The architecture of the optic lobes of insects is known from a relatively small number of studies. The lamina ganglionaris, or first synaptic region, is fairly well understood; the early accounts of its histology, by Cajal (1909, 1910) and Cajal & Sanchez (1915), which were derived from Golgi preparations, have been extended by the use of electron microscopy (Pedler & Goodland 1965; Trujillo-Cenoz & Melamed 1966; Trujillo-Cenoz 1965, 1966; Melamed & Trujillo-Cenoz 1967). The lamina of the lobster, *Homarus*, has been similarly described by Hamori & Horridge (1966). For knowledge of the medulla and lobula, which constitute the second and third synaptic regions respectively, workers are still dependent upon only two detailed accounts, that of Cajal & Sanchez (1915) for *Calliphora*, *Tabanus*, *Musca* and *Apis*, and that of Zawarzin (1913) for the *Aeschna* larva. The Spanish authors used the Golgi method of selective impregnation of which they were masters, while Zawarzin used both the Golgi and methylene-blue procedures. It is remarkable that they achieved so large a measure of agreement between the results they obtained with the three orders of insects, since these methods are known to be capricious. Their findings have been summarized and illustrated by Bullock & Horridge (1965).

Since the publication of these early researches, studies of the visual physiology of insects have progressed in three main directions: (i) There has been a considerable volume of work on the electroretinogram, and on the single-unit behaviour of the receptor layer (see Goldsmith 1964; Burkhardt 1964; Burtt & Catton 1966, for reviews). (ii) Attempts have been made to derive

the properties of the visual system from behavioural experiments designed in terms of cybernetic theory (for example Reichardt 1957, 1961, 1965; Hassenstein 1959; Hassenstein & Reichardt 1953, 1956; Jander & Voss 1963; Götz 1964; McCann & Macginitie 1965). (iii) A number of workers have investigated the properties of single, higher-order visual neurons with micro-electrodes (Burtt & Catton 1960; Ishikawa 1962; Blest & Collett 1965*a*, *b*; Collett & Blest 1966; Horridge, Scholes, Shaw & Tunstall 1965; Bishop & Keehn 1966; Bishop, Keehn & McCann 1968). All these studies have laboured under the disadvantage that there is no account of the connexions of the optic lobe which satisfies modern criteria of interpretation. The available descriptions of the deeper levels of the visual system do not allow much speculation about the ways in which the numerous types of visual neurons may be assembled into functional arrays. In particular, it has been the experience of neurophysiologists that small-field neurons are not readily accessible for recording; most records are taken from units with large fields, and must, presumably, stem from neurons with extensive dendritic spreads. Such cells are to be found in the tangential layers of the medulla and of the lobula complex, but these components were poorly realized in the histological preparations of early workers and have remained virtually unknown, except for a few species of neurons in *Tabanus* which were described by Cajal & Sanchez (1915) and fragments of cells recognized by Zawarzin.

During the last three years some modifications of the Golgi–Kopsch technique have been used to re-examine the lamina, medulla and the lobula complex of two species of Lepidoptera, *Sphinx ligustri* L. and *Pieris brassicae* L. *Calliphora vomitoria* L., *C. erythrocephala* Meigen. and three species of Syrphidae have also been examined; the former two species in order to obtain a direct comparison with the account by Cajal & Sanchez (1915) and to compare closely related species in the same families. The optic lobes of these Diptera are discussed in part II of this account.

The primary purpose of both papers will be the description and classification of the cells of the medulla, lobula and lobula plate. The lamina has not been examined in quite as much detail, and this account of it only deals with such matters as are relevant to its projections to the medulla. An analysis of the architecture of these three synaptic regions is meaningless unless one is prepared to consider the possible patterns of synaptic connexion of the units of which they are built, and silver preparations alone cannot give valid information about such contacts. Nevertheless, although silver preparations do not allow assertions that there are particular contacts between cells, they give the certainty that some contacts cannot exist and that others are unlikely to do so. They also can be correlated to some extent with the results of physiological recording. Both these aspects will be considered in the Discussion of this and the succeeding paper.

Cajal & Sanchez's (1915) paper on the nervous centres of insects is the most comprehensive description of the optic lobes that has been published. Kenyon (1896) and Zawarzin (1913) wrote detailed descriptions of the brain of the bee and the optic neuropil of the *Aeschna maculatissima* larva, respectively. However, the two latter works are not so detailed as that of the Spanish authors and many of the figures in Kenyon's work are schematic. Cajal & Sanchez's work covered many species of insects and contains a wealth of detailed information both in the text and in the figures. References to Cajal & Sanchez will refer to their 1915 work and references to Zawarzin will mean his 1913 paper. Zawarzin's account was published in German and much of the information in it was used in a later work (1925) on parallelisms in the morphology of nervous systems. The Cajal & Sanchez account was published in Spanish in

1915 following two shorter earlier works by Cajal (1909, 1910) on the retina and lamina of the Muscidae. An English translation was made of the 1915 work by Power & Truscott in 1942 but was never published.

Materials and methods

The majority of observations made in this paper have been derived from two species of Lepidoptera, *Sphinx ligustri* L. and *Pieris brassicae* L. Preparations of some other species of Lepidoptera have been made, including *Automeris aurantiaca* Weymer, and *Pieris napi* L. Brains of larval *Aeschna* sp. *Apis mellifica* L. and *Locusta migratoria* L. have been stained by silver impregnation procedures and some findings from these preparations are described in this paper. Five species of Diptera have also been studied in detail. They are: *Calliphora vomitoria* L., *C. erythrocephala* Meigen, *Eristalis tenax* L., *Syrphus elegans* Harris, and *S. nitidicollis* Meigen. Some details of their anatomy have been included in this paper.

The brains from about 1000 animals have been prepared by variations of the Golgi method: 100 brains were prepared from *S. ligustri*, 400 from *Pieris brassicae*, and 400 from the five species of Diptera and the rest from other species. Another 120 brains from all the species, except *Aeschna*, were stained by reduced silver and other methods.

Sphinx ligustri and *Automeris aurantiaca* were purchased as diapausing pupae, stored at 6 °C during diapause development and allowed to metamorphose and hatch at 20 °C. *Pieris brassicae* and *P. napi* were obtained as non-diapausing pupae from the Entomological Field Station, Cambridge, and from private dealers, and allowed to hatch at 20 °C. In addition, 20 *P. brassicae* pupae in diapause and 24 infected with an unidentified virus and reared on artificial food were obtained for experimental staining purposes. Adult *Apis mellifica* workers were obtained from the Zoological Society Gardens, London, and from the Zoologisches Institut, Frankfurt am Main, Germany. Larval *Aeschna* and the five species of Syrphidae were caught in the wild. *Calliphora erythrocephala* were obtained from continuously breeding stocks at the Zoological Institute, Frankfurt am Main, and *C. vomitoria* were purchased as larvae in this country and allowed to metamorphose and hatch at 23 to 25 °C.

(1) *Non-selective silver impregnations*

Brains, and in some cases, whole heads, were fixed in Dubosq-Brasil (1905) solution for at least 24 h. Variants of Blest's (1961) modification of Holmes's silver method were used for the impregnation of 10 to 15 μm wax sections. Fraser-Rowell's (1963) modification was used for tracts, for which it is admirable, but for these species of Lepidoptera and Diptera this technique yields a somewhat granular impregnation and does not reveal fine detail. The amounts of silver nitrate and of pyridine derivatives can be usefully increased for both methods: good results are obtained when each 500 ml of the incubating bath contains 20 ml of 1 % silver nitrate and *either* 7 ml each of 2,6-lutidine, and 2,4,6-collidine *or* 20 ml lutidine (from British Drug Houses, Ltd).

(2) *Selective silver impregnation*

A number of variants of the Golgi stain were tried without success. The technique finally adopted was devised by Colonnier (1964), and has been subsequently used, with good results, on the mammalian retina (Boycott & Dowling 1969). One part of crude gluteraldehyde solution (approximately 30 %, as supplied by the National Cash Register Company) is added to 4 parts of 2.5 % potassium dichromate in glass-distilled water immediately before use. The animals

are killed by intra-abdominal injections of this fixative. After movement has ceased the head is opened and as much of the musculature, air sacs and tracheation as possible is removed to leave the surface of the brain exposed. Whole open heads or removed brains are then fixed in this fluid for 2 to 3 days in the dark at 20 °C. After fixation the brains are washed several times in 0.75 % silver nitrate and left in that solution for 2 to 3 days in darkness. The temperature of fixation and of silver impregnation is, for most species, critical, and temperatures above or below 20 °C can give very poor results. Prefixation in gluteraldehyde buffered to pH 6.8 to 7.4† was subsequently used as part of the standard staining procedure. The animals are killed by injection of this prefixation solution and the brains or opened heads left in it for 6 to 48 h. The exact timing is different for each species. With few exceptions all the data about the types of neurons in the optic lobes have been obtained from preparations treated by this prefixation procedure. More cells are stained in prefixed material and, in addition, impregnation of the material is more consistent. Tangential cell projections to the mid-brain are rarely impregnated in brains which have not undergone prefixation.

In the experience of Cajal & Sanchez and other workers it was found that the success of the Golgi stain depended somewhat on the age of the animal concerned. This phenomenon has been found to apply to the species listed here. In addition, there are several other factors of importance which influence the success or failure of this staining procedure: (1) Young, newly emerged animals, or individuals taken from their puparia shortly before eclosion show more consistent impregnation than those stained several days after eclosion. Fixation of young animals in buffered gluteraldehyde resulted in excellent impregnation of a number of neurons localized within a small portion of a geographical region. Older animals tend to show impregnation of neurons more evenly spaced throughout the regions. Animals which are more than 5 days old (posteclosion) often show fewer neurons impregnated and dense crystallization within the fibre tracts of the first and second optic chiasmata and in tracts from the optic lobes to mid-brain regions. (2) Juvenile animals taken from the puparium 2 to 5 days before eclosion show species-specific peculiarities. At this time the brains are still undergoing development and nerve-cell growth and specialization (N. J. Strausfeld, unpublished). In young pupal animals only the transmedullary neurons of *Pieris* look like their adult counterparts. Other neurons, especially the tangential cells, have processes which are obviously different to those in the adult. Nevertheless, many of the tangential elements in the immature animals can be equated with those of the adult, they impregnate well, and their processes to other brain regions can be more readily determined in the pupal *Pieris* than in the adult. However, the topographical relationships of neurons have only been described from data taken from the adult animals of all the species studied. Pupal *Sphinx* are not receptive to the Golgi stain and early pupal *Calliphora* demonstrate, predominantly, clear impregnation of the tracheal system of the optic lobes. (3) The pH, molarity and duration of fixation in the buffered gluteraldehyde can be varied to give different results both in juvenile and adult animals. Preparations considered ideal for study should contain many recognizable cell types in several geographical regions, impregnated against a moderately clear background, and in addition provide information about their projections to other brain regions. Such results are most likely to be obtained when the prefixation solution is adjusted to the following ranges of pH: *S. ligustri*, pH 6.8 to 7.0; *E. tenax*, *C. erythrocephala* and *C. vomitoria*, pH 7.0 to 7.2; *P. brassicae*, *Syrphus elegans* and

† Gluteraldehyde, 17.5 ml, 0.8% sodium hydroxide, 25 ml, 2.7% potassium dihydrogen orthophosphate, 25 ml, and 32.5 ml of glass-distilled water. All reagents *must* be 'Analar'.

S. nitidicollis, pH 7.2 to 7.4. Changing the molarity of the prefixation solution was also tried and was found to show up cell-types in *Calliphora* which had been described by Cajal & Sanchez but had hitherto been missed in the present preparations. Another type of neuron in the lobula complex of *Pieris*, recognized in other species, was only found to be impregnated in animals that were infected with a virus and had been fed on artificial food.

Material was examined as thick (40 to 150 μm) celloidin (B.D.H. Necol-Collodion) sections mounted, serially, under coverslips in Permount (Vaisamurat & Hess 1953). Washing in distilled water for 6 to 8 h prior to dehydration in ethyl alcohol does not result in loss of impregnation or fading as has sometimes been supposed. Some preparations of *Sphinx* have suffered darkening of the unstained background. Such changes have also been observed in vertebrate tissue (Guillery 1966; Boycott & Dowling 1968).

Although the standard Golgi-Cox (1891) and Golgi-rapid procedures (Gatenby & Painter 1937) failed to give results, a modification of the double-impregnation procedure (Kallius 1910) resulted in the impregnation of only a few components clearly shown up against a deep brown background of unimpregnated tissue. For this method the tissue is fixed in buffered gluteraldehyde for 24 h and then washed in distilled water for 6 h prior to immersion in a solution containing 1 % OsO_4 5 ml, 2.5 % potassium dichromate 20 ml for 6 days (the solution is renewed every 24 h). The tissue is then washed for 6 h in distilled water before immersion in 0.75 % silver nitrate for 3 days in darkness, at 20 °C. The tissue is embedded in celloidin and sections are mounted in Permount under coverslips. Some suppression of staining has been achieved in 10 preparations of *Pieris* by a particularly bizarre variant of the Golgi–Colonnier procedure which involves injection of a 1 % methylene-blue solution for 6 h prior to prefixation in buffered gluteraldehyde. These preparations characteristically reveal a small number of neural elements which are more evenly distributed throughout the medulla. When tangential cells are favoured by this procedure they are ideal for determining the horizontal extent of their processes (figure 50, plate 8). The mechanisms of these effects is not understood.

Other general stains

Azan (Romeis 1948), Goldner's (1938) modification of the Masson trichromatic technique and Nissl stains (Lillie 1965) were used for general reconstructions of brain regions and in some cases cell-body counts.

Drawings were made with a Vickers or Watson camera lucida, and with the aid of a squared eye-piece graticule. Measurements were taken with the aid of a Vickers screw-micrometer eye-piece, or a calibrated Zeiss scale graticule. Most observations were made using a Vickers microscope or a Zeiss 'Photomicroscope'. Some photographs were made with a Zeiss Ultraphot microscope, on Kodak plus-X Professional Sheet Film, developed in D. 76, the others were made with a Zeiss Photomicroscope, on Adox KB14 or Recordak Microfile (for extreme enlargements of low-power photographs) and also developed in D. 76.

The general features of Golgi stained preparations and their interpretations

Although the mechanism of the Golgi stain is obscure it is known that the impregnating material is deposited inside the cell (Blackstad 1965; Stell 1965). All variants of the Golgi stain impregnate only a very small proportion of cells in the brain. This selectivity is the stain's

outstanding beauty and advantage; as Sholl (1956) pointed out, if all the cells were stained, none of them could be individually distinguished. The Golgi stain allows a neuron to be identified and traced over considerable distances.

Golgi staining procedures have been used in some quantitative analyses of nervous systems (Sholl 1956; Ramon-Moliner 1961, I and II; Colonnier 1964) and in particular for determining the topography of arrays of neurons in certain brain regions (see, for example, Eccles, Ito & Szentagothai 1967). Boycott & Dowling (1969) have emphasized that it is impossible to make worthwhile statements about the relative proportions of the different kinds of neurons in the vertebrate retina since this would necessitate procedures that stained the total population of a cell-type identified by the Golgi method. A similar situation prevails in the insect optic lobes, but it is nevertheless possible to match some endings in non-selective preparations with those identified by the Golgi method. For example, the endings of the long visual fibres of the Lepidoptera and Diptera can be stained by Blest's modification of Holmes's silver method and this provides a convenient indication of the numbers of this kind of ending in their medullae (see figures 13 and 15, plate 4, and figures 17 to 21, plate 5). Similarly, apparently all the line tangentials of *Pieris brassicae* (p. 113) are stained selectively by the Fraser-Rowell modification of the Holmes silver procedure (figure 61, plate 9). However, it is rare to find this type of information by such direct comparisons.

The Golgi stain can never provide the certainty that all the species of cells in the optic lobes are receptive to impregnation by selective silver methods. All the cell types described by Cajal & Sanchez have been identified. Most types described by Zawarzin have also been seen, but certain neurons described in his account may be erroneous since in some of his illustrations he has probably interpreted two cells closely apposed to one another as a single entity. Cajal also expressed some doubt about a few of the cell-types described by the Russian author. In addition to the cell types described by the earlier workers many others have been identified which were either hitherto unappreciated or unimpregnated in their preparations. It is therefore particularly important to define the kinds of evidence upon which the findings in this account are based.

Other variants of the Golgi technique have failed to demonstrate that the Golgi–Colonnier procedure, using buffered gluteraldehyde, is consistently 'missing' certain cell types in the optic lobes. But, this is not a conclusive argument: tissues treated with Golgi procedures that employ fixation in osmic acid show up fewer cell-types than procedures which employ fixation in buffered gluteraldehyde. It could be claimed that osmium fixation results in a consistent absence of certain cell types and that fixation in gluteraldehyde stains more types. Argument could have it that another method, yet to be devised, could show up even more species of neuron and so on *ad infinitum*. In the preceding section it was pointed out that variations of the pH and the molarity of the buffer solution could alter the affinity of the stain for a certain species of neuron. These variations in pH and molarity have now been exhausted and it could be claimed that all the cell-types receptive to impregnation by the Colonnier technique have been seen. However, there may be neurons which cannot be stained at any pH, molarity or temperature and others may be simply too small to be resoluble by light microscopy. The fibre diameters of some endings appear larger or smaller in material fixed in osmic acid than do those observed in material treated by the Golgi–Colonnier technique. Such discrepancies are most notable in the case of the long visual fibre endings in the medulla of *Pieris*; the linking-fibres of these cells have a larger diameter in the osmium-fixed material than they do in

gluteraldehyde-fixed brains, whereas the reverse situation applies to the terminal processes. However, all the components of a cell-type observed in tissue treated with osmium or gluteraldehyde are readily identifiable and there are none seen in brains treated with the former fixative that are not seen in those treated by the latter.

In this account the descriptions of neurons and their geographical and topographical arrangements and relationships have been determined by observations of material treated by the Golgi–Colonnier procedure. There are two exceptions; an ending in the medulla of *Pieris* which is derived from a cell-body between the lamina and the medulla, and an amacrine cell deep in the medulla of the same species.

Although processes appear to be closely applied against one another there are, as yet, no criteria from these preparations to prove synaptic contact. Intimacy between neurons is suggested during this account, but direct proof is lacking. Cajal (1937) argued that in the vertebrate retina rods and cones must be separately connected to other neural elements since, if it were otherwise, separate scotopic and photopic elements would be superfluous. He pointed out that there was no clear anatomical proof of this because the receptor and underlying neural elements could not be stained together with each other. In the insect material receptor and neural elements are sometimes stained so that they appear in contact with each other in the lamina (figure 23, plate 14: part II). In three species of Diptera some synaptic relationships between them have been determined by electron microscopy (Trujillo-Cenoz & Melamed 1966; Melamed & Trujillo-Cenoz 1967). Other endings in the lamina, whose connexions have not yet been determined by this technique, have been observed in Golgi–Colonnier preparations and it is tempting to suggest definite synaptic relationships for them, but an assertion of this kind is, nevertheless, a presumption. For instance, Boycott & Dowling (1969) point out that the classic axosomatic junctions for rod bipolars to ganglion cell perikarya described by Cajal (1933), from Golgi preparations, in the retina of many mammals and fishes has not been substantiated by electron microscopy; no typical synaptic specializations have been found between those two components, although tight junctions have been detected (Cohen 1967; Raviola & Raviola 1967).

Blackstad (1965) and Stell (1965) have shown that the relationship of one cell to another can be determined by the electron microscopy of Golgi impregnated cells. This technique, although out of the scope of this light microscopy investigation, may yield valuable information from insect material. At present most arguments for junctions between particular cells must be made with reference to previous electron microscopy studies of the receptor layer (see, for example, Pedler & Goodland 1965; Trujillo-Cenoz 1965; Trujillo-Cenoz & Melamed 1966; Melamed & Trujillo-Cenoz 1967). In deeper levels of the optic lobe likelihoods of connexions between cells can only be suggested on the basis of somewhat circumstantial evidence.

There are four levels of reliability in identifying the cell types in the optic lobe: (1) A cell is seen, well impregnated and in a clear or moderately clear field, in its entirety. This is true, for example, in many preparations which show up perpendicular and amacrine cells. (2) Sometimes a cell can be traced in its entirety through a background of many other well-impregnated fibres. Such preparations are usually derived from brains that have been prefixed. Cells recognized in them include the ones mentioned above and, in addition, many of the narrow-field tangential cells of the medulla and lobula complex. Some projections to the mid-brain and especially the optic tubercle have been seen on one section or reconstructed from a series of sections of this type of preparation. (3) Only parts of cells can be visualized in any one section, either because of incomplete impregnation, failure to follow their processes when these

extend over more than one section, or because a cell-type appears in brains so densely impregnated that part of it is always lost in crystals or deposits which prevent its resolution. This is particularly the case for large-field neurons, but has been largely overcome by careful control of the pH of the prefixation solution. However, for most cells in this category, and even for the tangentials with extremely large fields which extend over the whole of a regional surface, it is possible by careful measurement and the study of a large number of serial sections and preparations to piece together neurons in what is believed to be a reliable way. In addition, those cells which are only partially impregnated usually show the uncoloured portions of their processes as resoluble unimpregnated 'ghosts'. (4) Only fragmentary portions of cells are available for study and the cells to which they belong cannot be entirely reconstructed. This category is particularly liable to provoke erroneous interpretation through failure to recognize fragments of cell types with which one is already familiar. In most cases we have avoided classifying observations in this category, but the more outstanding components which could not be traced out and assigned are listed as *incerta sedis*.

Observations in the first two categories are impregnable, and so also are many reconstructions in the third. Wherever these latter are open to doubt this has been noted. Those in the fourth are best ignored; however, most observations of the tangential systems are necessarily incomplete because these neurons send branches to distant points of the mid-brain and in some cases the contralateral optic lobe, suboesophageal ganglion and ventral nerve cord. These projections are still being analyzed and will be fully described in a separate section (N. J. Strausfeld, in preparation).

With regard to fine detail, a certain amount depends on the techniques of impregnation. The Colonnier method produces a degree of shrinkage, and fibres may often appear dilated with blebs, possibly due to swelling of the mitochondria induced by gluteraldehyde. There is, though, some evidence that blebs are not necessarily artifacts, for blebs are characteristic of particular species of neurons and do not occur in others. Secondly, there is evidence from electron microscopy that the antennal nerves of *Bombyx* are naturally blebbed and that the mitochondria appear large in relation to the fibre diameter whether the tissue is fixed in gluteraldehyde or not (R. A. Steinbrecht, personal communication). Preparations fixed in a mixture of osmic acid and potassium dichromate characteristically show up very few cells against a background which does not clearly reveal stratifications or striations characteristic of the optic lobe neuropil. Golgi–Colonnier preparations reveal more elements with good definition of fine processes and unimpregnated background features. By and large fixation in the prefixation fluid seems less 'aggressive' to the tissue than direct fixation in unbuffered gluteraldehyde or a mixture of osmic acid and potassium dichromate.

Terminology

The terms lamina, medulla, lobula and lobula plate have been used to describe the first, second, third and fourth geographical regions of the optic lobes. This nomenclature conforms to that used by Bullock & Horridge (1965) and Larsen (1966). In the Lepidoptera, as in the Diptera, the lobula plate is a posterior discoid component of the lobula complex. The locations of some of the regions in the mid-brain and suboesophageal ganglion have been described by earlier workers (see, for example, Viallanes 1886, 1887*a*, *b*; Kenyon 1896; Pflugfelder 1937; Power 1943*a*; Jawlowski 1960) using species other than those of the Lepidoptera whose medial

lobes and protocerebrum have not been adequately described. The corpora pedunculata of the Lepidoptera are relatively small and the intrinsic cells are somewhat differently arranged from those of *Apis* which Kenyon (1896) described from Golgi preparations (N. J. Strausfeld, unpublished). However, the central body and the optic tubercles appear to have similar organizations in the Lepidoptera to those of the Diptera and of the bee. An attempt has been made to equate some mid-brain regions in the Lepidoptera with those in the Diptera and other orders of insects (Power 1943*a*; Larsen 1966; Vowles 1955; Goll 1967). This is though, an unsatisfactory procedure and future knowledge of the precise projections of fibres to the mid-brain from the optic lobes and between different mid-brain regions may make necessary a revision of the old nomenclature of these regions. Precise information about the central projections of the optic lobe tangential endings cannot be offered in this paper and only the general direction in which they project is described.

The stratification of each geographical region in the optic lobes is precise. Lateral processes of the various neuronal components are characteristically situated within specific strata. Some details of lamina organization have been described by Trujillo-Cenoz & Melamed (1966) and the terminology they use for the zones of this region has been adhered to in this paper. The medulla and the lobula complex present difficulty. Each species has a particular number of distinct strata in these regions and it is most convenient to define these by one characteristic type of ending. Thus the second layer of the medulla of *Pieris* is derived from the deepest monopolar cell endings, likewise in *Sphinx*. Three layers of gross stratification can be seen in the medulla of all the species studied; an outer, middle (serpentine) and inner layer. These will be referred to when comparisons of the gross organizations of the medullae are made between species, or, on very rare occasions when a cell component lies within one of these layers but is not invariably located at a particular stratum within it.

The difficulty in devising criteria for synaptic relationships from Golgi preparations has already been mentioned. Furthermore, Dowling & Boycott (1965, 1966) have demonstrated reciprocal contacts between primate retinal elements. Anatomical and physiological evidence has also been found of reciprocal contacts between mitral cell dendrites and granule cell processes in the mammalian olfactory bulb (Rall, Shepherd, Reese & Brightman 1966). Similarly, Trujillo-Cenoz (1965) figures reciprocal contacts between receptor and second-order neurons in the eyes of lycosid spiders. Thus it is dangerous to imply from light microscopy that a cell process is specialized to receive or to donate information. Terms that imply direction of transmission have also been avoided. Specification of particular processes as dendrites or axon terminals has been avoided in the absence of physiological information as to their function. Instead they are called axis-fibres, linking-fibres, lateral processes and so on, and the neutral terms 'process' and 'ending' are used. A guide to these terms is shown in figure 1. Many cell components are composed of characteristically shaped processes. These may, for example, be spiny or blebbed. The commonest forms of specializations, typical of insect optic lobe elements, are illustrated in figure 1*d*. The sections have been cut predominantly in three planes: vertically, horizontally and tangentially. Field-sizes of groups of processes have been given as the maximal extents in both horizontal and vertical planes, unless otherwise specified.

Camera lucida drawings (using a grid graticule eye-piece) have been used for all the line illustrations of neurons. Cells were illustrated from adult animals, shortly after eclosion, with the exception of figure 73. Unless otherwise stated in the figure legends all the illustrations in plate and text figures are derived from Golgi–Colonnier stained material cut horizontally.

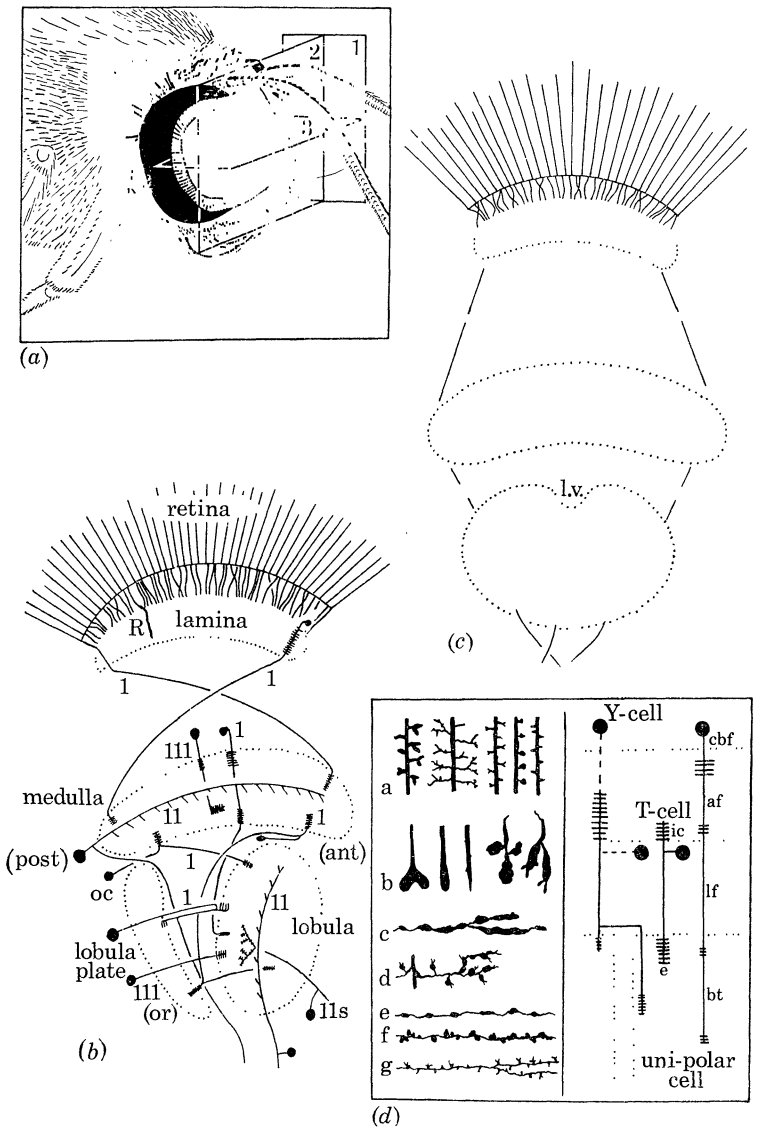

FIGURE 1. Introductory diagram: terminology.

(*a*) The three main planes of section: 1 = tangential; 2 = vertical (dorso-ventral); 3 = horizontal (antero-posterior) ≡ to plane of first chiasma cross-over.

(*b*) A schematic diagram illustrating optic lobe terminology (horizontal plan): 1 = class I (perpendicular) cells (R = retinula cell); 11 = class II (tangential) cells; 111 = class III (amacrine) cells; 11S = small-field subclass of II (these elements are exclusive to the lobula complex). The cross-over of fibres between the lamina and the medulla constitutes the first optic chiasma. The cross-over between the medulla and lobula constitutes the second optic chiasma. Fibres which connect the lobula and lobula plate form the intra-complex tract. Medulla orientation: ant = anterior; post = posterior. Lobula complex orientation: or = oral edge of the lobula; oc = ocular edge of the lobula. The outer face of the lobula and lobula plate are opposite one another; thus the anterior edge of the lobula means its inner face, and the anterior edge of the lobula plate means this region's outer face.

(*c*) Vertical sections of the optic lobe reveal the lamina, medulla and usually either the lobula or the lobula plate. No cross-over of fibres is visible in this plane. l.v. = lobula valley.

(*d*) Left hand inset: some camera lucida drawings of processes and their specializations. Row a: forms of lateral processes from perpendicular axis-fibres. From left to right: short varicose processes, spiny branching processes, branched lateral spines, unilateral knobs, unbranched spines. Row b: left: three forms of simple endings; a forked, club and plug ending. Right: varicose and varicose spiny terminal swellings of the processes of some class II cells. c. varicose processes. d: lateral processes with tubers (thick swellings) and spines. e: a blebbed process. f: tuberous processes. g: a spiny process. Right hand inset: The three arrangements of class I cell components. The Y-cell invests three regions. Its cell-body may be linked to the outermost axis-fibre either from above the outermost region that this cell invests or from between one of the inner regions. The T-cell has a characteristic T arrangement between its axis-fibre and cell-body fibre. The group of processes nearest this T-junction is here termed the initial component. The cell-body fibre of the unipolar cell is continuous with its axis- and linking-fibre (cells of this type include monopolar cells and transmedullary cells). cbf = cell-body fibre; ic = initial component; e = ending; af = axis-fibre; lf = linking-fibre; bt = a bistratified ending (or terminal).

Scales have not been drawn for each plate figure: in both accounts where one plate contains several figures at the same magnification the scale has been drawn over only one of them. Figure scales and their calibrations are listed at the end of the legend to each plate.

Classification of the cell-types in the optic lobes

A more serious problem concerns the classification of the types of neurons. With so many distinct forms it would be merely confusing to use descriptive terminology throughout. Some neurons lend themselves to such terms as: 'pi-cells', 'line fibres', 'T-cells' and 'Y-cells', all expressing the *gestalts* of distinct categories of neurons when seen in profile. A more satisfactory approach is to number cell-types off from the periphery, relative to the positions of their cell-bodies. This is the method used by Bullock & Horridge (1965). Unfortunately they chose to enumerate the cell-types 1 to 29, and this leads to difficulties. Certain cell-types described by them have not been identified in the Lepidoptera, either because they are absent in this order or because they have not been impregnated; but others have been found which they have not listed. In *Calliphora* all the cell-types described by Cajal (1909, 1910) and Cajal & Sanchez (1915) have been found as well as additional ones. There are at least six species of T-cells against two described in the Bullock and Horridge account. The Y-cells described in this paper do not seem to have been hitherto recognized, apart from an ambiguous drawing in the Cajal & Sanchez account of what appears to be the lobula and lobula-plate components of a Y-cell in *Tabanus*.

Cajal & Sanchez divided the neurons of the optic lobe into three categories which were based on the distribution of the processes arising from the cell-body. Type 1 had a single unbranched fibre projecting to a geographical region where it gave rise to one or more groups of lateral processes. Type 2 had a cell-body fibre which bifurcated outside a geographical area so that the cell-body fibre and the two branches derived from it formed a typical T-shape (see figure 1*d*); these two branches together form the linking-fibre between two geographical regions. Type 3 had a single cell-body fibre which projected to one geographical region where its diameter considerably enlarged before projecting into it: this prolongation, here called the 'axis-fibre' projected through a region and then to one or more others, as a 'linking-fibre', and had groups of processes from it in each.

This classification was further complicated by Cajal's definition of the amacrine cell in insects. In his 1909 paper he described a species of neuron in the lamina of *Musca* as having several branches, arising near the perikaryon, which project into the lamina. He called these cells spongioblasts or 'cellulas amacrinas'. In Cajal & Sanchez's account the type 1 neurons are also called amacrines, as were any others that were situated intrinsically within a geographical region. It is possible that he was making an analogy between the amacrines of the vertebrate retina when he described the 'cellulas amacrines' in 1909 but later decided to include all neurons which invested one region only under the same heading. His definition of amacrines in the vertebrate includes all neurons lacking an axonic component (Cajal 1933). We have altered the Cajal & Sanchez classification so that their types 2 and 3 neurons are included under the same heading and the relatively unknown large tangential cells put in a separate one. In this and subsequent accounts the neurons of the optic lobe have been divided into three classes: *Perpendicular*, *Tangential* and *Amacrine* cells. These are characterized by the orientation of their long axis to that of the outer and inner surfaces of the geographical region in which they lie and by their projections to other brain regions.

Class I. The perpendicular cells. These cell-types are arranged so that their axes are at right angles to the surfaces of a geographical region in which they lie.

Class II. The tangential cells. These have optic lobe fields which are arranged so that their axes are parallel to the surfaces of their geographical region. The ratio of the perpendicular and tangential field-spreads of the two classes is also different; for the former class the ratio is between 1 : 1 and 6 : 1 and for the latter 1 : 40 and 1 : 1000. Both class I and class II cells send fibres from one geographical region to another.

Class III. Amacrine cells. This class of neurons shares orientation and field-size characteristics with the former two classes but differs in one important respect: it does not have any members which link one geographical region with another. Type 1 cells of the Cajal & Sanchez classification fall into this third class. Types 2 and 3 fall into the perpendicular class of neurons.

Each class has been divided into groups, or in some cases subclasses. For example class I cells are further divided into transmedullary cells, T-cells, Y-cells, among others. An abbreviation is assigned to each cell-type and a further letter precedes it to indicate some specific characteristic such as the outermost of the geographical regions in which the processes lie. The individual cell types are then numbered off. Thus the wide-field tangential in the medulla of *Pieris* (figure 50, plate 8), whose processes form a plexus of characteristic fibres (figure 49, plate 8) at the level of the shallow monopolar cell endings in the medulla stratum 2 is designated M : tan 2—i.e. as the second of a series of medullary tangential fibre types. Cell types and their abbreviations are given in the appropriate figure legends. Most cells are described from whole elements on single sections.

RESULTS

The gross features of the optic lobe

The supra-oesophageal ganglion is flanked at both sides by an outgrowth of the brain, which is directly surmounted by the retina. This outgrowth is called the optic lobe. The lobe is *Sphinx* is connected to the mid-brain via a stalk, the optic peduncle. In *Pieris* the peduncle is not macroscopically distinct.

There are three main geographic regions in the optic lobe; the lamina, the medulla and the lobula complex. In the Lepidoptera and Diptera, the lobula complex has two components; the lobula, and posterior to it, the lobula plate (figures 1*b* and 42). These areas are characteristically similar in shape, though not in size, in all species of the two orders and for this reason *Pieris* will be cited as the representative example.

The lamina

This has a shallow, vertically elongated, dome-like structure whose peripheral convex surface lies directly under the retina, separated from it by the basement membrane. The depths between the outer convex surface of its external plexiform layer and the inner concave surface is more or less uniform throughout, varying from 35 μm at the edges, to 50 μm in the middle.

First optic chiasma

This separates the lamina from the medulla. It is composed of fibres linking these two regions. Fibres from the anterior portion of the lamina end in the posterior portion of the medulla and vice versa. There is only one apparent plane of cross-over, roughly antero-

posteriorly, seen in horizontal sections; the projection of these fibres is further discussed in the succeeding account.

The medulla

In horizontal section this has the appearance of a blunt aerofoil, whose anterior portion has a maximum depth of 150 μm, its median portion 165 μm, and posterior portion 130 μm. In vertical section the outer convex surface is more or less parallel to the inner, concave surface (figures 55 and 56).

The lobula complex and the second optic chiasma

The lobula lies centrally to the anterior portion of the medulla. In horizontal section its convex outer surface is clearly demarcated, whereas there is no clear margin between its inner face and the background of tangential linking-fibres, cell-bodies and cell-body fibres. In vertical section the lobula appears scallop-shaped, with a smooth outer surface, divided by a medial 'valley', through which run a number of linking-fibres to the mid-brain, derived from medullary tangential components.

In horizontal section the lobula plate is crescent-shaped, and in vertical section its outer surface appears as a symmetrical, shallow curve. Again there is no clear demarcation between its inner, posterior, face and the background.

There is a second optic chiasma between the medulla and the lobula and lobula plate: anterior medullary fibres destined for the lobula enter the oral portion of the lobular arc (see figure 42). Fibres from the mid-portion of the medulla enter the mid-portion of the lobula, and so on. Medullary fibres destined for the lobula plate obey the same pattern of dispersion. Again this chiasma, seen in horizontal sections, has only one apparent plane of cross-over. In addition, there are fibres running between the two components of the lobula complex: those that leave the ocular portion of the arc of one enter the ocular portion of the arc of the other, and so on. This lobula-lobula plate dispersion is here termed the intra-complex tract.

Gross distribution of other fibre tracts leaving the optic lobe and the distribution of cell-bodies

Tracts, derived from tangential endings, leave the anterior margin of the medulla to pass dorsally over, or in between, the lobula and lobula plate. Tracts leaving the inner face of the medulla may also pass through the lobula 'valley' towards the mid-brain; others may project directly through the lobula to emerge from its anterior face as one of two tracts comprising the anterior optic tract to the optic tubercle (figure 67, plate 10). There are several other distinct tracts which leave the inner face of the lobula, that can be traced to the mid-brain or contralateral optic lobe. The central edge of the lobula plate is perforated by a single tract, which later divides into several small ones towards the mid-brain; the fate of these tracts will be described in a subsequent account in greater detail (N. J. Strausfeld, in preparation).

The cell-bodies of the neurons are distributed as follows: monopolar cells in the lamina are confined to a discrete layer between the external plexiform layer and the basement membrane. The first optic chiasma is surrounded by cell-bodies of some perpendicular cells, in particular transmedullary cells, linking the medulla and lobula, and T-cells linking the medulla and lamina, and also some amacrine cell-types. Interspersed between chiasmal fibres, above the outer face of the medulla, are cell-bodies of other amacrine cells and some medullary tangential elements. Most tangential elements in the medulla are derived from cell-bodies situated at its

anterior edge (figures 56 and 57). These 'globuli' cells (Larsen 1966) are comparatively large, having a diameter of over 30 μm compared with the average 15 μm diameter of the perpendicular neurons and some small-field tangentials. The remainder of the tangential elements are derived from cell-bodies situated centrally, in the mid-brain, ventral nervous system or in the contralateral lobe. Doubtlessly some tangential components in the lobes are terminal components different from other central nervous system regions. Cell-bodies of T- and Y-cells which link the medulla to the lobula regions lie posteriorly to the lobula plate as a sheath over its inner face, or between the lobula plate and the portion of the medulla external to it. Interspersed between fibres of the second optic chiasma of *Sphinx* are cell-bodies of amacrine cells whose prolongations penetrate the inner face of the medulla. Anterior to the inner face of the lobula are the cell-bodies of some lobula tangentials. In the Lepidoptera lobula amacrine cell-bodies are most probably situated dorsally above this region.

The disposition of glia in the optic lobes has not been closely examined. Glia cells are occasionally impregnated by the Golgi stain but usually only in pupal animals. Glia invests both the outer and inner faces of the medulla. Their somata are situated closely adjacent to these two margins. Glia somata are also situated behind the posterior face of the lobula plate; these send prolongations into the lobula plate neuropil where they branch profusely. These branches give rise to extremely fine processes which pass across the intra-complex tract to penetrate the outer face of the lobula. They subsequently give rise to similar branches which form a dense mass in the lobula neuropil. For detailed accounts of the glia cells of insect brains the reader is referred to Cajal & Sanchez (1915) and Sanchez (1935). There is no evidence from the present species that cell-bodies lie within the medulla, lobula, or lobula plate neuropil.

The neurons of the lamina

Introduction

There are two major zones of laminal stratifications between the basement membrane, which corresponds to the 'membrane limitante posterieure' (Ciacco 1876), and the first optic chiasma (Cajal & Sanchez 1915): (1) an outer (cell-body) layer containing the cell-bodies of the monopolar neurons, and (2) an inner (external plexiform) layer which is striated perpendicularly in a way which reflects the columnar arrangements of the monopolar cells and retinula cell endings. The lamina has been divided more specifically into three strata (Cajal & Sanchez 1915; Trujillo-Cenoz & Melamed 1966); the fenestration layer lies directly under the basement membrane and is characterized by numerous tracheae. The cell-body layer lies below this. The third stratum is called the external plexiform layer and lies beneath the cell-body layer. In some species such as *Schistocerca* and *Sphinx* the fenestration layer is wide (figure 2, plate 3) and obviously demarcated, while in others, for example *Eristalis*, the fenestration layer and the cell-body layer are almost indistinguishable.

In addition to the first-order receptor fibres, which include the long visual fibres, Cajal & Sanchez described four other types of cells in the lamina. These comprise the monopolar cells, amacrine cells (their spongioblasts, which they also termed brush cells), centrifugal elements and tangential elements. There is no evidence that all the elements they listed as centrifugal conduct from the medulla to the lamina. Thus their so-called centrifugal fibres have here been listed under the *gestalt* names given to them by the Spanish authors and infer only a morphological implication of fibre growth directed peripherally from a more centrally positioned cell-body.

Observations on fibres derived from the retina

There are two main types of visual cell fibres which project from ommatidia to regions in the optic lobe; these are the retinula cell fibres which end in the external plexiform layer of the lamina, and the so-called long visual fibres which ultimately terminate in the outer layer of the medulla. The retinula cell fibres of *Pieris* end in discrete groups of between six and eight, which with the accompanying axis-fibres of two monopolar cells make up an optic cartridge. This arrangement agrees basically with observations on other species (for example, Cajal & Sanchez 1915; Braitenberg 1966; Trujillo-Cenoz 1966). In the Diptera, however, two or three other types of endings, derived from cell-bodies situated at deeper regions of the optic lobe, can be associated with each optic cartridge (see part II, p. 143). The long visual fibres project through the external plexiform layer to end, via the first optic chiasma, in the medulla. Long visual fibres derived from anterior ommatidia end in the posterior region of the medulla, those derived from posterior ommatidia end anteriorly in the medulla. There is no evidence of a cross-over in the vertical plane. The Lepidoptera have been variously described as having 8 to 10 receptor elements within each ommatidium (Johnas 1911; Yagi & Koyama 1963; Fernandez-Moran 1958). Nowikoff (1931) describes *Pieris napi* as having nine and the Sphingid, *Manduca sexta*, is reported to have 7 or 8 retinula cells in each ommatidium plus a basal rhabdomere (Carlson, Steeves & VandeBurg 1967).

In *Pieris* there are three forms of retinula cell ending in the lamina:

(*a*) *The type 1 retinula cell ending*. This is a stout plug terminal that lacks resoluble lateral processes, but which may appear pitted along its length in the external plexiform layer. Its diameter is between 1 and 2 μm (figure 25; figure 3, plate 3).

(*b*) *The type 2 retinula cell ending*. This fibre is extremely slender, with a diameter less than 0.6 μm, and has a single collateral near its laminal end. This terminal branch has a diameter of less than 0.3 μm and extends between 2 and 4 μm laterally (figure 25).

(*c*) *The type 3 retinula cell ending*. This has dimensions like those of the type 1 ending but in addition has two branches in the cell-body layer. These are between 3 and 7 μm long, and arranged horizontally and bilaterally with respect to the axis of the retinula cell ending (figure 25; figure 9, plate 3). Discrete groups of retinula cell endings of an optic cartridge have been observed containing one type 2 and two type 3 endings. In addition, there are probably three or four type 1 endings in each group. Collections of retinula cells in *Sphinx* contain two type 3 endings and at least four type 1 endings. No type 2 endings have been seen in this species. The optic cartridges cannot be clearly defined in *Sphinx*. One giant monopolar cell may have a lateral spread through over 100 retinula cell endings (figure 75).

Pairs of long visual fibres leave the retina to end finally, in pairs, in the medulla. The Spanish authors (Cajal 1909, 1910; Cajal & Sanchez 1915; Sanchez 1916, 1918) recognized one type of long visual fibre in most of the species they described. Zawarzin did not see this fibre in *Aeschna* but there is some evidence that it exists, even though it has only been detected in relation to the fenestration layer, cell-body layer and external plexiform to first optic chiasma (unpublished data). The earlier authors failed to recognize the paired arrangement of these fibres. In *Sphinx*, *Pieris*, *Automeris* and the species of Diptera (part II) both members of a pair are resoluble in clearly impregnated preparations. They are, though, closely apposed to one another for most of their length and can easily be interpreted as a single fibre.

In *Sphinx* and *Pieris* there are three distinct forms of long visual fibre recognizable in the lamina, and three form-variants of long visual fibre endings in the medulla: these will be described in the section dealing with medullary components. The lamina characteristics of long visual fibres are as follows:

(*1a*) *The spiny long visual fibre.* This has a diameter of between 2 and 3 μm and has spines, less than 0.5 μm long, arranged radially down its length in the cell-body and external plexiform layer (figures 25 and 26; figure 4, plate 3).

(*1b*) *The smooth long visual fibre.* The dimensions of this variant are the same as the preceding one; however, it lacks spines.

(*2*) *The wide-field long visual fibre.* This has a similar diameter, between 1 and 3 μm, and has characteristic recurrent lateral processes arranged uni- or bilaterally along its length in the cell-body and external plexiform layer (*Pieris*, figure 25) or arranged radially down part of its length near the inner margin of the external plexiform layer (*Sphinx*) (figure 5, plate 3). These lateral processes are between 5 and 10 μm long. Each pair of long visual fibres that has been detected consists *either* of two smooth fibres, a smooth and wide-field fibre or a smooth and a spiny fibre. A similar situation exists in species of Diptera.

Observation on monopolar cells

These class I, first-order inter-neurons, providing the first relay between the visual cells ending in the lamina and higher-order neurons in the medulla (see Trujillo-Cenoz & Melamed 1966) were termed 'monopolar' cells by the Spanish authors. Their perikarya lie above the external plexiform layer and form the cell-body layer of the lamina. There is characteristically a single prolongation from each cell-body which eventually projects perpendicularly through the external plexiform layer to end in the medulla after crossing-over via the first optic chiasma. Again this cross-over is seen only in the antero-posterior plane. Lateral processes arise from the axis-fibres of these cells at some or all levels of the external plexiform layer. In the Diptera one form of monopolar cell, with diffusely arranged branches that extend radially from the axis fibre through the external plexiform layer, is supposed to be synaptically intimate with all the receptor endings in a cartridge (Trujillo-Cenoz 1965; Trujillo-Cenoz & Melamed 1966).

Cajal & Sanchez divided the monopolar cells into two main groups. Those that had lateral processes arranged down the whole length of the axis-fibre in the external plexiform layer were called giant monopolars. Other forms were called small monopolars. It is felt here that this is an ambiguous basis for the classification of these cells, since in some species the so-called giant monopolars have lateral processes restricted to one optic cartridge while in others the processes may extend through many groups of retinula cell endings. In this account monopolar cells are reclassified as follows: (1) giant monopolar cells have lateral spreads through more than one discrete group of retinula cell endings; also their fields overlap, (2) small monopolars have lateral spreads confined to one optic cartridge, though some processes, in the cell-body layer, extend over the perimeter of an immediately adjacent optic cartridge.

Giant monopolar cells

There are two types of giant monopolar cell. Radial diffuse giant monopolars have lateral processes at all levels in the external plexiform layer. They have been observed in *Sphinx* and *Automeris* and were figured by Cajal & Sanchez in the lamina of *Aeschna*. Stratified giant monopolar cells have been seen in *Apis* and in the locust (N. J. Strausfeld, unpublished): these

cells have wide-field lateral processes restricted to a narrow layer at the inner margin of the external plexiform layer. There are three variants of monopolar cells in *Sphinx* characterized only by their lateral extent (see figures 6 to 8, plate 3). Processes are arranged radially from the axis-fibre through the whole of the external plexiform layer.

Small monopolar cells

There are four forms of these elements: two types of radial monopolars, bilateral monopolars and midget monopolars. In many respects the monopolar cells of *Pieris* are similar to those of the Diptera. There are no large-field monopolars in any part of the lamina, although some radial cells have outermost processes that are sometimes seen to extend into the immediately adjacent cartridge either side of it. Three distinct species of small monopolar cells are recognizable in the lamina of *P. brassica* and *P. napi*. Two of them have processes restricted to within the confines of an optic cartridge (they have lateral spreads of between 9 and 11 μm) and the third (radial) has processes that extend as far as the immediately adjacent cartridge. The narrow-field cells are comprised of (*a*) small-field diffuse cells that have both branched and unbranched processes arranged bilaterally down the whole length of the axis-fibre in the plexiform layer; and (*b*) small-field bistratified bilateral cells which have their processes confined to the cell-body layer and the plexiform layer. A fourth, infrequently stained cell, has unilaterally arranged processes from its axis-fibre just beneath the cell-body or at the outer margin of the plexiform layer. This cell has been here termed the midget monopolar cell. There is also a variant of the radial monopolar cell which has its processes in lower half of the plexiform layer. These elements are illustrated in figures 25 (*Pieris*) and 26 (*Sphinx*). Their medullary endings are described on p. 101.

Observations on class II elements in the lamina

Only one form of tangential component has been seen in its entirety in the laminae of either of the present species. In *Pieris* this element is bistratified with processes that are disposed in two layers; thick fibres, oriented vertically and horizontally give rise to finer branches that arborize over the outer and inner face of the plexiform layer. Thus, seen in tangential section, this region appears covered by a dense network of multi-branched processes. Short collaterals

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DESCRIPTION OF PLATE 3

The lamina

FIGURE 2. *S. ligustri* (Holmes–Blest preparation). The lamina stratification: Ret = retina; F = fenestration layer (outer stratum, note the bundles of receptor fibres); Fb = fenestration layer (inner stratum); C = cell-body layer; EP = external plexiform layer; 1st OC = first optic chiasma (outermost 20 μm). The fenestration layer is indivisible into two strata in *Pieris* and the Diptera.

FIGURE 3. *S. ligustri*. Type 1 retinula cell endings in the external plexiform layer.

FIGURE 4. (*S. ligustri*.) Spiny long visual cell fibres.

FIGURE 5. *S. ligustri*. Wide-field long visual fibre.

FIGURE 6. *S. ligustri*. Small-field radial giant monopolar.

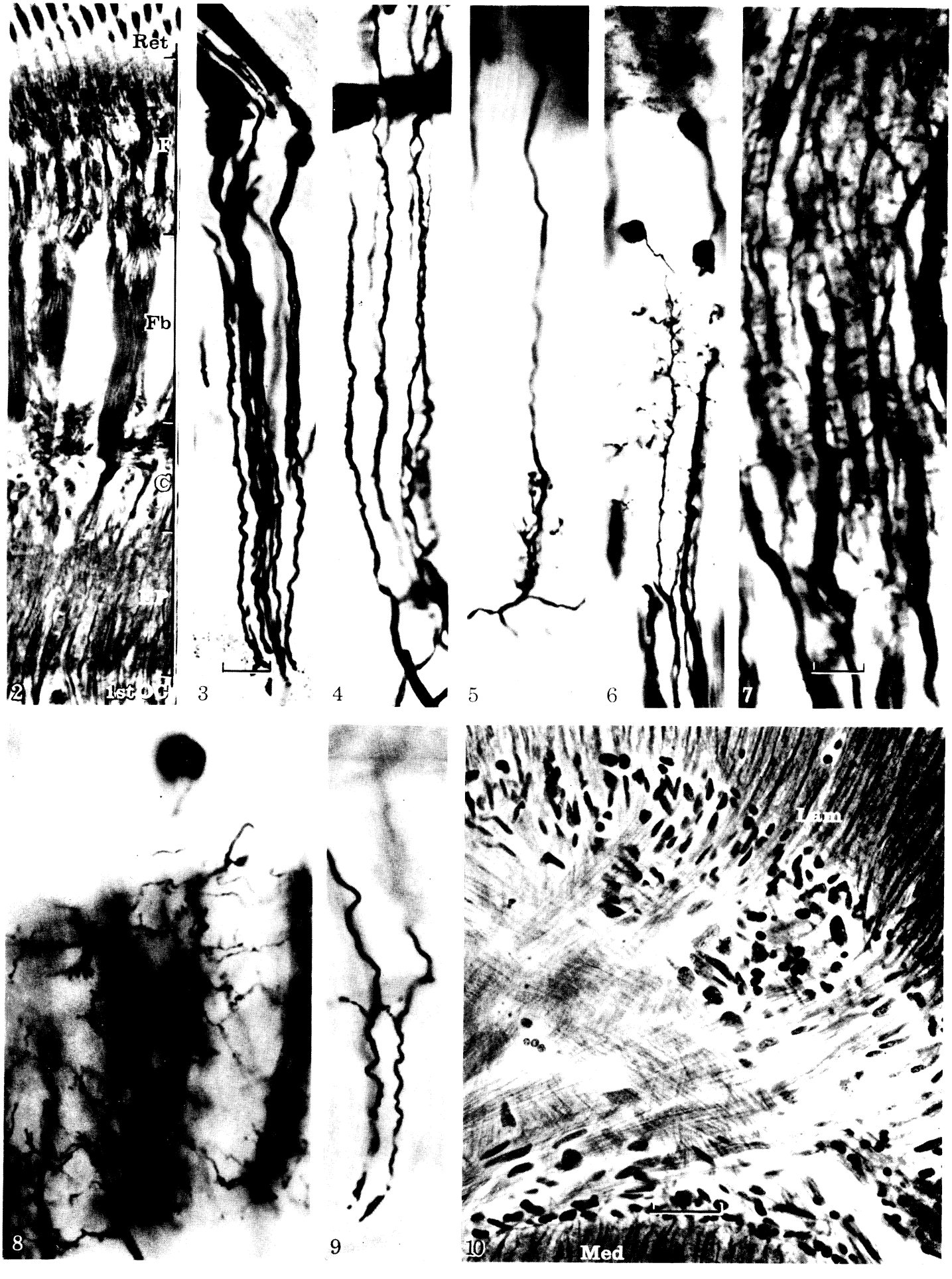
FIGURE 7. (Holmes–Blest preparation). The axis-fibres and lateral branches of giant monopolar cells in *Sphinx*.

FIGURE 8. *S. ligustri*. The spiny processes of wide-field monopolar cells.

FIGURE 9. *P. brassicae*. Two type 3 retinula cells.

FIGURE 10. *S. ligustri* (Holmes–Blest preparation). The first optic chiasma showing the distribution of small perikarya directly beneath the plexiform layer and glia (multipolar) cell-bodies.

Lam = lamina; Med = medulla. Figure 2, external plexiform layer = 70 μm; figures 3 to 9, scale = 10μm; figure 10, scale = 20 μm.



(*Facing p. 98*)

are also derived from the thicker fibres, at the outer margin of the plexiform layer, which project peripherally as far as the level of receptor fibres derived from interommatidial sensory hairs (N. J. Strausfeld 1968). The layers of the tangential fibres are arranged so that any class I or class III cells could contact them at the outer margin of the plexiform layer, and all, bar some retinula cells, contact them at its inner margin.

Three or four large cell-bodies are situated at the extreme mid-line anterior edge of the lamina. One preparation of *P. brassicae* shows a perikaryon joined to a large proportion of the tangential network in this region: from serial sections it seems that a single cell-body gives rise to fibres which form a very wide (about one-fifth to one-quarter of the horizontal lamina extent) vertical strip-field that extends through the whole dorsoventral extent of this region. Branches at the inner margin of the plexiform layer give rise to collaterals that project across the first optic chiasma to the surface of the medulla. Each branch extends along its outer face for between 100 and 180 μm, horizontally. Unilateral collaterals, derived from these, pass perpendicularly through all the medulla strata and end just above its inner margin (figure 25). Lateral spines arise from these processes in strata 3, 4, 5, 7 and 8.

A tangential element has been impregnated in only four preparations of *Sphinx*. From the fragmentary evidence that is available it seems that a fine arborization of blebbed fibres invests only the cell-body layer of this region. The processes stem from a thick (between 4 and 7.5 μm diameter) linking-fibre that can be traced from the anterior edge of this region towards the mid-brain, but which bypasses the medulla *en route*. Its cell-body location is not known, nor have we been able to trace the fibre to another neuropil destination. There is no evidence, from these preparations, that collaterals invade either the plexiform layer or medulla from this element.

Centrifugal endings in the lamina

Some elements in the lamina are derived from cell-bodies situated either beneath the external plexiform layer, or above the outer face of the medulla, or between the medulla's inner face and the lobula complex. Cajal & Sanchez described a few of these cells in the Diptera; they termed fibres that invaded the lamina from cell-bodies directly beneath the external plexiform layer 'cellulas amacrinas' (lamina amacrine cells). Other components to the lamina, from more centrally placed perikarya, and which also enter the medulla were named centrifugal endings. We have used the same terminology in this account; it must be stressed that the terms centrifugal and centripetal have here only a morphological meaning. The former implies that a class I terminal is peripherally located with respect to its cell-body and initial component, and the latter implies that centrally placed cell components are derived from a more peripherally placed cell-body.

Amacrine cells have never positively been identified in *Pieris*, *Sphinx* or *Automeris* in these preparations although oval, small-diameter, cell-bodies can be seen beneath the plexiform layer in reduced silver preparations (figure 10, plate 3). However, they have frequently been observed in the various species of Diptera and conform to the descriptions of them by the Spanish authors. They also described the lamina of an unidentified sphingid in which they figured several diffuse endings in its lamina which were listed as centrifugal elements. Fragments of endings like those figured by Cajal and Sanchez have been seen at this level in *Sphinx*† but not in *Pieris*. These may be derived from a tangential system or from some other cell-type and must

† At the time of going to press an amacrine cell has been seen, in its entirety, in the lamina of *Sphinx* (L. Pearson personal communication).

at present be included as *incerta sedis*. The large multipolar cell-bodies situated below the external plexiform layer and amongst the fibres in the first optic chiasma have only been seen in reduced silver preparations and may possibly be glia somata.

There are two variants of T-cell endings, derived from the medulla, in the Diptera ('basket endings', see part II). Both may be topographically associated with single optic cartridges. The lamina endings of similar T-cells have been identified in both *Sphinx* and *Pieris*, but are rarely impregnated. In *Sphinx* they have a wide-field with a spread through between 25 and 30 retinula cell endings (figure 26). In *Pieris* there are two types of basket endings in the lamina, one with a lateral spread equivalent to one optic cartridge the other equivalent to at least two (figure 25). In the Lepidoptera these endings do not have terminal processes arranged as a basket, enclosing the components of an optic cartridge, as they do in the Diptera: they spread through, rather than embrace, single groups of retinula cell endings. The wide-field endings, in both species, consist of between 8 to 12 varicose processes which fan out into the plexiform layer: in *Pieris* a single ending fans through between two and three optic cartridges in the horizontal plane. There is no evidence for other types of centrifugal endings in the Lepidoptera which are analogous to either the climbing or capped endings in the Diptera (part II).

The neurons of the medulla

Introduction: the parallel stratification of the medulla

Even in unstained frozen sections cut at between 10 and 30 μm, it is clear that the medulla is highly stratified. These layers must presumably correspond to the distribution of processes and synapses. Bullock & Horridge (1965) described the medulla as being divided into 10 layers (A to J), which they derived from the account of Cajal & Sanchez. The stratification of the medulla has been re-examined by a comparison of Holmes's and Golgi preparations. For this purpose over 100 Golgi preparations were examined. Whenever a clearly recognizable neuron was found, measurements were made of the depths in the medulla at which its components occurred, and the total depth of the medulla at that point. It proved possible to obtain between 15 and 100 readings for each major cell type, and as far as possible, readings were obtained from medial sections of the medulla in the two planes of section; horizontal and vertical. This eliminated errors arising from its curvature. These readings were then averaged and expressed as percentages of the total depth of the medulla. The medulla was then searched for sections in which two or more cell-types lay close to one another in the same plane of section, and within a restricted portion of the total medullary arc. This was usually less than one-twentieth of the total arc of the section of the medulla, and never more than one-sixteenth. The components of each group were measured and the arrangement of each group was again expressed at percentages of the total medulla depth. The results derived from the data from measurements of single neuronal components, and from measurements obtained from groups, were found to agree to within 2 %.

These results from Golgi preparations allowed the reconstruction of a plan of the stratification of this region. This plan was then compared with the results obtained from measuring the gross stratification of ten preparations made by variants of the Holmes–Blest silver techniques. Further measurements were made from sections stained by the Goldner's triple coloration methods.

A comparison between the plans derived from the Golgi preparations and Holmes–Blest

silver method is given in figures 41*a* and *b*. The two schemes correlate well, and it is possible to state the components which contribute to any one stratum with some degree of reliability. Unfortunately, the strata which are defined for species of Lepidoptera and Diptera cannot be wholly related to the scheme of Bullock & Horridge (1965); undoubtedly this is a consequence of species difference and the limited information available to them from earlier authors.

Class I cells: endings derived from the retina

The long visual fibres provide a direct projection from the ommatidia to the outer layer of the medulla. If the medulla is considered as the second integrative layer of the visual system this arrangement is in contrast to that of vertebrates, where receptor elements usually project only as far as the outer plexiform layer (Cajal 1933; Polyak 1941; Boycott & Dowling 1969), and probably that of cephalapods (Young 1962). However, some outer strata of the medulla could possibly contain combination of neuronal elements which may perhaps be analogous in function to similar combinations in the outermost synaptic regions of vertebrate and cephalopod visual systems. Parallelism of function in vertebrate and invertebrate visual systems, if it exists, may not necessarily be reflected by similar topographical neural arrangements. Some endings in the medulla have not been traced back to their corresponding components in the lamina and vice versa. Thus this description is, in part, incomplete. But by a process of elimination most components in these two regions can be correlated with each other.

There are three major forms of long visual fibre endings in the medulla. The cone-shaped endings in stratum 4 have lateral spreads which overlap marginally. The narrow endings are separate from one another. Pairs of long visual fibres in the lamina of *Pieris* and *Sphinx* keep their paired arrangement in their medulla. The same is true for *Apis* (N. J. Strausfeld, unpublished). Cone and narrow-field endings are usually detected together even if they are derived from a pair of smooth long visual fibres or a pair composed of a smooth and spiny variant. The wide-field endings end shallowly in the medulla between strata 3 and 4 (figure 20, plate 5). Other variants are illustrated in figures 25 and 26.

Pairs of long visual fibre endings are characteristically accompanied by a pair of monopolar cell terminals (figure 27). These four endings together are here termed a 'Quad'. Quads end in regular arrays throughout the region giving the outer layer a distinctly columnar appearance (figures 13 and 15, plate 4). Their distribution is extremely clear in tangential sections where the medulla can be seen stippled by groups of four fibres (figures 14 and 16, plate 4). Each group defines the axis of discrete clusters of neural elements (serially arranged in the medulla in a way analogous to the repetitive arrays of optic cartridges in the lamina), which are here termed medullary columns. Each column axis is extended further into the serpentine layer by at least one fibre that eventually passes from the outer surface of the region to the second optic chiasma. The lateral extents of all class I cells can be conveniently expressed as a spread through a particular number of medullary columns.

Class I cells: endings derived from the lamina; monopolar cells

The two variants of monopolar endings in *Sphinx* are characterized only by their different depths of penetration into the medulla. Both are situated between the surface of the medulla and stratum 3. The endings are characterized by a terminal swelling of the axis-fibre between 4 and 6 μm wide, from which radiate tuberous or extremely slender processes, less than 0.3 μm diameter, each terminating as a spherical swelling (figure 24, plate 5; figure 26). The total

lateral spread of any one of these endings is equivalent to 25 to 30 μm, that is between 1 and $1\frac{1}{2}$ medullary column widths. In *Sphinx*, the differences of lateral extent of the monopolar cells in the lamina is not reflected, morphologically, by their endings in the medulla.

Four variants of monopolar endings have been found in *Pieris*. Two of them, similar in form to the *Sphinx* monopolar endings, end in stratum 1. The outermost of these belong to the small radial diffuse monopolars (figure 18, plate 5). The other endings consist of lateral processes radiating from between 10 and 15 μm from their axis-fibres in stratum 2 (figure 25; figure 19, plate 5), but the relationships of these endings with the monopolar components in the lamina is not yet wholly clear. There is evidence from the study of serial sections that the midget and radial monopolar cells end as shallow terminals, and that the other forms end deeply (see also, figure 27).

Class I cells: T_1 components in the outer strata of the medulla

A T-cell characteristically consists of a linking-fibre between two regions from which arises a slender side branch that leaves it more or less at right angles to the cell-body. However, the cell-body fibre of the medulla-lamina T-cell of the Lepidoptera is sometimes seen as a continuation of the linking-fibre (figure 26). There are two T_1 cell endings in the lamina of *Pieris*. The wide-field ending is derived from a wide-field component in the medulla. The small-field ending is derived from a minute component at the surface of the medulla. These two forms are illustrated in figures 25*a* and *b*.

Only one form of T_1 cell ending has been detected in the lamina of *Sphinx*. This is derived from a deep wide-field component in the medulla (figure 26*b*). However, osmium-fixed material (a variant of the Golgi-repeat procedure: see Methods) has also revealed a fragment of a small-field T-cell component at the surface of the medulla which is similar in appearance to the surface T-cell component in *Pieris* (figure 26*b*). Whether this is an artefact of this Golgi variant or is truely representative of a second form of medulla-lamina T-cells is not known. We have failed to find this component in Golgi–Colonnier preparations and see no evidence for a corresponding ending in the lamina.

Class I cells: components linking the medulla with the lobula and lobula plate

Both species of Lepidoptera have a similar perpendicular distribution of neurons. The medullary strata of *Sphinx* are not so strongly defined as they are in *Pieris*. Components of the latter show better impregnation by the Golgi stain and superior fixation of the unstained back-

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DESCRIPTION OF PLATE 4

The medulla

FIGURES 11, 12. *P. brassicae*. (Masson trichromatic procedure.) Multipolar glia cell-bodies. The processes from these elements invest the cell-body cortex around the first optic chiasma and the fibres in the chiasma.

FIGURE 13. *P. brassicae* (Holmes–Blest preparation). The columnar organization of the medulla. OL = outer layer; SL = serpentine layer; IL = inner layer.

FIGURE 14. *P. brassicae* (tangential section of the medulla surface; Holmes–Blest preparation). The stippling of the medulla.

FIGURE 15. *P. brassicae* (Holmes–Fraser Rowell preparation). The axes of medullary columns in the outer layer of the medulla ('Quads' from the lamina). The arrows indicate the two approximate levels of endings of monopolar cells (outer) and long visual fibres (inner).

FIGURE 16. *P. brassicae* (tangential surface section; Holmes–Blest preparation). Cross-sections of regularly spaced 'Quads' showing the four axis-fibres of each stipple. *Scales:* Figures 11, 12, 10 μm; figures 13, 14, 25 μm; figure 15, 10 μm; figure 16, 10 μm.

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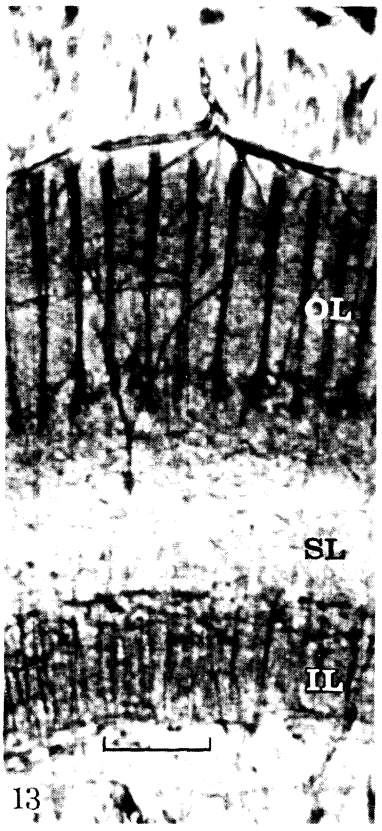

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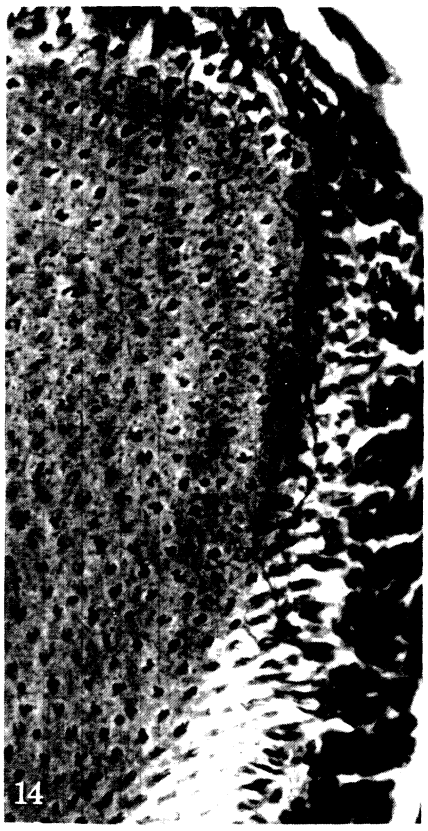

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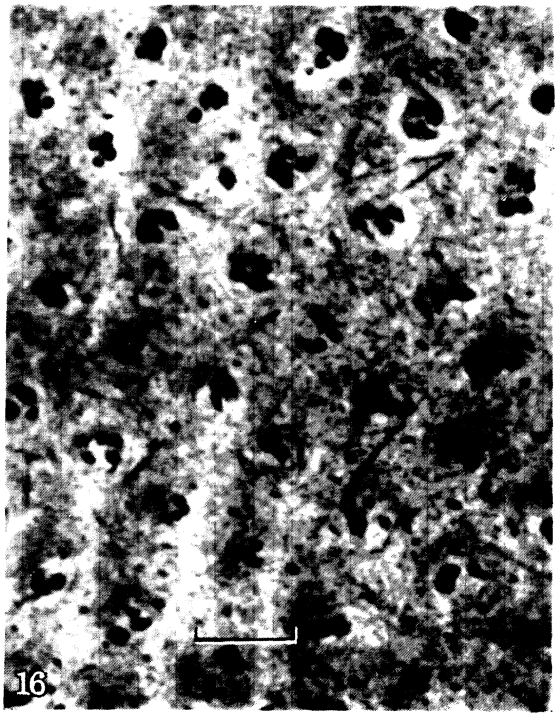

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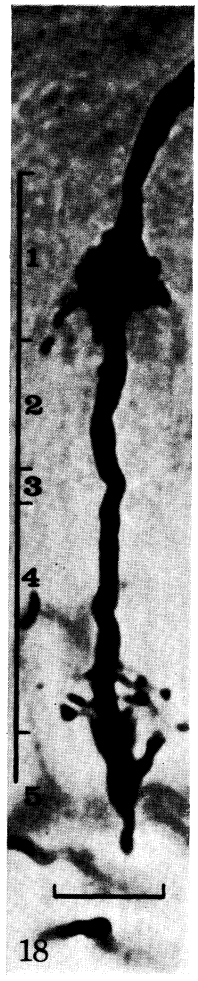
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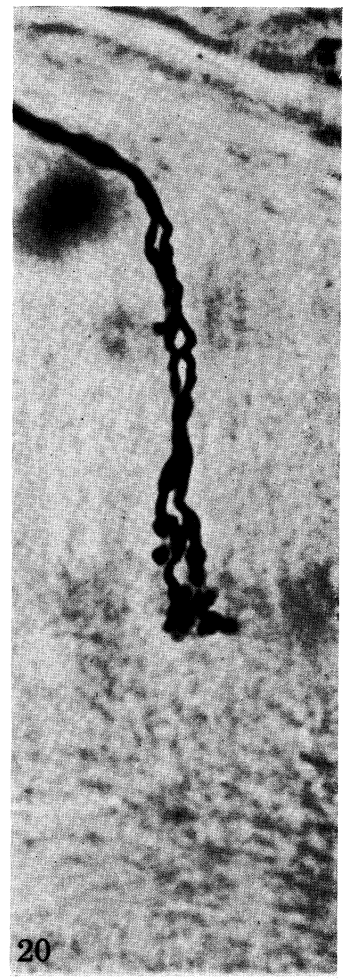
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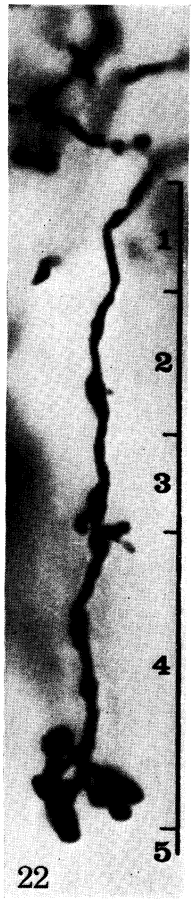
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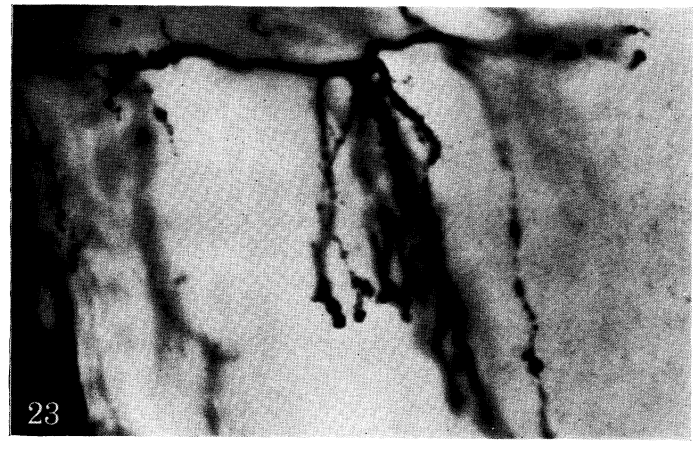
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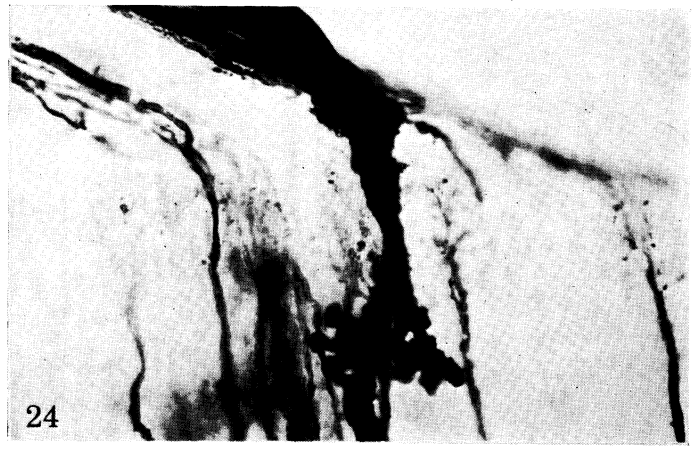
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ground. For these reasons the cell-types of *Pieris* have been described in detail. They are also more amenable to precise measurements. Comparisons of cell-types in the two species are given when there is an outstanding difference in their layer relationships, and when there are cell-types in one that are absent in the other and vice versa.

There are three groups of neurons that link the medulla to the lobula complex: transmedullary cells, T-cells and Y-cells. Both species have only one type of transmedullary cell which has three size-variants based on the differences in their lateral extents. The perpendicular extent of their processes in the medulla strata is similar in both species and they have analogous patterns of branching. The Diptera, on the other hand, have several distinct forms of these cells which are characterized by the different shapes, field-spreads, strata relationships and penetrations into the lobula (see part II).

Observations on the type 1 transmedullary cells (Tml)

The cell-bodies of transmedullary cells are situated above the medulla in the cell-body cortex which surrounds the first optic chiasma. The cell-body fibre prolongates into an axis-fibre which traverses all the medullary strata to leave its inner face for another optic lobe region, the lobula (figure 44, plate 7). In both species this cell has lateral processes confined to absolutely specific strata. Holmes–Blest preparations stain the axis-fibres of these cells and occasionally their lateral processes in the outer layer of the region. Such preparations show that the transmedullary cells are regularly spaced throughout the medulla and that there is at least one of these cells to each medullary column. In *Pieris* the outer group of branched processes of these cells has a lateral spread of between 10 and 15 μm. The perpendicular spread of this group is confined to strata 1 and 2. Thus they are on the same level as all the outermost monopolar cell endings (and some deeper ones) and the tangential ending M : tan 2. In stratum 3 the axis-fibre of each transmedullary cell gives rise to a restricted band of between five and six spines each between 2 and 3 μm long. This band is always at the same level as the predominantly dorsoventrally orientated 'line tangential' fibres (see p. 113). This class II element has a perpendicular field-spread which corresponds exactly to that of the band of spines. Whenever these two cell components are seen together in the same section they are invariably at the same level. Likewise the lateral processes of the antero-posteriorly orientated tangential elements in stratum 8, are at the same level as the deepest group of branched

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Description of plate 5

The medulla

Figure 17. *P. brassicae* (Holmes–Blest preparation). The anterior edge of the outer layer of the medulla showing the different sizes of the 'Quads'.

Figure 18. *P. brassicae*. Two long visual fibre endings and a shallow monopolar cell ending. The fourth component of this Quad is unimpregnated (strata 1 to 5).

Figure 19. *P. brassicae*. The four elements of a Quad: two outer monopolar cell endings, and two deep long visual fibre endings.

Figure 20. *P. brassicae*. The ending of a wide-field long visual cell fibre.

Figure 21. *S. ligustri*. Two adjacent long visual fibre endings.

Figure 22. *S. ligustri*. This element is analogous to the cone-shaped long visual fibre ending of *Pieris*.

Figure 23. *S. ligustri*. The pi-cell; so-called because of its similarity to the Greek letter π. This cell is a form of tristratified diffuse amacrine which is characteristic of the Lepidoptera.

Figure 24. *S. ligustri*. A shallow monopolar cell ending.

Scales: Figures 17, 10 μm; figures 18 to 20, 10 μm; figures 21 to 24, 10 μm.

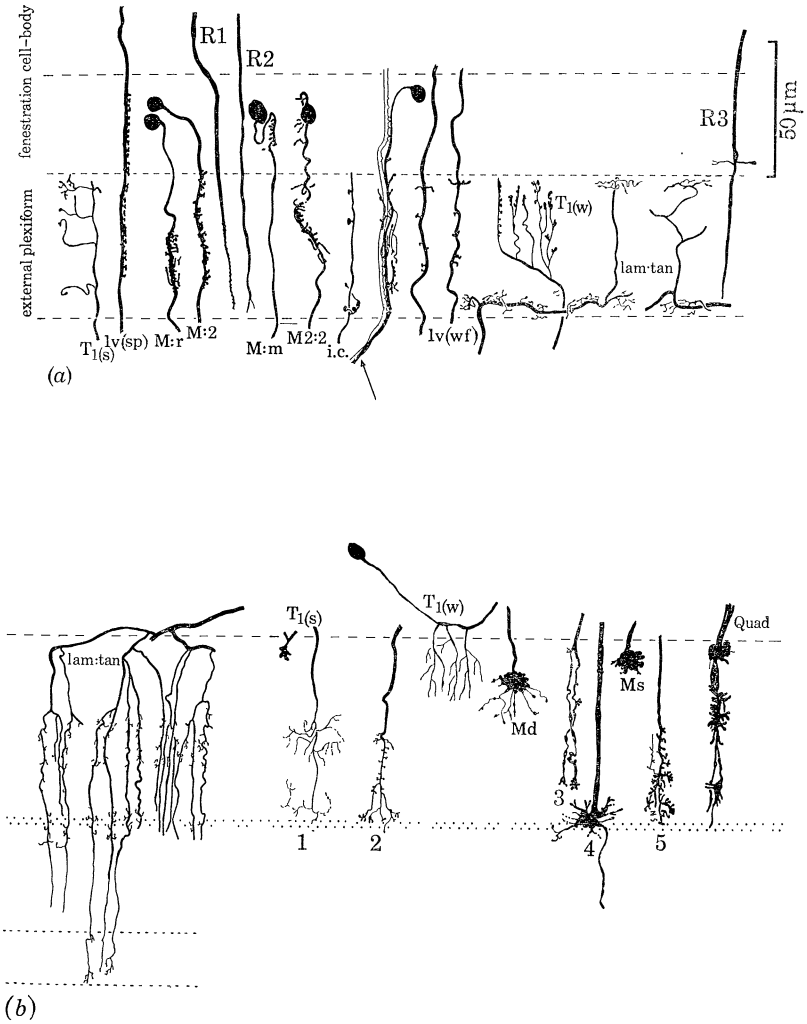


FIGURE 25. *P. brassicae.* Summary diagram of the receptor elements in the lamina and neurons linking the lamina to the medulla.

(*a*) Lamina elements. R 1 to R 3 = types 1 to 3 retinula cell endings. Lv sp = spiny long visual cell fibre. Lv wf = wide-field long visual cell fibre (smooth long visual cell fibre stippled). M: r = small radial monopolar cell. M: 2 = small bilateral monopolar cell. M2 : 2 = small bistratified bilateral monopolar cell. A wide-field bilateral monopolar cell (arrowed) is shown closely applied to a smooth long visual cell fibre. M: m = a midget monopolar cell. $T_{1(s)}$ = small-field T_1-cell ending. $T_{1(w)}$ = wide-field T_1-cell ending. Lam: tan = some processes of the inner and outer strata of the tangential element in the lamina. Ascendent processes in the fenestration layer have not been shown in this diagram.

(*b*) Medullary components. 1 = a diffuse ending of a long visual fibre (seen in one osmium-fixed preparation). 2, 5 = two diffuse long visual fibre endings derived from spiny long visual fibre elements in the lamina. 3 = the shallow ending of a wide-field long visual fibre. 4 = a pair of long visual fibre endings: the shallower ending is derived from a smooth long visual fibre, the deeper ending is derived from a spiny variant. Some deep endings, like the one figured here, have been traced to smooth variants in the lamina. Md = deep wide-field ending of a wide-field radial monopolar cell. Ms = shallow ending of a monopolar cell. The deeper endings are invariably derived from radial monopolar cells. Shallow endings are derived from bilateral and midget monopolar cells. Quad = four closely apposed endings from the lamina in the same medullary column. The outer part of each column has been here termed a medullary cartridge. $T_{1(s)}$ = a small-field initial component of a T_1-cell (seen rarely in osmium fixed material, and once in Golgi–Colonnier material). $T_{1(w)}$ = a wide-field initial component of a T_1-cell. Lam: tan = part of the field of the stratified diffuse ending of the lamina tangential. The tangential subfields in the lamina are oriented vertically. They give rise to several strip subfields in the medulla. Their horizontal extent is equivalent to the cross-sectional extent of the outer subfields. Double row of dotted lines = outer limit of stratum 6. Deepest dashed lines indicate stratum 8.

processes of the transmedullary cells (figure 44, plate 7). The transmedullary cell also has a second band of spines whose range of stratification lies between strata 4 and 6. The topographical relationship of these to another element is not known.

The axis-fibres of the transmedullary cells leave the inner face of the medulla to cross over to the lobula, via the second optic chiasma, where they terminate in its outermost stratum (figure 42, and figure 44, plate 7).

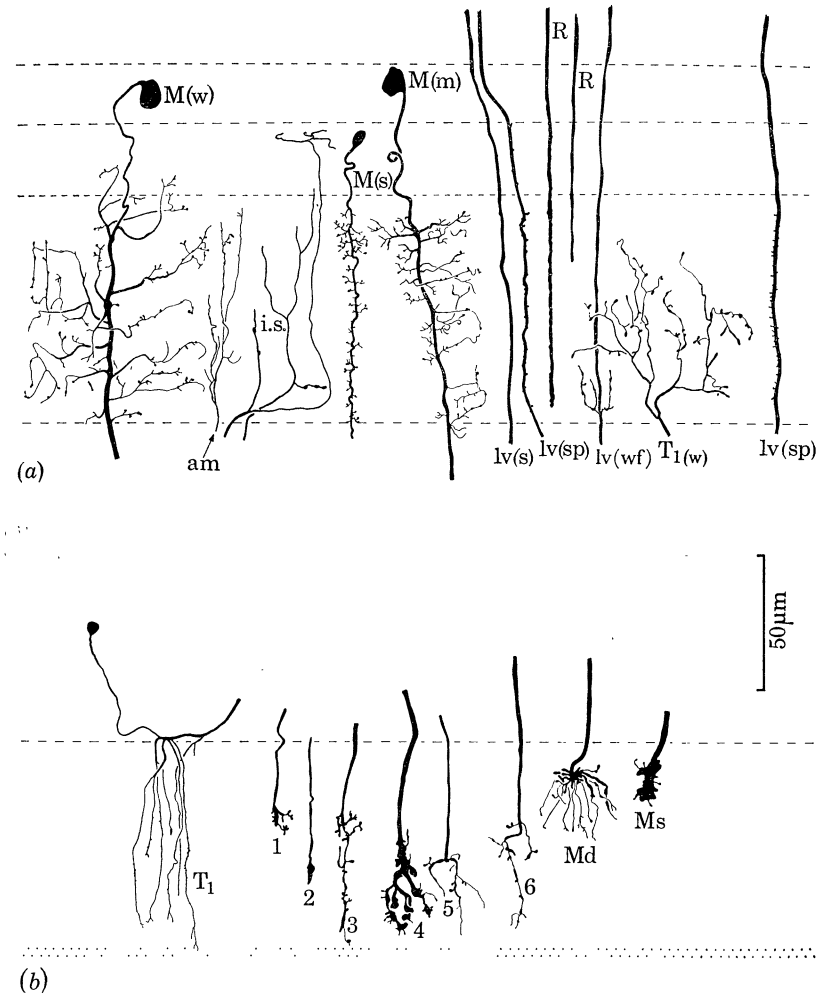


FIGURE 26. Summary diagram of the lamina and medulla receptor and first-order interneuron elements (*S. ligustri*).

(*a*) Lamina elements. R = retinula cell endings. Lv(s) = smooth long visual cell fibre. Lv(sp) = spiny long visual cell fibre. Lv(wf) = wide-field long visual cell fibre. M(w), M(m), M(s) = large, medium and small-field giant radial monopolar cells. $T_{1(w)}$ = wide-field ending of a T_1 cell. The lateral extents of these components vary to the same extent as those of giant monopolar cells. i.s. = *incerta sedis*. This may possibly be derived from a lamina tangential or may be a smaller field T_1 cell ending. However, the form of its processes is markedly different from those of $T_{1(w)}$. am = these processes may possibly be derived from an amacrine cell-body below the inner face of the lamina.

(*b*) Elements in the medulla derived from the lamina. 1 to 6 = variants of long visual fibre endings. The fourth form has only been detected in Osmium fixed preparations. Md = deep monopolar cell endings. Ms = shallow monopolar cell endings. The stratification of the medullae of *Sphinx* and *Automeris* are less definable than those of *Pieris* and the Diptera. However, it does seem, from Golgi preparations, that the shallowest endings in these moths are invariably derived from small-field giant radial monopolar cells. T_1 = the initial component of a medulla-lamina T-cell.

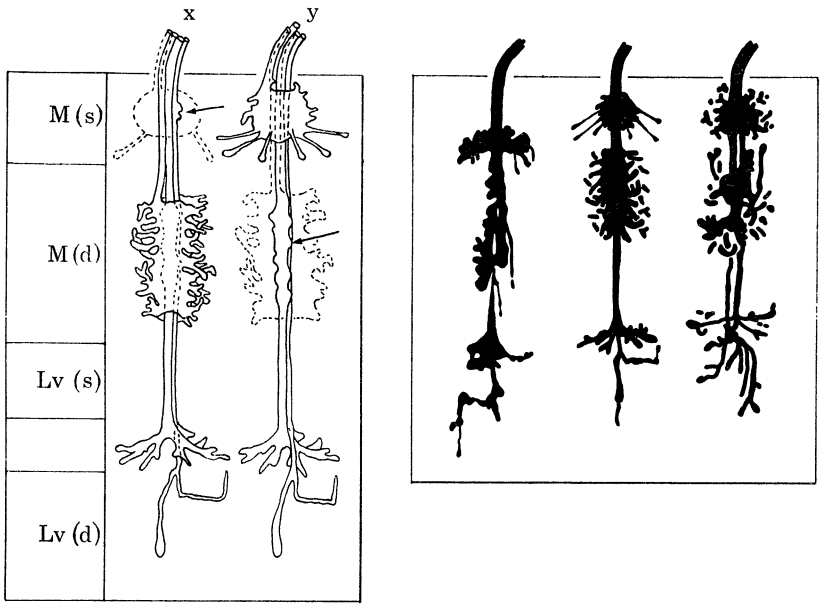


FIGURE 27. *Pieris brassicae.* (*a*) The reconstructions of a Quad from wholly or partially impregnated input groups from the lamina. The deepest long visual fibre has a lateral swelling in stratum 1 at the same level as a shallow monopolar cell ending (M(s); arrowed). The shallow long visual fibre ending has swellings (arrowed) at the same level as the deeper monopolar cell M(d) ending (dotted outline in Y). Both monopolar cells can be seen wrapped around the pairs of long visual fibre axis-fibres. These swellings may possibly be indicative of functional contacts between monopolar cell and long visual fibre components in the same medullary cartridge. Similar swellings have been seen at corresponding levels in the medullae of Diptera (see part II).

(*b*) Camera lucida drawings of completely impregnated components of Quads. Each invariably contains two monopolar cell endings at two distinct levels and two long visual fibre endings. (See also figures 18 to 20, plate 5.)

DESCRIPTION OF PLATE 6

The medulla

FIGURE 28. *S. ligustri.* The bistratified medullary component of a transmedullary cell. Two cells in this figure lie in adjacent medullary columns. Note the overlap between the outer processes in stratum 1 and the separated fields of the recurrent inner processes (s = serpentine layer).

FIGURE 29. *S. ligustri.* A high magnification of the outer processes.

FIGURE 30. *S. ligustri.* A detail of the inner recurrent processes. Note the terminal knobs.

FIGURE 31. *P. brassicae.* Strata 1 to 8 of the medulla, a = the spatial relationship between a cone ending of a long visual fibre and the outermost processes of a small disk subfield of the type 12 medullary tangential cell (b).

FIGURE 32. *P. brassicae.* The bistratified component of a transmedullary cell.

FIGURE 33. *P. brassicae.* The tristratified wide-field medullary amacrine cell.

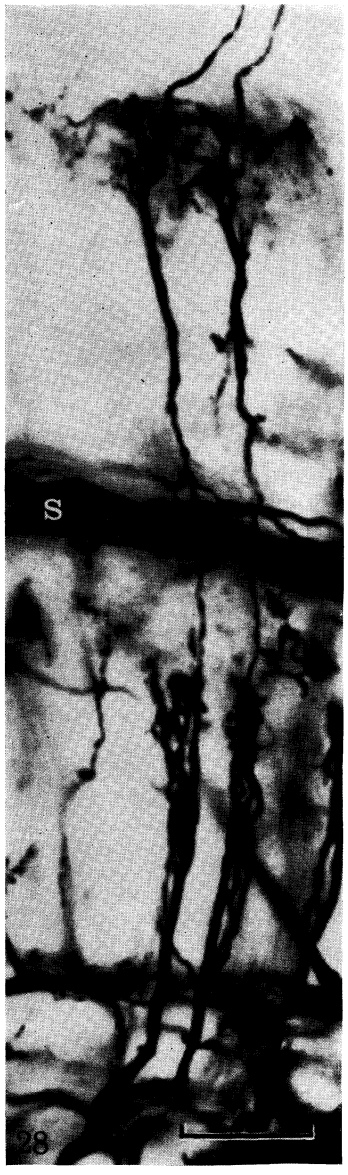
FIGURE 34. *P. brassicae* (methylene blue—Golgi–Colonnier procedure). The bistratified-diffuse medullary component of a Y_1 cell.

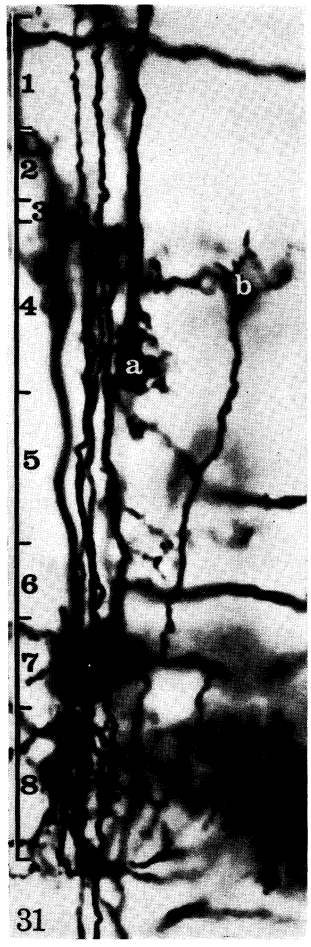
FIGURE 35. *S. ligustri.* The initial (medullary) component of a T_4 cell.

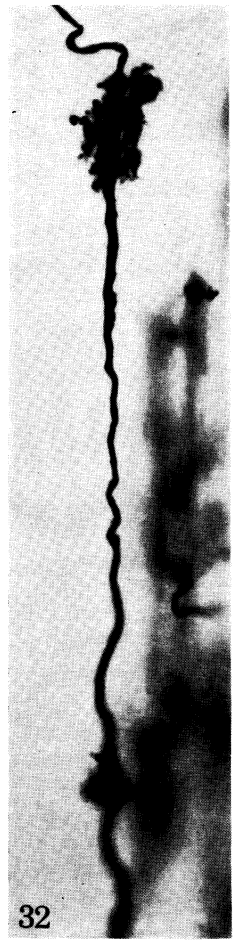
FIGURE 36. *P. brassicae.* Two T_4 components in adjacent medullary columns.

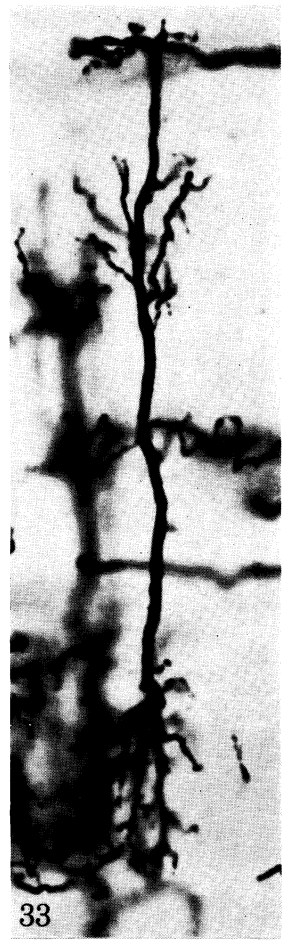
FIGURE 37. *P. brassicae.* The bistratified small-field amacrine cell. The two inner branches in stratum 7 appear to embrace the initial component of a T_3 cell in the same column.

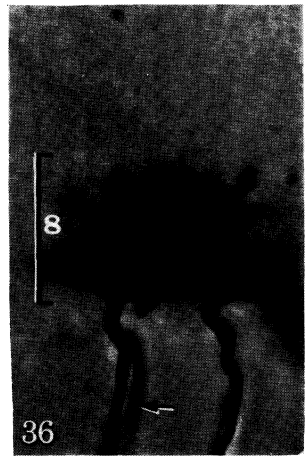
FIGURE 38. *P. brassicae.* The medullary component of a T_2 cell. Note its depth of penetration into the medulla with respect to the other types of T-cells. Figures 28 to 38; scale = 25 μm.

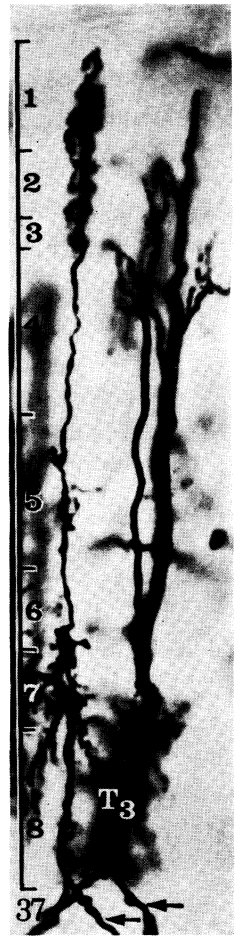


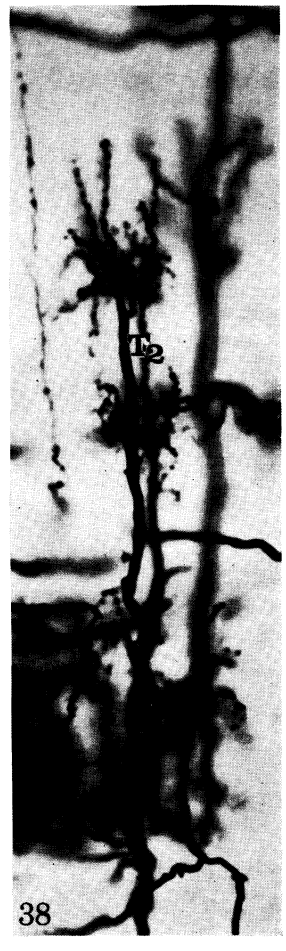












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In *Sphinx* the shape of this cell is somewhat different to that of *Pieris* (figure 28, plate 6). Its deepest processes in the medulla are unbranched and have characteristic knobs at their ends (figure 29, plate 6). The lateral processes of this cell have similar distributions in the strata of the medulla to those of *Pieris*. There are, however, no spines from the axis-fibre in stratum 3, although fragments of a line tangential element have been detected at this level.

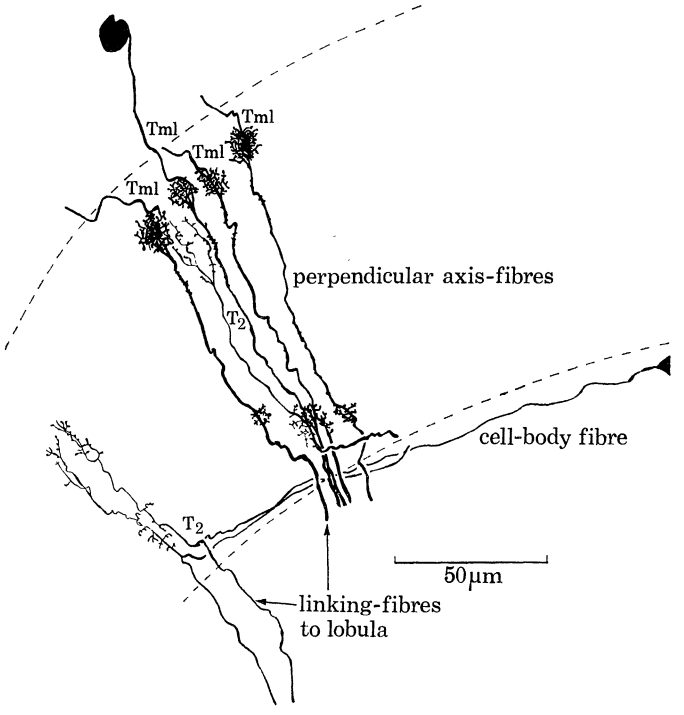


FIGURE 39. The medulla. *P. brassicae*. Tm 1 = the type 1 transmedullary cells. T_2 = bistratified components of deep T-cells.

Giant forms of transmedullary cells have an outer lateral spread which is twice as wide as the 'ordinary' form; 'miniature' variants have lateral spreads which are less than half as wide. There is no corresponding variation in the lengths of the spines nor in the lateral extent of the inner groups of processes in stratum 8. The 'giant' variant has only been seen at the anterior edges of the medulla disk. This is not a water-tight indication that it is restricted to this portion of the medulla, since the Golgi stain may be preferentially staining only those at the edge: but they have never been seen placed otherwise in any of the preparations. On the other hand, the ordinary form of this cell is distributed throughout the medulla. The miniature form has only been seen once in *Pieris* and twice in *Sphinx* (figure 41). Possibly it stains infrequently due to the thinness of its axis-fibre and lateral processes. The inner group of processes in the medulla of all the variants of the type 1 transmedullary cell have a lateral spread of between 7 and 10 μm, in *Pieris* and between 10 and 15 μm in *Sphinx*. All variants of the transmedullary cell have the same form of ending in the lobula, consisting of a terminal swelling of the axis-fibre (figure 42). There is very little variation of the width or depth of penetration of this ending, which is strictly confined to the outer stratum.

Observations on T-cells linking the medulla, lobula and lobula plate

The perikarya of these neurons lie along the posterior face of the lobula plate and as far as the posterior inner margin of the medulla (figure 42). Each neuron links two geographical regions, with a group of processes in each. The linking-fibre and the cell-body fibre have a characteristic T-arrangement (figure 1*d*). Three main types of T-cell, with an outermost group of processes in the medulla, have been found in the present species.

(*a*) *The deep T-cell*, T_2

The medullary component is bistratified: perpendicular processes extend through strata 8 to 2; lateral (spiny and varicose) processes are derived from them in the outer half of stratum 8, the inner half of stratum 7 and also within strata 4, 3 and 2. They are especially abundant in strata 7, 3 and the inner quarter of stratum 2 (figures 39, 41, 42). The lobula ending consists of a unistratified diffuse group of processes in the third (innermost) stratum of this region (see figure 41). The lateral spread of the medullary component rarely exceeds 20 μm except at the perimeter of this region. The lobula ending is not wider than 15 μm (*Pieris*). In *Sphinx* the extents are correspondingly larger, 30 and 23 μm respectively.

(*b*) *The shallow T-cell*, T_3

The unistratified medullary components of these cells are situated within strata 7 and 8. The processes are thin, branched and radiate equilaterally from the axis-fibre. The lateral extents of these medullary arborizations do not exceed 12 μm. The lobula endings of this cell-type are quite different in *Sphinx* from those in *Pieris*. In the former species the linking-fibre gives rise to four or five terminal branches which penetrate the lobula to terminate as simple club-endings in stratum 3 (figure 42). In *Pieris* the linking-fibre penetrates the lobula and projects to the inner margin of stratum 2 where it gives rise to a tightly packed group of club-endings which extend as far as the middle of stratum 3. The lateral spreads of these endings do not exceed 15 μm in *Pieris* or 25 μm in *Sphinx*.

(*c*) *The bushy T-cell*, T_4

This cell-type has a group of bushy processes in the medulla which are characteristically biased to one side of the short axis-fibre and flattened in the dorsoventral plane (figures 35 and 36, plate 6). The antero-posterior spread never exceeds 20 μm, and the dorsoventral spread does not exceed 10 μm (*Pieris*). The linking-fibres from these elements project to the outermost stratum of the lobula plate where each gives rise to a bushy ending with a lateral spread equal to that of its medullary component (figure 42).

Incerta sedis

A fourth type of T-cell ending in the medulla has been seen exclusively in *Pieris brassicae*. It consists of a set of diffuse processes which project from the inner face of the medulla as far as the outermost stratum. This ending has a total lateral spread of 40 to 60 μm in both the antero-posterior and dorsoventral planes. Each process has a series of swellings which are distributed at regular intervals along its length. The cell-body fibre has been traced to a position behind the posterior margin of the lobula plate but its ending in other regions is not known.

Observations on Y-cells linking the medulla to the lobula and lobula plate

Y-cells link three regions, having a group of processes in the medulla, lobula plate and the lobula. The medullary (initial) component is joined to that in the lobula plate by a stout linking-fibre, between 2 and 4 μm in diameter.

The lobula plate component is, in turn, linked to a group of fibres in the lobula by a further extension of the linking-fibre that crosses over to this region via the inter-complex tract. The locations of the Y-cell-bodies vary; cell-body fibres join the Y_1 linking-fibres between the medulla and the lobula (*Pieris*) from perikarya that are situated behind the lobula plate. However, in *Sphinx* Y_1 cell-bodies have been seen posteriorly, above the outer surface of the medulla, as well as behind the lobula plate region. Perikarya of Y_2 cells are situated between the lobula plate and medulla, or behind the lobula plate: their cell-body fibres join the linking-fibres at the surface of this latter region (figure 42). All the measurements in the following description of Y-cells, are quoted from *Pieris*: but the same cell-types exist in *Sphinx*; these are correspondingly larger, and are illustrated in figure 42.

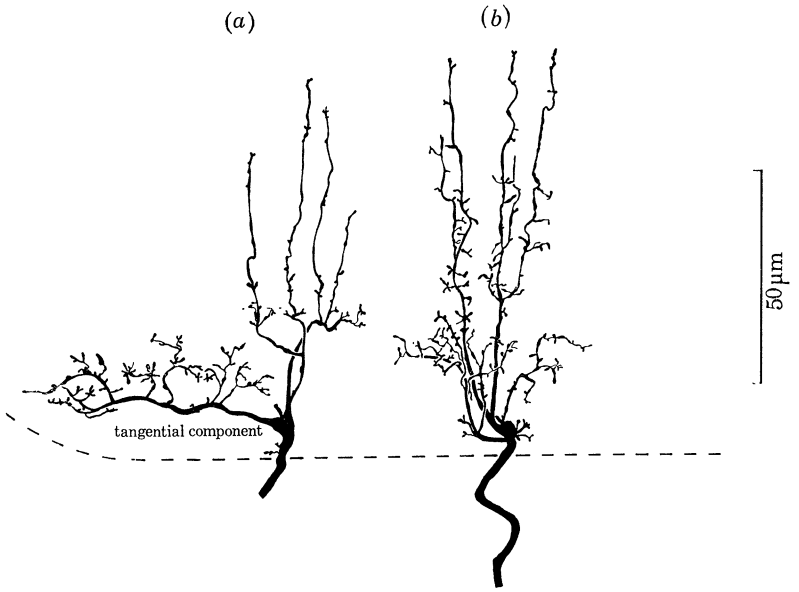


FIGURE 40. *P. brassicae*. The two variants of Y_1 cell components in the medulla. The tangential variant has only been seen near the margin of the medulla disk.

(*a*) *Deep Y-cell*, Y_1. The medullary component is bistratified and diffuse (figure 34, plate 6): the linking-fibre penetrates the inner face of the medulla where it gives rise to a deep group of processes in strata 7 and 8. These have a total lateral spread, at the middle of the medulla disk, of 40 to 50 μm in both planes. However, at the periphery of the medulla disk the deep processes are derived from a single tangential branch of the axis-fibre in stratum 8 (figure 40). This is invariably orientated dorsoventrally. Several perpendicular processes arise from the lower group of processes and project as far as stratum 3. The lateral extent of these does not exceed 50 μm, irrespective of their horizontal or vertical location in the medulla disk. The processes at both levels are varicose. These swellings are particularly noticeable in strata 7 and 8 and in 6 and 5 but are absent in the serpentine layer (figure 34, plate 4; figures 40 and 41). The lobula plate component of this cell is a narrow-field ending which extends through this region as far as its posterior face. Its lateral extent does not exceed 15 μm.

The lobula component is bistratified. It consists of an axis-fibre (a prolongation of the linking-fibre from the lobula plate component) which extends as far as the middle of stratum 3. One or two branches are derived from it, unilaterally, in stratum 1. Thin branched processes are also derived from it, radially, in stratum 3. These sometimes have tuberous swellings along their length and may be arranged perpendicularly or tangentially with respect to their fibre of origin. However, their lateral extents rarely exceed 30 μm (see figure 70).

(*b*) *The tangential Y-cell*, Y_2. The medullary component of this cell is unistratified. It consists of a long tangential branch restricted to stratum 8 which gives rise, unilaterally, to slender processes regularly arranged along its length; these extend as far as the interface between strata 6 and 7. The processes are characterized by terminal double-swellings giving the end of each process the appearance of a swollen Y. Each perpendicular process serves a very narrow field, up to 6 μm laterally. The field of the whole medullary component is less than 25 μm wide dorsoventrally but may extend through a quarter of the medulla arc in the antero-posterior plane. The lobula plate component of this cell is diffuse with a widespread ending over the surface of a restricted portion of this region and, in addition, sends a narrow plug-ending through it. The total lateral spread of the lobula plate component is between 30 and 40 μm. The linking-fibre from the posterior component gives rise to a narrow-field ending deep in lobula stratum 3; its lateral extent does not exceed 25 μm. This cell-type is illustrated in figures 42 and 54. Functionally the Y_2 call might be considered as a quasi-tangential element in the medulla arranged to interact with one or more of the lobula plate tangential systems over its surface and one or more other types in the lobula.

Class II cells: the tangential endings in the medulla: introduction

The earlier workers included outlines of the tangential endings in their figures of the optic lobe regions, but did not commit themselves to describing their exact distribution or projections to other mid-brain and contralateral optic lobe regions.

Cajal & Sanchez figured a few of the class II elements in the lobula complex and indicated that they projected towards the mid-brain rather than to another ipsilateral optic lobe region. Zawarzin (1913, 1925), using both methylene blue and Golgi preparations of *Aeschna* larvae, published beautiful drawings of fragments of tangential components in the medulla. He also gave a system of stratification of the optic lobe regions based on the different densities of the background texture (Marksubstanz) shown up by non-selective procedures. His account gives a good indication of the layer relationship of some elements.

Hanström (1924*a*, *b*, 1928) figured and described several tangential neurons in the optic lobes of some crustacea, and also figured their central projections. Unfortunately much of his work is schematic and omits the precise morphology. At any rate these cells cannot yet be homologized with elements in the insects. The tracts and regions of the mid-brain of insects have been described by several authors who used non-selective procedures, notably by Viallanes (1884, 1885, 1887*a*, *b*), by Bretschneider (1913, 1914, 1921, 1924) and Power (1943*a*). Recently similar studies have been carried out by Vowles (1955), Satija (1957, 1958), Jawlowski (1960) Guthrie (1961) and Larsen (1966). The terminology is confusing; many authors have used different terms for the same tract or region. However, that favoured by modern authors is one used by Power (1943*a*) and has been adopted for this account.

This present study is still incomplete. In all cases it has been possible to determine the morphological characteristics of tangential elements and the depths at which they lie.

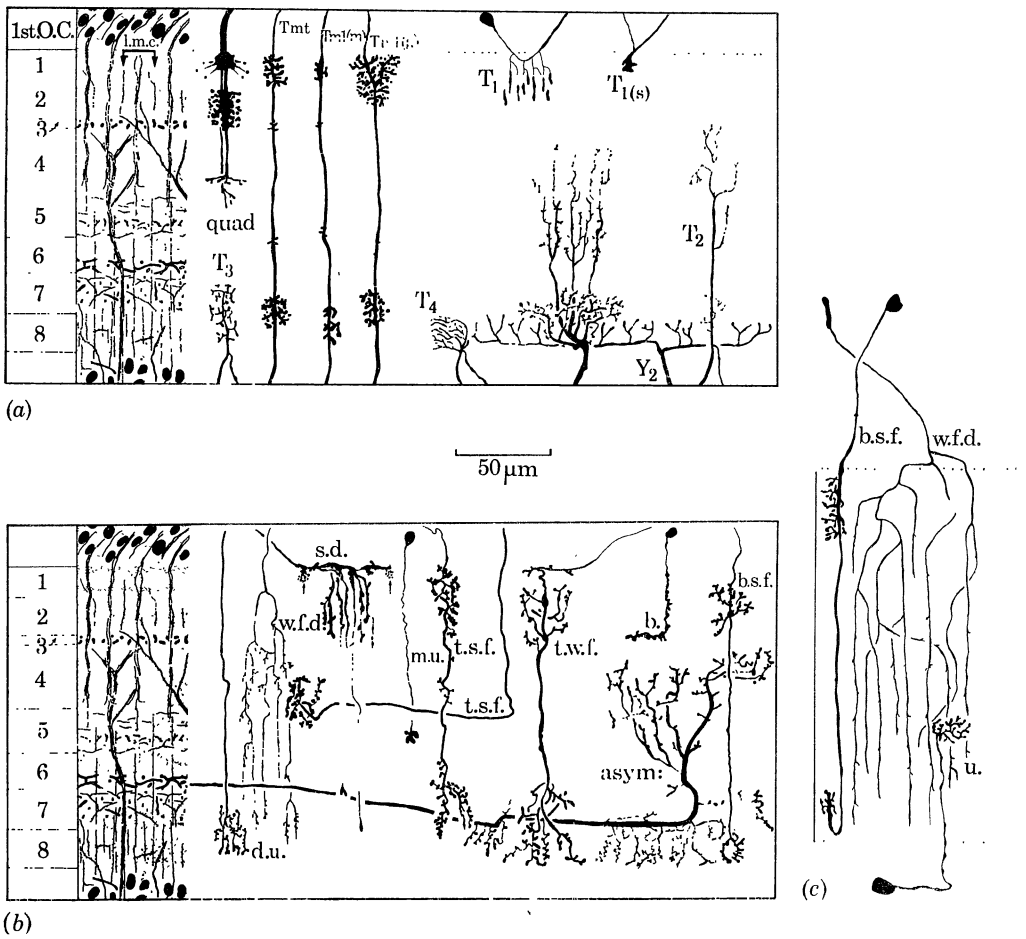


FIGURE 41. *P. brassicae.* Summary diagram of classes I and III elements in the medulla and their layer relationships with one another.

(*a*) A comparison between the stratification of the medulla as seen in reduced silver preparations (left) and class I elements revealed by selective silver techniques. There are eight strata apparent in Holmes–Blest silver preparations. These can be related to the levels of neuronal components from Golgi preparations; for example, stratum 1 contains the shallow endings of monopolar cells and the outer processes of type 1 transmedullary cells. Stratum 3 is defined by vertically oriented fibres (in Holmes–Blest preparations) which are revealed as line tangential processes in Golgi preparations (see figures 60 to 64, plate 9). The spines of the transmedullary cells are also restricted to this level (see also figure 54). T_4 components are restricted to stratum 8 and T_3 components characteristically extend into the medulla as far as the middle of stratum 7. 1st O.C. = inner most 25 μm of the first optic chiasma. 1–8 = strata of the medulla. T_1 and $T_{1(s)}$ = medulla-lamina T-cell components. Quad = input group of four endings from the lamina. Tm1 = the type 1 transmedullary cell. Tm1(m) = miniature (narrow-field) form of Tm1. Tm1(w) = giant form of Tm1. T_2 = the initial component of a deep T-cell ending. T_3 = the initial component of a shallow T-cell ending. T_4 = the initial component of a 'bushy' T-cell ending. Y_1 = the medullary component of a deep Y-cell. Y_2 = the medullary component of a tangential Y-cell. 1 m.c. = the lateral extent of 1 medullary column.

(*b*) Amacrine cells (see text). d.u. = deep unistratified amacrine cell. w.f.d. = wide-field diffuse amacrine cell. s.d. = tristratified diffuse amacrine cell (pi-cell). Note the characteristic 'bunches' of knobs (arrowed) at either end of the surface tangential component. This cell is invariably oriented horizontally. m.u. = midget unistratified amacrine cell. t.s.f. = tristratified small-field amacrine cell. t.w.f. = tristratified wide-field amacrine cell. b = bent amacrine cell. b.s.f. = bistratified small-field amacrine cell. t.s.f. = tangential small-field unistratified amacrine cell. asym. = the terminal component of an asymmetric amacrine cell.

(*c*) *Sphinx ligustri.* Three amacrine cells seen in *Sphinx.* Similar forms have also been seen in *Automeris.* u = unistratified amacrine cell.

Connexions to other regions and to their cell-bodies have been determined for a minority of these elements. This study is still in progress: unfortunately the central projections of these cells are only resoluble in their entirety in late pupal animals and it is necessary to recognize the adult cells from their pupal shape. It is clear that a great many of the components of class II cells in

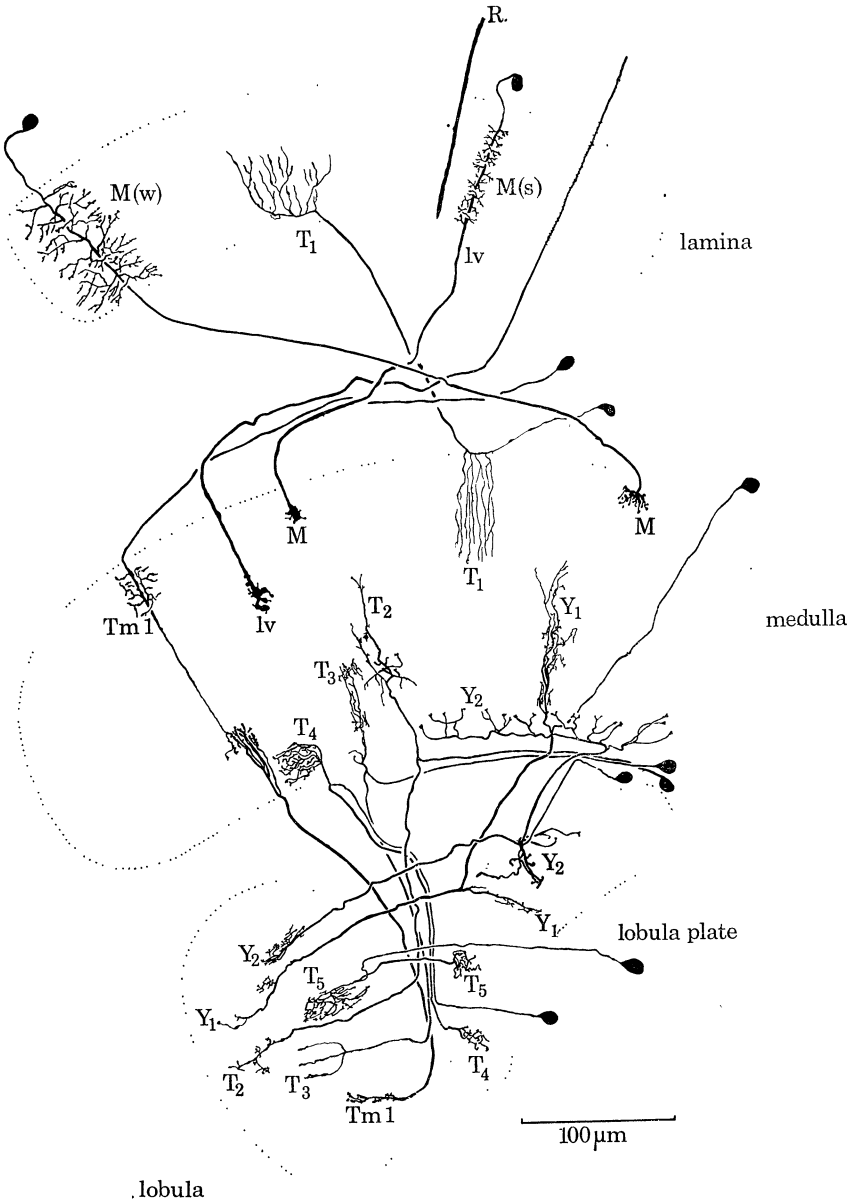


FIGURE 42. *Sphinx ligustri.* Summary diagram of its class I cells in the optic lobe. *Lamina:* Mw = wide-field giant monopolar cell; Ms = small-field giant monopolar cell; R = retinula cell; Lv = long visual fibre; T_1 = medulla-lamina T-cell ending. *Medulla:* Tm1 = type 1 transmedullary cell; Lv = long visual fibre ending; M = monopolar cell endings; T_1 = the initial component of a medulla-lamina T-cell; T_2 = the initial component of a deep T-cell; T_3 = the initial component of a shallow T-cell; T_4 = the initial component of a 'bushy' T-cell; Y_1 = the medullary component of a deep Y-cell (note the location of the cell-body above the outer face of the medulla); Y_2 = the medullary component of a tangential Y-cell. *The lobula complex:* T_5 = the lobula-lobula plate T-cell; Tml = ending of transmedullary cell; T_2, T_3 = endings of deep and shallow T-cells; Y_1 = deep ending of Y-cell; Y_2 = ending of tangential Y-cell. *Lobula plate:* T_4 = ending of bushy T-cell; Y_1 = lobula plate component of Y-cell; Y_2 = lobula plate (surface) component of tangential Y-cell.

the optic lobes, and their central morphologies, look very different in the pupae to those of the adult. For instance, the wide-field multi-stratified element described by Sanchez (1919) looks somewhat similar to the pupal form of the surface unistratified wide-field element in the adult. This pupal tangential element has lateral processes at several levels in the medulla; however, during late development these grow peripherally to the outer surface of this region where they become incorporated into the unistratified large-field element of the adult.

The tangential elements in all the optic lobe regions have either wide, strip or small oval fields of processes within particular strata. In the medulla strip-field elements have total fields composed of several strip subfields: the sub- and total fields of adjacent elements overlap marginally or not at all. Some oval field elements overlap, others do not; these tangentials are not usually formed from several subfield components. Wide-field tangentials also only overlap marginally.

Some of the tangential elements have similar field-shapes and forms to class II elements in the medullae of the Diptera (see part II). We do not know the precise central destinations of these cells in either order. This lack of knowledge complicates a simple system of terminology. Transmedullary cells, Y- and T-cells have been identified, in their entirety, in both the Diptera and Lepidoptera and are thus classifiable in the same way in both orders. There are very similar forms of wide- and strip-field class II elements in the lobulae of the two orders and some, if not all of them project to the same locations in the mid-brain. These too can be similarly classified. Some, but not all, of the class II elements in the medulla have similar morphologies and field characteristics to those in the Diptera. They have, in addition, similar layer relationships. Thus the three wide-field elements in the outer layer of the medullae of both orders have been classified as M : tan 1–3. The small-field and strip-field elements have been classified with reference to their morphological counterparts in the Diptera. There are fewer elements in the Lepidoptera and their classification is not sequential.

The class II elements of the medulla are diagrammatically summarized in figures 55 and 56. A short description of each is given in the accompanying figure legend. Four elements need special consideration.

(1) Single sections of newly eclosed *Pieris* show that the surface tangential (M : tan 1) (figure 43, plate 7 and figures 47 and 48, plate 8) and the wide-field tangential (M : tan 2) (figure 43, plate 7 and figures 49 and 50, plate 8) beneath it are derived from the same cell-body. Between two and five large perikarya (30 to 40 μm in diameter) are situated at the anterior margin of the medulla. Each gives rise to a large-diameter linking-fibre (see table 1) which projects to the surface of the contralateral medulla. It may reach the surface through the inner face of the medulla or by climbing over its antero-dorsal edge. It ends as M : tan 1. Each cell-body also gives rise to a large-field ipsilateral component in stratum 2 (M : tan 2). A single collateral is derived from the linking-fibre in the mid-brain at a position just central to the margin of the calyx of the ipsilateral mushroom body. This component projects antero-ventrally as far as the dorsal margin of the protocerebral bridge. Short stubby processes are derived radially from its entire length.

(2) The optic tubercle is linked to the medulla. In the Diptera there is some evidence that two neurons may be involved in this arrangement. A line amacrine in stratum 3 may be contiguous with a unilateral class I element. This cell projects to the tubercle via the anterior optic tract (see part II). In *Pieris* there is a line tangential at the same level in the medulla; between 8 and 10 tangential fibres are derived from a single axis-fibre. Each tangential fibre

gives rise to short spiny processes along most of its length. These invariably lie at the same level as the outer band of spines of the transmedullary cells: the two components have been seen closely applied to one another. The tangential fibres are predominantly oriented horizontally (figures 61, 63 and 64, plate 9), but at the margin of the medulla disk they may be oriented at any plane (figure 62, plate 9). Each linking-fibre projects from the inner face of the medulla through the lobula neuropil to the optic tubercle where it has a distinct and characteristic ending (figures 67 and 68, plate 10; figure 69). The 8 to 10 tangential fibres from each linking-fibre in the medulla invade a narrow strip field in stratum 3 whose horizontal extent is equivalent to the whole of the medulla arc. The vertical extent is less than 12 μm (figure 70).

(3) The strip-field tangential in stratum 8 is unique in that it is perpendicularly asymmetrical. Each cell-body gives rise to a short axis-fibre which divides between 4 and 6 times in the serpentine layer. Each of these subsequent fibres gives rise to a small strip subfield (figure 44, plate 7, figure 57, and figure 58, plate 9). The four or five subfields together make a total strip field in stratum 8 whose horizontal lateral extent is equivalent to the arc of the medulla. The vertical extent does not exceed 20 μm. The axis-fibre also gives rise to an ascendant arborization which is restricted to the anterior margin of the medulla and ramifies through strata 6–2 (figure 57).

(4) *The giant optic lobe tangential.* The medulla, lobula and lobula plate contain some characteristic subfields which are composed of processes derived from a single large-diameter linking fibre (Table 1). These components are part of an extremely complex cell which at present is only known from the brain, though it most probably sends processes to the ventral nerve cord and its ganglia.

In *Pieris* the suboesophageal ganglion gives rise to a pair of giant fibres which extend into the ventral nerve cord. These apparently are derived from a single cell-body fibre; however, this could possibly represent several finer fibres closely apposed to one another. One of the giant fibres gives rise to a thick recurrent branch that projects to, and then bifurcates within, the tritocerebrum. Single sections have demonstrated that two resultant fibres project bilaterally, each finally arriving at an optic lobe where it gives rise to the wide-field component: a dense tangle of processes, that covers the lobula plate surface. This arborization in turn gives rise to collaterals which end in the lobula and the medulla. Those in the lobula form narrow vertical strip-fields in the lobula stratum 1: those in the medulla give rise to a plexus of processes which extend throughout the whole of the interface between strata 7 and 8. Further ascendent

Description of plate 7

The medulla

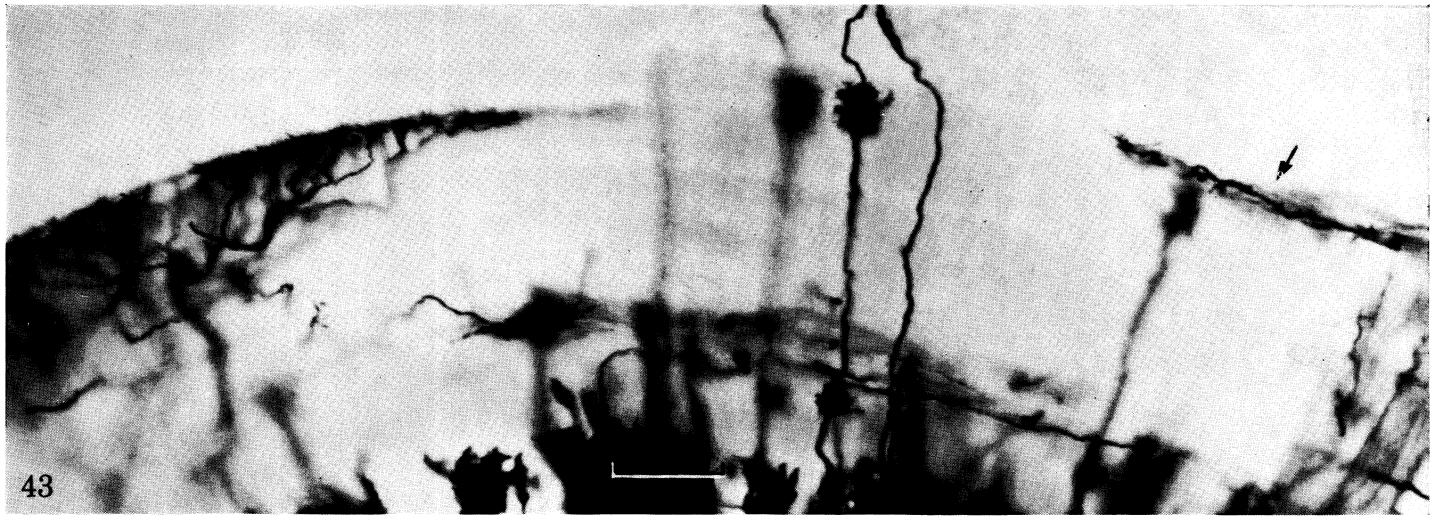
Figure 43. *Pieris brassicae.* The stratification of the medulla. This illustration shows the processes of M: tan 2 (left) in stratum 2 and M: tan 1 at the surface of the medulla (right; arrowed). The outermost processes of the transmedullary cell (centre) are sandwiched between these two class II components.

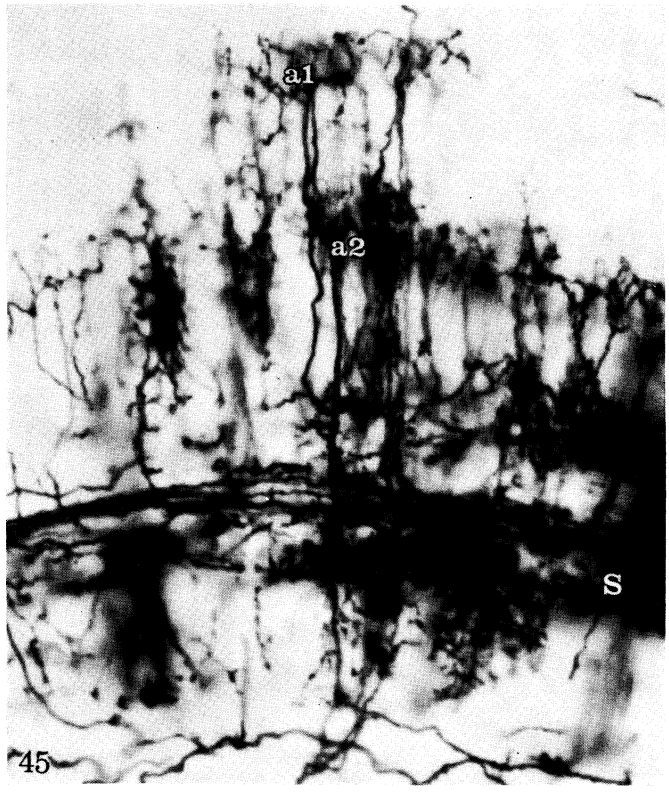
Figure 44. *P. brassicae.* The transmedullary cell shown in its entirety (cell-body is at the extreme top right-hand corner). Outer and inner spines arrowed. The strip field (sf) tangential (M: tan 11) is at the same level as the inner group of Tml processes in strata 7 and 8 (arrowed). The transmedullary cell axis-fibre extends to the lobula (L) as a linking-fibre. Note the convergence of transmedullary cell endings at the outer stratum of the lobula.

Figure 45. *P. brassicae.* Processes of small-field tangentials in the medulla (M: tan 12 and M: tan 7). S = serpentine layer. a1 and a2 = outer processes of a tristratified amacrine cell. (Note that the second group (a2) are situated at the same level as the tangentials outer branches.)

Figure 46. *P. brassicae.* M: tan 11 processes and an initial component of a T_2 cell.

Scales: Figure 43, 20 μm; figures 44 to 46, 20 μm.





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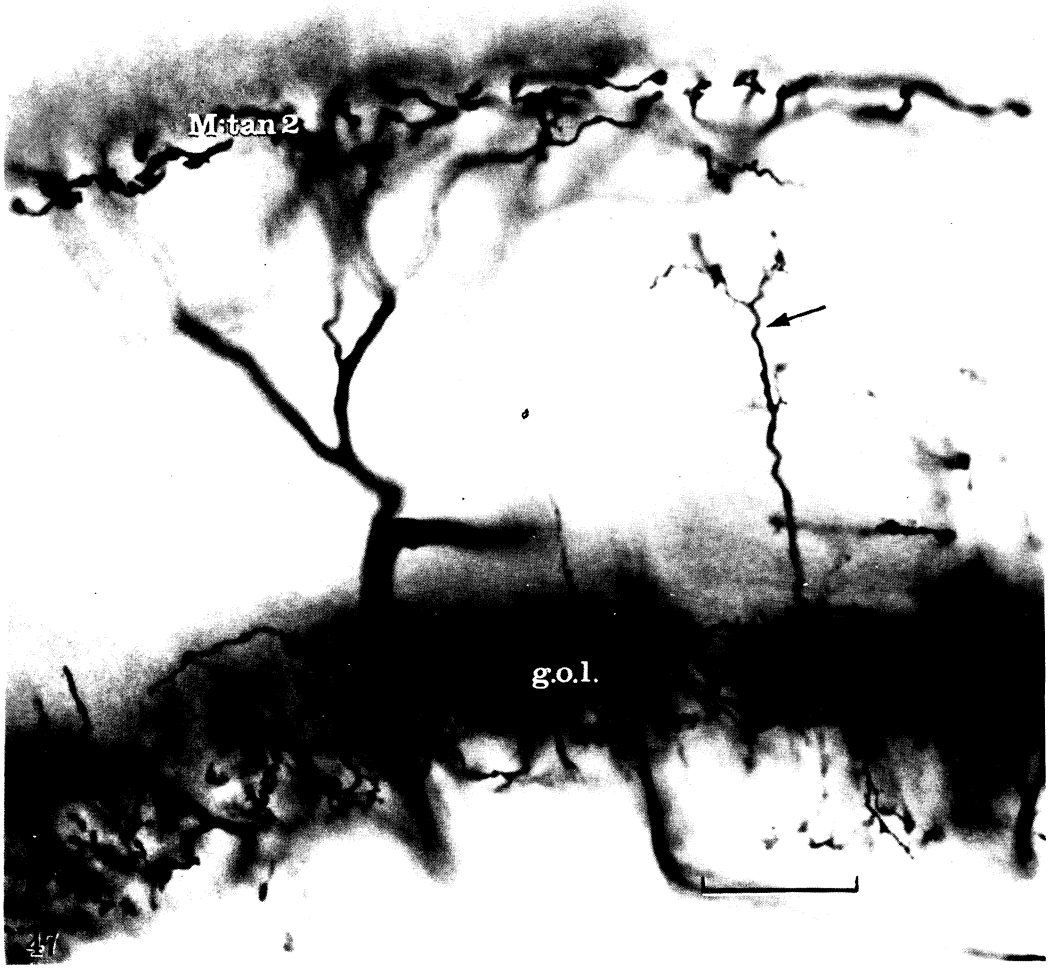

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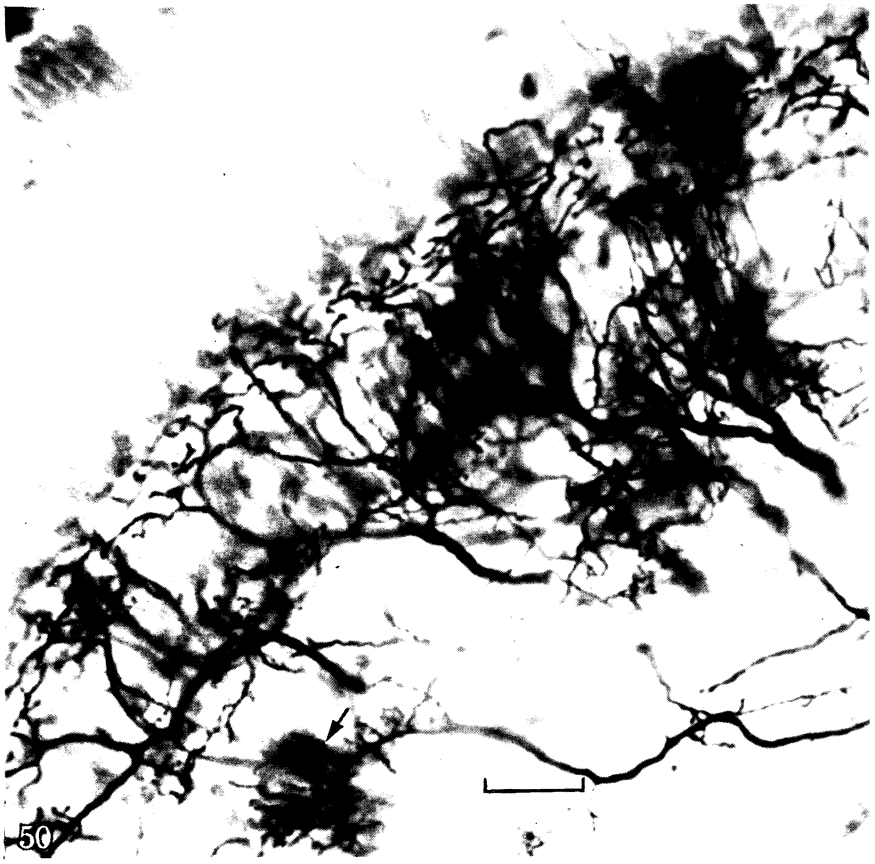

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processes extend to stratum 3 where they end as small disk subfields (figures 47 and 51, plate 8). The neuron has not been traced further in the ventral nerve cord, but its ramifications in the brain also extend to other mid-brain regions. These other projections are still under investigation. The morphology of this element in the optic lobes of *Sphinx* is similar to that of *Pieris*. However, its oval subfields in stratum 3 of the medulla are wider, and adjacent subfields overlap by as much as one half of their lateral extent.

Amacrine cells of the medulla

Introduction

Amacrine cells (figures 41*b* and 41*c*) are neurons whose processes lie within only one geographical region. They are divided into three subclasses: diffuse amacrines, stratified amacrines and asymmetric amacrines. Cajal (1933) made similar distinctions between the various types of amacrine cell in the vertebrate retina. In mammals and in insects amacrine cells have processes which are disposed in particular layers (stratified amacrines) or which ramify through a region without giving rise to branches concentrated in strata (diffuse amacrines). Cajal (1888) described a third form of amacrine cell in the pigeon retina; this has a group of diffuse or stratified processes in one part of the retina which give rise to a long lateral prolongation to another part where it ends as a diffuse or stratified bundle of processes with a different morphology from the first. This form of cell has been positively identified in the two species of Syrphidae and in the Calliphorinae (see part II). There is evidence, from the study of serial sections, that its analogue exists in *Pieris*.

Diffuse amacrine cells

(*a*) *Wide-field diffuse amacrine cells*

There is only one species of this amacrine cell in the medulla of *Sphinx*, *Pieris* and *Automeris*. Its cell-bodies are situated above the outer face of the medulla amongst the fibres of the first optic chiasma. They give rise to an extremely slender cell-body fibre which projects as far as stratum 3 where it gives rise to many slender branched processes which extend through the medulla to its inner face. The processes are characteristically blebbed down their length. Some cells of this type, seen in *Pieris*, do seem to have rather more blebs concentrated in strata 4 and 8 but this cannot warrant the classification of an extra species of stratified diffuse amacrine

Description of plate 8

The medulla

Figure 47. *Pieris brassicae*. M: tan 1 (wrongly labelled on the plate as M: tan 2) = processes of the type 1 medulla tangential element (surface) derived from thick axis-fibres in strata 7 and 8. G.o.l. = the medullary component of the giant optic lobe element: linking-fibres from the lobula plate give rise to many processes in stratum 8 which form a network throughout this level. Ascendent processes also extend from these as far as stratum 3 (arrowed).

Figure 48. *P. brassicae* (tangential section). Processes of M: tan 1 showing the characteristic thick swollen endings.

Figure 49. *P. brassicae* (tangential section). Processes of M: tan 2.

Figure 50. *P. brassicae* (methylene blue-buffered gluteraldehyde prefixation. Sections cut at 200 μm). M: tan 2 processes extend throughout stratum 2. Arrow indicates the vertical component of a perimeter variant of a Y_1 cell.

Figure 51. *P. brassicae* (vertical section). The layer relationships between line tangential processes (arrowed), T_2 cell processes and deep processes of the giant optic lobe element in strata 7 and 8.

Scales: Figures 47, 51, 25 μm; figures 44, 48, 5 μm; figure 50, 15 μm.

TABLE 1. FIBRE DIAMETERS (AXIS-FIBRES IN REGIONS, LINKING-FIBRES OUTSIDE REGIONS: FROM GOLGI-PREPARATIONS)

		diameters (μm)			
		Pieris brassicae		*Sphinx ligustri*	
cell types	location of measurement	max.	min.	max.	min.
type 1 retinula cell ending	external plexiform layer	2.0	1.0	2.0	1.4
type 2 retinula cell ending	external plexiform layer	0.5	0.2	—	—
type 3 retinula cell ending	external plexiform layer	2.0	1.0	1.7	1.0
spiny long visual fibre	external plexiform layer	3.0	1.9	3.0	2.0
	1st optic chiasma	2.6	2.5	3.0	2.5
smooth long visual fibre	external plexiform layer	3.0	2.0	3.1	2.2
	1st optic chiasma	3.0	2.5	3.0	2.7
wide-field long visual fibre	external plexiform layer	3.0	1.0	2.5	1.3
	1st optic chiasma	2.1	2.0	2.0	2.0
T_1	1st optic chiasma	1.0	0.3	?	?
small monopolar cells	external plexiform layer	2.0†	1.0†	—	—
	1st optic chiasma	2.6	1.4	—	—
giant monopolar cells	external plexiform layer	—	—	2.5	1.7
	1st optic chiasma	—	—	2.5	2.1
type 1 transmedullary cell	medulla	1.5	0.8	2.0	1.7
	2nd optic chiasma	1.6	1.0	1.8	1.2
T_2	2nd optic chiasma	1.7	1.0	1.6	1.0
T_3	2nd optic chiasma	1.5	0.8	1.9	1.4
T_4	2nd optic chiasma	2.5	1.0	1.7	1.0
T_5	intra-complex tract	1.8	1.3	1.8	1.6
translobula plate cell	intra-complex tract	1.2	0.8	?	?
M: tan 2	medulla (ant. edge)	4.0	3.5	5.2	3.8
M: tan 1	medulla (inner face)	4.5	3·7	5·0	3·5
M: tan 3	surface processes only	3·0	?	?	?
M: tan 7	medulla (ant. edge)	2.1	2.0	2.5	2.0
M: tan 9	medulla (ant. edge)	2.0	2.0	2.0	1.9
M: tan 10	medulla (inner face)	1.5	0.75	?	?
M: tan 11	medulla (ant. edge)	4.0	2.5	?	?
line tangential	medulla (strata 3-inner face)	1.1	0.8	?	?
line tangential	fibres to and through lobula	0.8	0.5	?	?
line tangential	fibres in anterior optic tract to optic tubercle	1.2	0.5	?	?
giant optic lobe element	linking fibres between lobula plate and medulla	1.8	1.5	1.7	1.5
giant optic lobe element	linking fibres from lobula plate to lobula	1.5	0.7	1.5	0.7
giant optic lobe element	linking fibre from lobula plate subfield to mid-brain (at oral edge of lobula plate)	4.8	3.0	5.8	5.5
LP wf 1	inner face of lobula plate	3.5	2.7	4.0	3.5
LP sf 1	oral edge of lobula plate	2.5	1.2	2.5	2.0
LP sf 2	oral edge of lobula plate	2.8	2.0	2.5	2.3
L wf 1	inner face of lobula	3.5	2.0	3.0	2.5
L wf 2	inner face of lobula	4.0	3.5	4.0	3.0
L sf 1	oral edge of lobula	3.0	2.8	3.0	2.4
L sf 3 and 5	oral edge of lobula	2.0	1.8	3.3	2.5
L sf 4	inner face of lobula	4.0	3.4	4.2	3.0
small-field lobula element to optic tubercle	anterior optic tract	2.8	2.2	?	?
IIS (stratum 2)	inner face of lobula	2.5	2.3	—	—

† Midget monopolar cell = 1.1 (max. in lamina), 0.7 (min. in lamina), 1.0 (max. in first chiasma), 0.8 (min. in chiasma).

— = not seen; ? = no data.

cell. In *Sphinx* some processes may arise from the cell-body fibre in the outer-most stratum of the medulla (figures 41*b*, *c*).

(*b*) *Stratified diffuse amacrine cells*

This form of neuron (*Gestalt* name = 'pi-cell') seems to be characteristic of the Lepidoptera. It has never been seen or described in any other order. The perikaryon of this element is situated in the cell-body cortex which surrounds the first optic chiasma. The cell-body fibre projects to the outer surface of the medulla where it gives rise to a thick process, about 3 μm in diameter, 40 μm long in *Pieris*, which lies on the outer surface and is orientated in the antero-posterior plane. Characteristic bunches of knobs 'hang' from its ends within the outermost

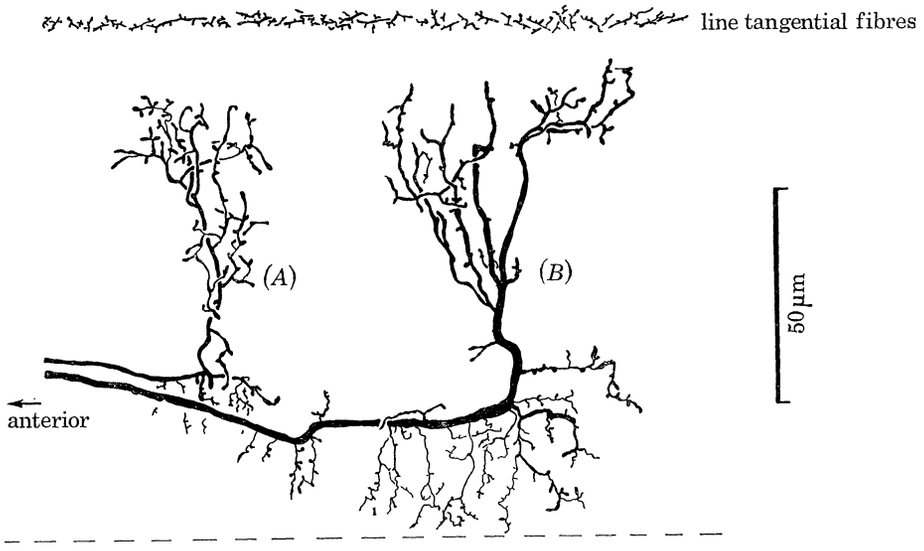

FIGURE 52. *Pieris brassicae.* A camera lucida drawing of two terminal components of two asymmetrical amacrine cells (in horizontal section). The vertically oriented line tangential processes are also shown in stratum 3.

stratum of the medulla (figure 23, plate 5; figure 41*b*). The tangential process gives rise, at about its mid-point, to several perpendicular processes which descend as far as the inner most stratum. In strata 2 to 4 these processes are thick, 2 to 3 μm wide and are varicose. The perpendicular processes below this level are thin, between 0.7 and 1.0 μm in diameter, and end as long irregularly shaped tubers. The lateral spread of these descending fibres is equivalent to two medullary columns.

Unistratified amacrine cells

(*a*) *Deep unistratified amacrine cells*

Thin cell-body fibres derived from the cell-body cortex around the first optic chiasma give rise to single groups of processes in stratum 7. Each has a very small lateral field, between 8 and 12 μm in extent, formed by the bifurcation of the axis-fibre and the several short spiny processes derived from it.

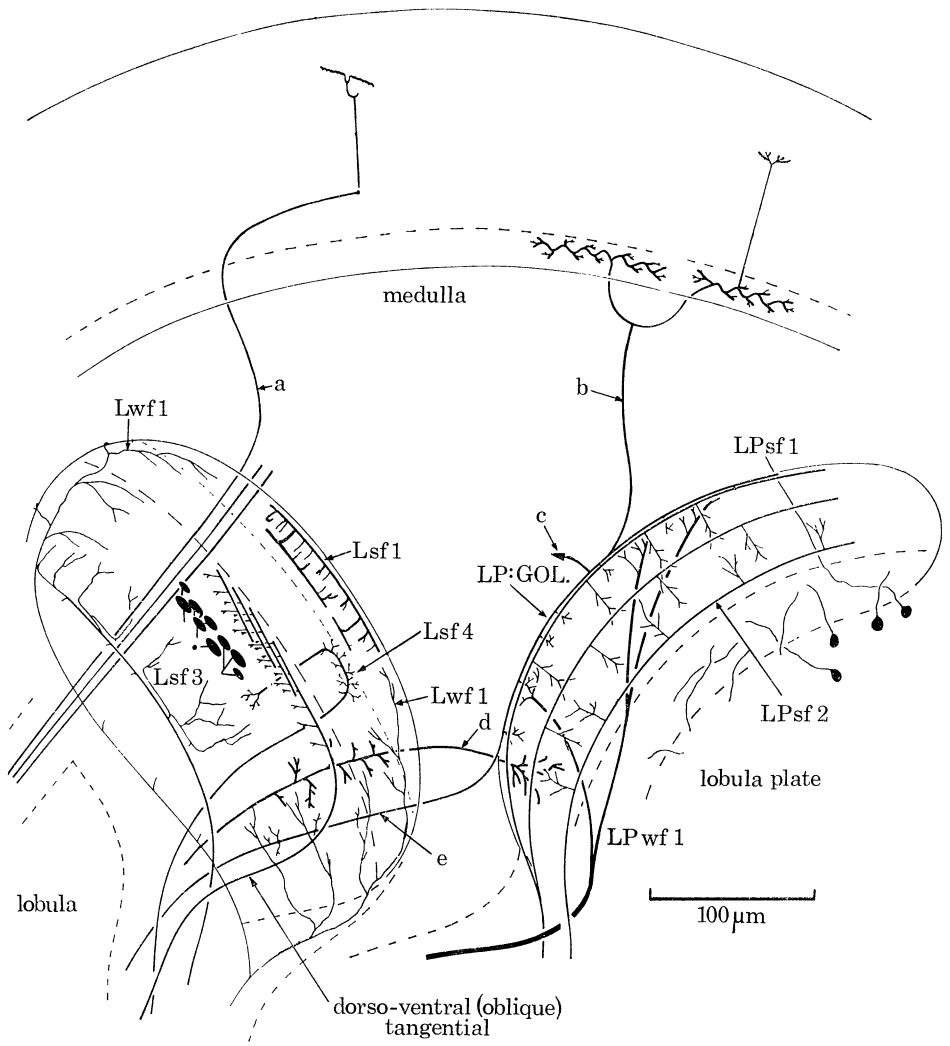

FIGURE 53. *Pieris brassicae.* Schematic diagram of lobula complex tangential elements. The lobula plate characteristically contains two strip-field elements, LP.sf 1 and 2, which extend through the whole oral-ocular arc of this region. The wide-field element LP.wf 1 covers the surface of the lobula plate.

The lobula of the Lepidoptera contains many elements which have similar forms and dispositions to those in Diptera; for example, the strip-field elements Lsf 1, 3, 4 and 5; and the wide-field elements Lwf 1 and Lwf 2 (figures 71 and 74). Lsf 2 of the Diptera has not been seen in the Lepidoptera. However, the obliquely antero-posterior, dorsoventrally oriented strip-field elements (*incerta sedis*) in the Lepidoptera may be analogous to it.

The outermost strip-field element extends through the whole oral-ocular arc of stratum 1. Types 3 and 5 are restricted to stratum 3 (see figure 73). Type 4 (figure 71) is characteristically oriented vertically in both Lepidoptera and Diptera.

a = linking-fibres from the line tangential in the medulla, through the lobula, into the anterior optic tract. They terminate in the optic tubercle. b = one of many linking-fibres of the giant optic lobe element from the lobula plate subfield to the medulla. LP: GOL = the lobula plate component of the giant optic lobe element. c = one of many linking-fibres from LP: GOL to the lobula, each gives rise to a vertically oriented strip subfield (see figures 69 and 74). d = a small-field IIS element which invests both the lobula and lobula plate. This cell has been seen in only one viral infected preparation. Its form is analogous to IIS: 9 in the Diptera. e = linking-fibre from the giant optic lobe element to the mid-brain.

(*b*) *Miniature unistratified amacrine cells*

The cell-bodies of these elements are situated above the outer face of the medulla amongst the fibres of the first optic chiasma. Each gives rise to a stout cell-body fibre which projects as far as the serpentine layer of the medulla where it ends as a group of six or eight terminal swellings. These have a total lateral spread of between 5 and 10 μm. This amacrine cell has only been seen, in its entirety, in *Pieris*. Part of a similar element has been seen in the corresponding strata of *Sphinx* but has not been traced to its cell-body.

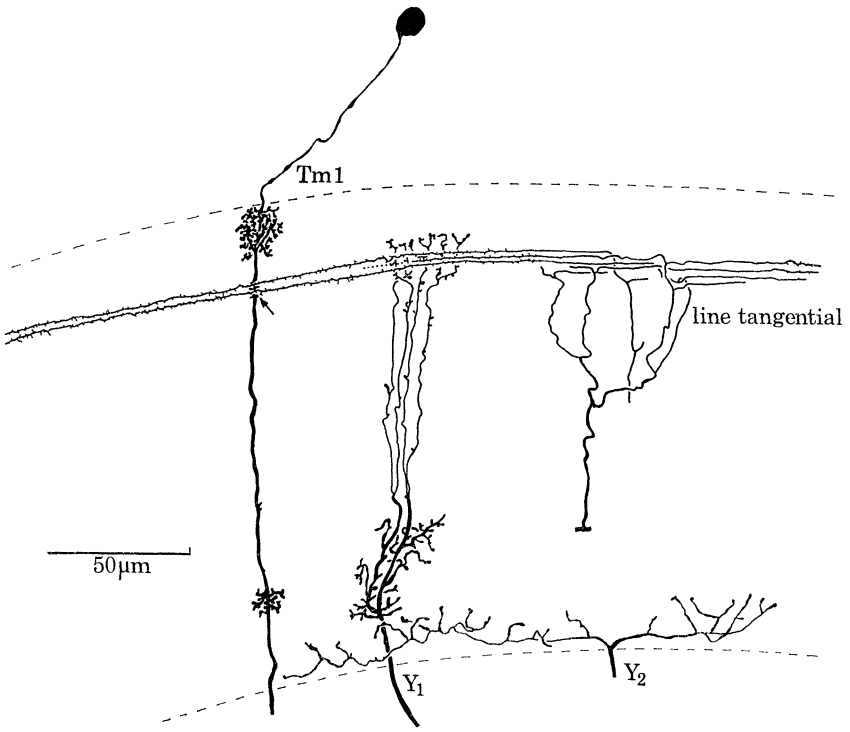

FIGURE 54. *Pieris brassicae* (vertical section). Camera lucida drawing of some elements in the medulla. Note the layer relationship between the transmedullary cell spines (arrowed) and the line tangential cell processes.

(*c*) *Tangential small-field amacrine cells*

Cell-body fibres penetrate the outer face of the medulla and project as far as strata 4 or 5 where they curve sharply at right-angles and project tangentially at the same level for a distance of between 100 and 150 μm. Each terminates as a dense group of branches in the serpentine layer or in stratum 5. Each branch has lateral swellings at regular intervals along its length, and ends as a single or double swelling. The lateral spread of a group of branches is between 15 and 25 μm. These cells are characterized by the bent appearance of their cell-body fibres, and the unbranched prolongation of each of them. Its significance is not known but possibly the tangential fibre may receive or donate information somewhere along its length, although there are no lateral specializations to indicate this. This cell has only been seen in its entirety in *Pieris* (figure 41*b*). There is a similar ending at a similar location in *Sphinx*. Its cell-body, however, is situated below the medulla amongst the fibres of the second optic chiasma.

Bistratified amacrine cells

(*a*) *Bistratified narrow-field amacrine cells*

The medulla components of these cells have frequently been seen but the cell-body fibres have never been traced to their corresponding perikarya. This may possibly be the consequence of the extreme thinness of the cell-body fibres (less than 0.3 μm in diameter), and a resultant failure to impregnate it.

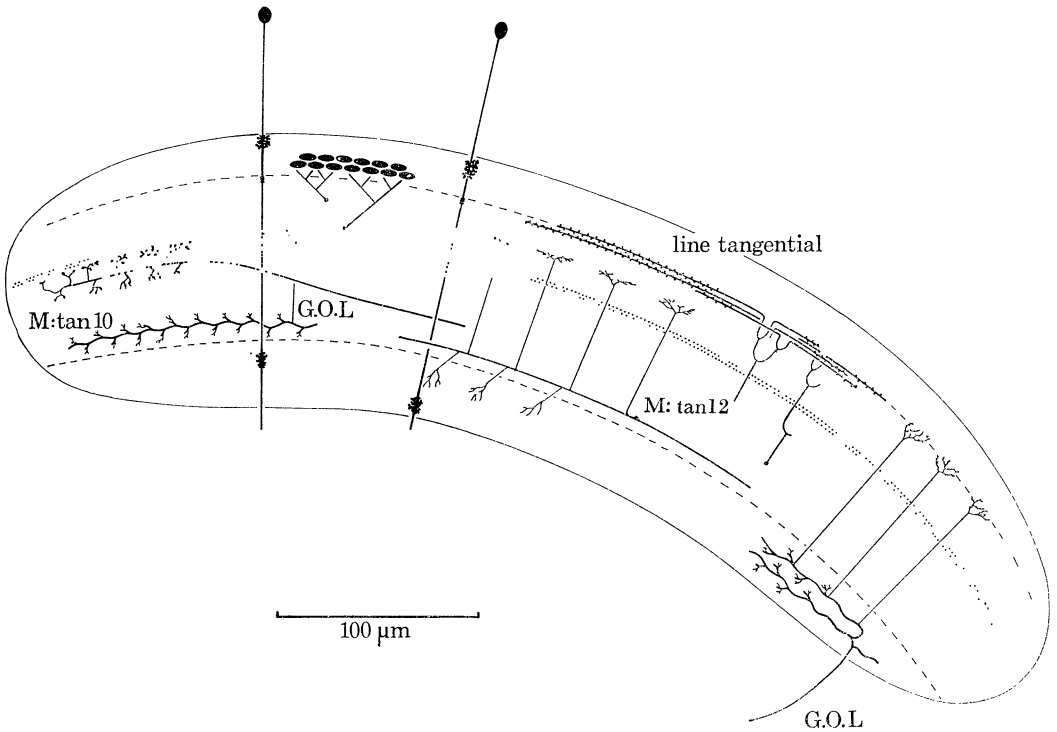

FIGURE 55. *Pieris brassicae* (vertical section). Schematic summary diagram of the layer relationships of class II elements in the medulla. Vertically oriented small-field tangentials, M: tan 10 and M: tan 12 consists of many small oval subfields from axis-fibres in the serpentine layer. The giant optic lobe tangential (G.O.L.) subfields in the medulla are bistratified. The ascendent processes from strata 7 and 8 extend as far as the level of the line tangential cell processes or as far as the endings of long visual cell-fibres (dotted lines).

Each cell-body fibre gives rise to an axis-fibre which projects as far as stratum 7. There are two groups of processes; an outer group in strata 2 and 3 which consists of about 6 to 8 branches derived from a restricted zone of the perpendicular axis-fibre. The axis-fibre itself, in these strata, is characteristically spiny as are the lateral branches. The inner group of processes in stratum 7 arises from two extremely short, stout, terminal branches. The processes are slender, short (3 to 5 μm long) and are sometimes blebbed with a terminal swelling. The outer group of processes has a lateral spread of between 10 and 15 μm, the inner group a spread of between 8 and 10 μm.

Both *Sphinx* and *Pieris* have somewhat similar species of amacrine cells which are situated at structurally homologous layers. The bistratified amacrine cells are an exception. In *Sphinx* the outer group of processes ramifies through only a single stratum (figure 41*c*); the stratification of the *Sphinx* medulla is not so obvious compared to that of *Pieris*, and the only components that this group of processes can be related to, on the basis of layer relationships, are the

outermost monopolar endings. Similarly, the outer group of processes of the bistratified amacrine cell of *Pieris* seems to lie in strata which also contain monopolar endings. The deep component of the bistratified cell in *Sphinx* has a recurrent prolongation of the perpendicular axis-fibre, from which arises fine lateral processes. The same form of deep component has been seen in the Diptera.

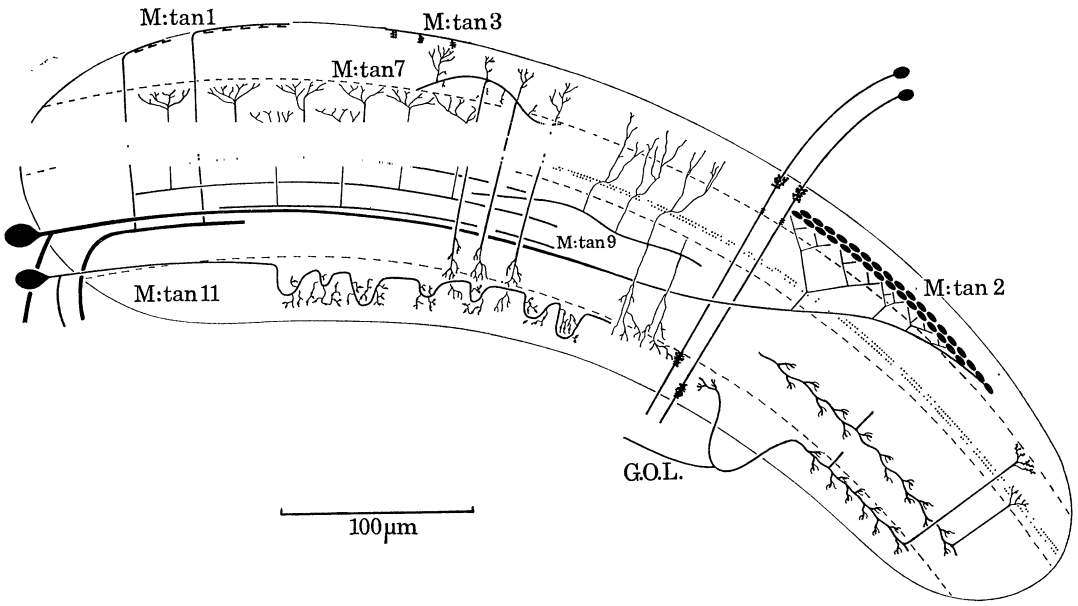

FIGURE 56. *Pieris brassicae.* Schematic summary diagram of the layer relationships of class II elements in the medulla (horizontal section). Wide-field tangentials = M: tan 1, 2 and 3. Small oval field tangentials = M: tan 7 and 9. Strip-field tangentials in strata 7 and 8 = M: tan 11. Medullary components of two trans-medullary cells are also shown in figures 55 and 56.

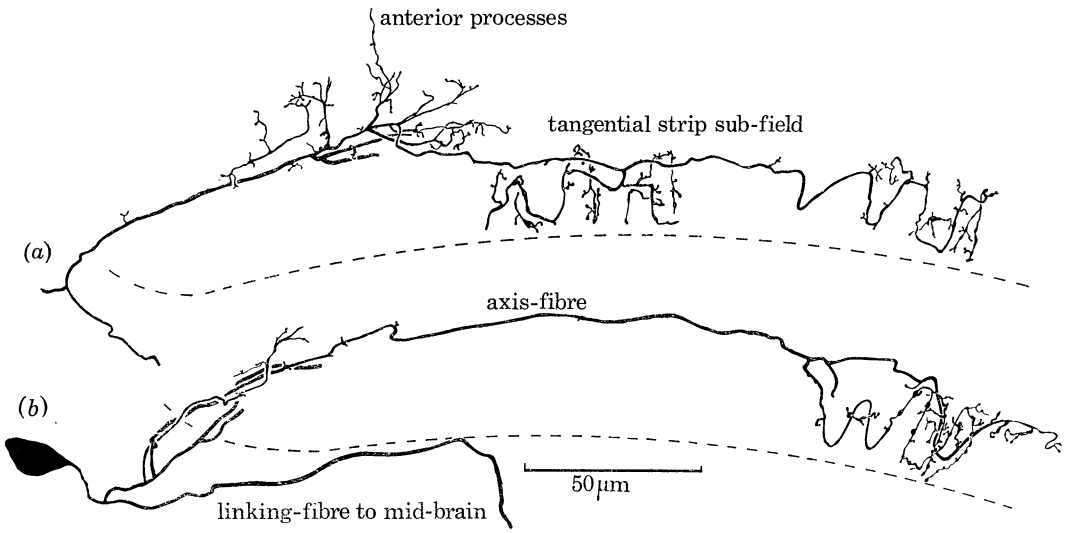

FIGURE 57. *Pieris brassicae.* Two camera lucida drawings of M: tan 11 showing the anterior ascendent processes in strata 6 and 5 and the tangential processes in strata 7 and 8.

Multistratified amacrine cells

(*a*) *Tristratified narrow-field amacrine cells*

This species of cell is derived from perikarya in the cell-body cortex surrounding the first optic chiasma. Each cell-body fibre gives rise to a perpendicular axis-fibre which projects as far as the middle of stratum 7. There are three groups of processes arising from this fibre: in strata 1 to 3 there are many short, branched lateral processes with a lateral extent of 10 to 12 μm: at the interface of strata four and five several unbranched processes with terminal spines contribute to the second group of processes. The axis-fibre bifurcates in stratum 7; the two branches give rise to many slender processes all of which have a total lateral spread of between 18 and 20 μm antero-posteriorly and between 8 and 12 μm dorsoventrally. This deepest group is sometimes seen closely associated with the shallow T-cell components (T_3) in the medulla (figure 37, plate 6).

(*b*) *Tristratified wide-field amacrine cells*

The cell-body fibre is extremely slender and has never been traced to its corresponding perikaryon. The axis-fibre is directed tangentially in stratum 1 for about 20 μm; it gives rise to several branched processes each with a terminal swelling. The axis-fibre then projects perpendicularly as far as stratum 7. A tangled group of multi-branched processes is located in strata 2, 3 and part of 4: this has a lateral spread of between 25 and 35 μm. The deepest group of processes arises from a bifurcation of the axis-fibre and has a similar field size in stratum 8 (figure 33, plate 6). The outermost group of processes is compressed dorsoventrally with a lateral spread of between 4 and 6 μm in that plane. The innermost processes are sometimes seen closely applied to the processes of the bushy T-cell ending in the medulla or to the strip field-tangential processes at the same level (figure 58, plate 9).

Other forms of amacrine cells

(*a*) *Linear asymmetric amacrine cells*

This cell (figure 52) was originally included as *incerta sedis* but must now be considered as an amacrine cell. The axis-fibre is directed tangentially in the serpentine layer. It terminates as a fan of multi-branched processes which may extend through the whole of the medulla's depth except for the outermost three strata. The orientation of the axis-fibre gives this cell the

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DESCRIPTION OF PLATE 9

The medulla

FIGURE 58. *Pieris brassicae*. A detail of M: tan 11 processes and the deepest processes of the small-field bistratified amacrine cell at the same level (arrowed).

FIGURE 59. *Sphinx ligustri*. M: tan 2. The tangential axis-fibres are disposed in two layers (arrowed). The ascendent processes from them terminate in stratum 2.

FIGURE 60. *P. brassicae* (tangential section). Detail of the lateral spines of a line tangential process.

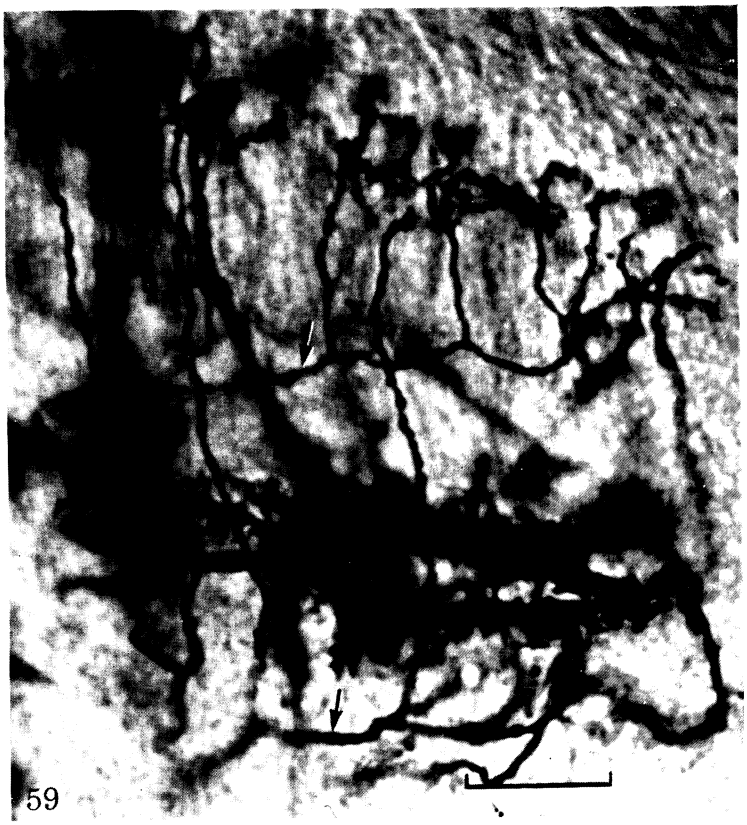
FIGURE 61. *P. brassicae* (Holmes–Rowell preparation: vertical section). The line tangentials are shown up selectively by this procedure.

FIGURE 62. *P. brassicae* (tangential section). Line tangential processes near the perimeter of the medulla disk.

FIGURES 63, 64. *P. brassicae* (vertical section, osmium fixation). Ascendent fibres and the spiny line tangential processes from them.

Scales: Figures 58, 60, 10 μm; figure 59, 25 μm; figures 61 to 64, 20 μm.


58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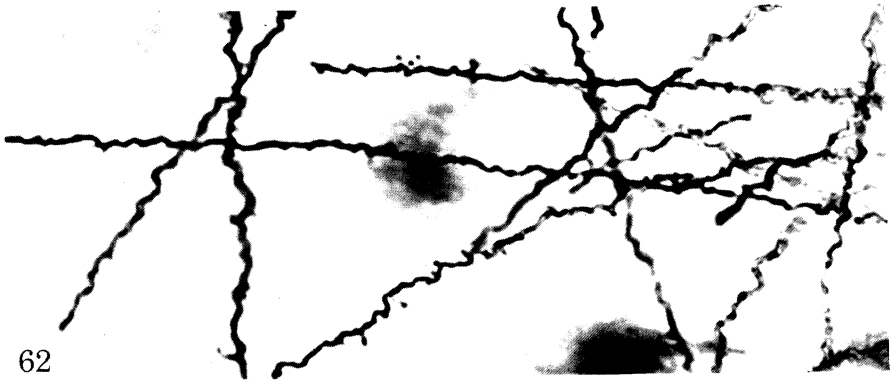
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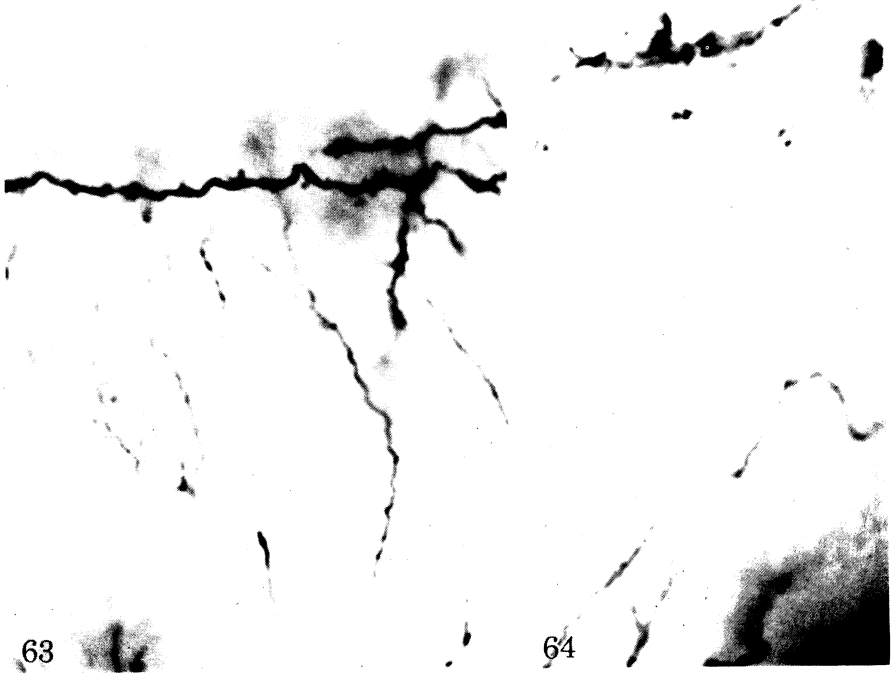
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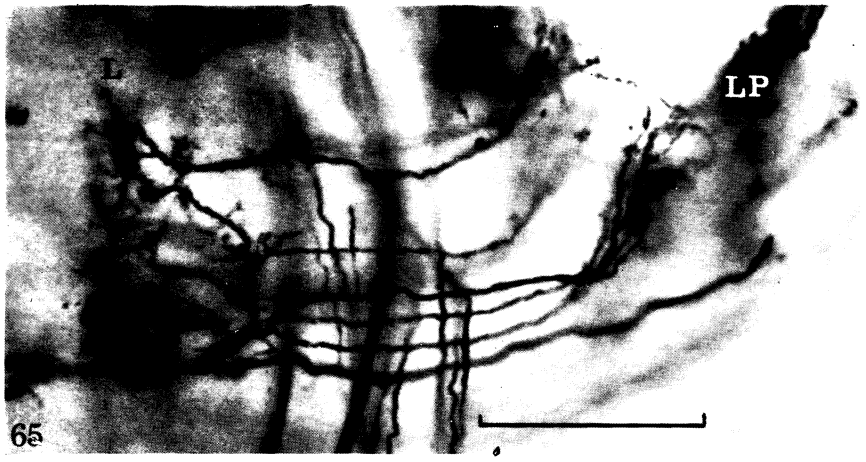

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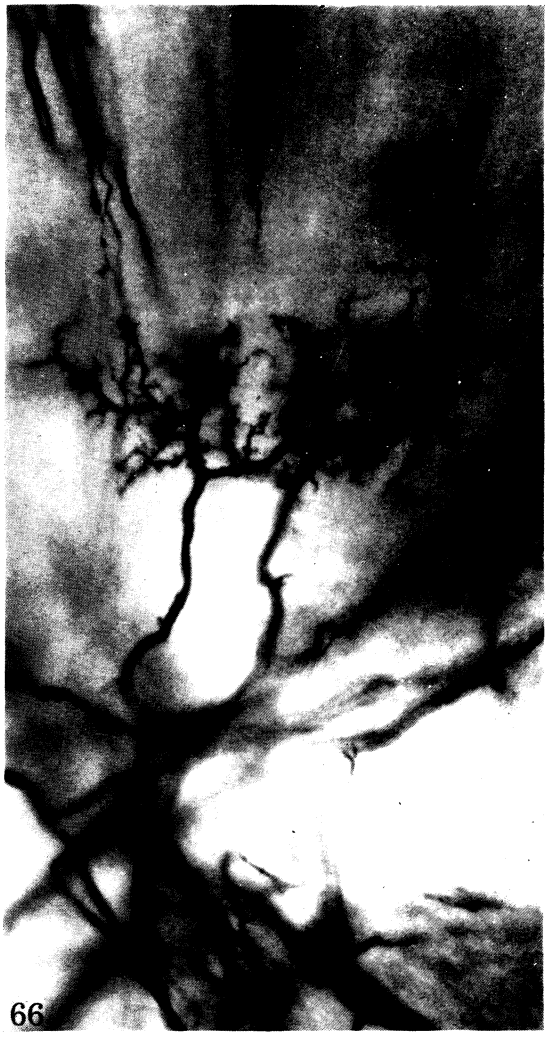

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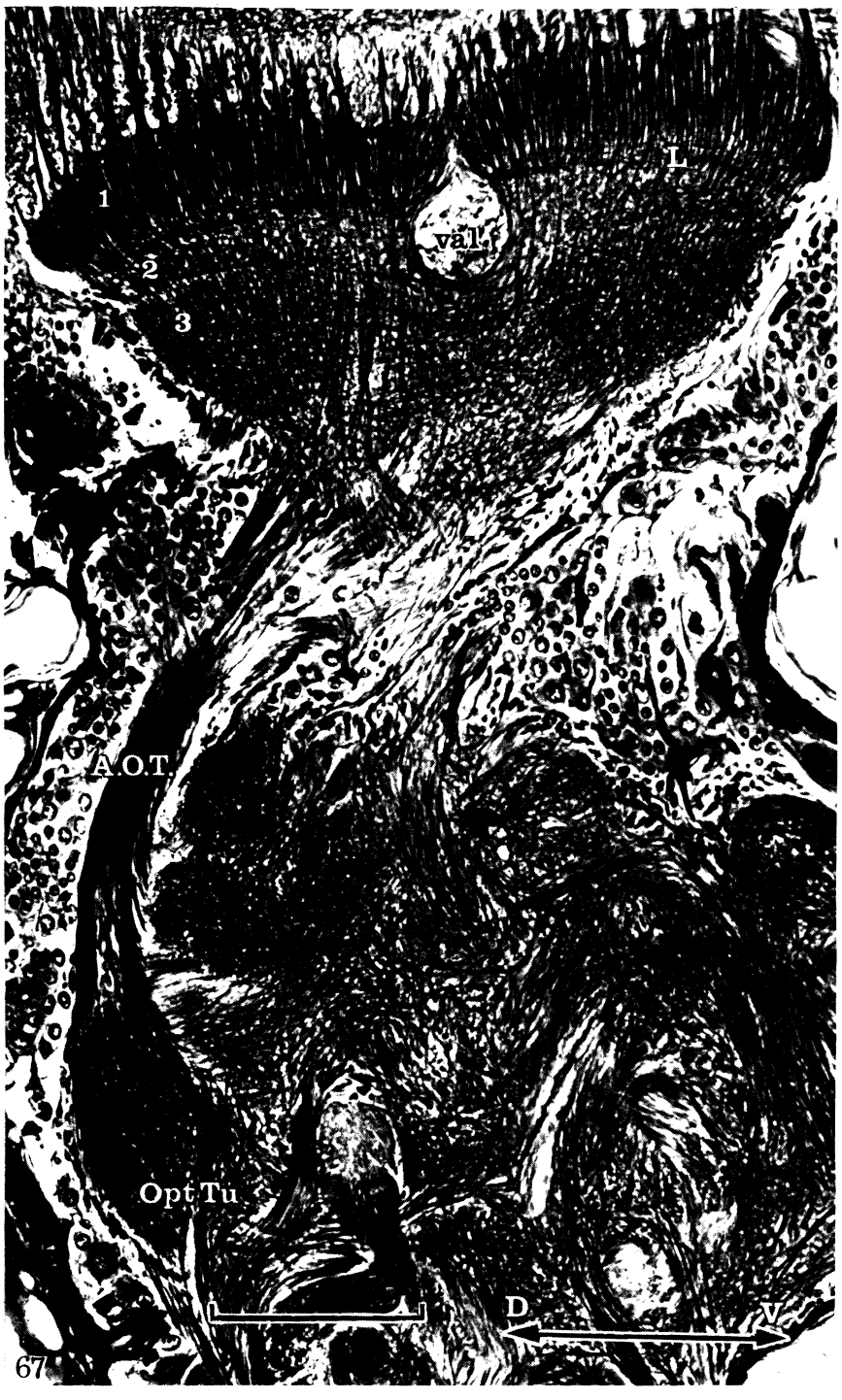

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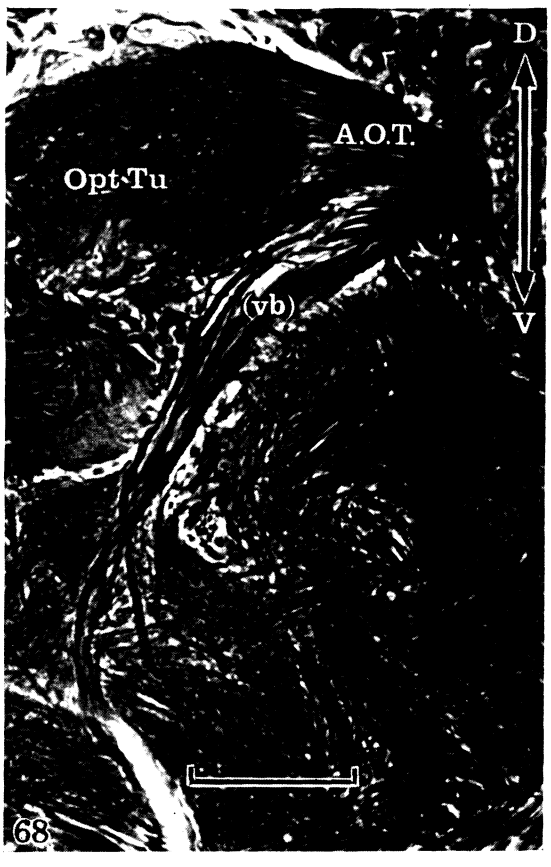


68

appearance of a class II element, but the disposition of its terminal processes are quite unlike any other cell-type. Its tangential fibre is between 2 and 4 μm thick in the median medulla but becomes extremely thin and blebbed anteriorly, and has all the appearances of a cell-body fibre. Tangential components in the medulla have axis-fibres that are invariably larger at this anterior location than elsewhere.

(*b*) *The bent amacrine cells*

This species of cell is extremely difficult to observe and was first realized in the medullae of the Syrphidae. Subsequently it has been found in other species of Diptera (part II) and in *Pieris*. Figure 41 summarizes the general disposition of this amacrine cell in the medulla. An L-shaped extension of the cell-body fibre gives rise to short lateral processes along its length. These are either spiny or blebbed. Its total lateral extent is equivalent to two medullary columns. It is usually detected at the same level as the monopolar cell endings or long visual fibre endings.

Asymmetric cells have not been seen in *Sphinx* or *Automeris*. Both these species show a large proportion of their neurons impregnated and these rather complex cells may be 'lost' amongst a dense tangle of other fibres derived from other neurons.

The neurons of the lobula and lobula plate

Introduction

The strata of the lobula and lobula plate were determined by the same procedure used for distinguishing medullary stratification. The lobula plate is simple, having only one clearly distinct stratum which is striated perpendicularly near its outer face: these parallel lines represent thick perpendicular fibres that contribute to the endings of the bushy T-cells (T_4), the T-cells linking the lobula and lobula plate (T_5) and the lobula plate components of the Y_1 and Y_2 cells.

The lobula is more complex: like the lobula plate it has an obvious outer layer, striated perpendicularly (figure 67, plate 10). The deeper lobula strata differ from those of the medulla in that they are not clearly demarcated in Holmes's silver preparations by the arrangement of their perpendicular and tangential components. In determining the lobula stratification there has been a greater reliance on measurements from Golgi preparations of the various tangential components and their topographical relationships. However, three main strata are definable in the present series of Lepidoptera. Similarly, in the Diptera three strata can be clearly delineated.

Description of plate 10

Figure 65. *Pieris brassicae*. Two translobula plate cells which invest the lobula plate and lobula.

Figure 66. *P. brassicae* (vertical section). Small field tangential elements in the lobula (subclass IIS cells; see page 183, part II).

Figure 67. *P. brassicae* (Holmes–Blest preparation: vertical section). The optic tubercle and anterior optic tract. L = lobula. 1 to 3 = lobula strata. val = lobula valley. A.O.T. = anterior optic tract. Opt Tu = optic tubercle. D–V = dorsoventral orientation.

Figure 68. *P. brassicae*. A detail of the optic tubercle and the ventral branch of the anterior optic tract which by-passes it. Opt Tu = optic tubercle. A.O.T. = anterior optic tract. vb = ventral branch of A.O.T.

Scales: Figures 65, 66, 30 μm; figure 67, 100 μm; figure 68, 50 μm.

Observations on class I endings in the lobula plate derived from the medulla and the lobula

Only two endings are solely confined to the striated portion of this region; these are the terminals of the T_4 cells from the medulla and T_5 cells from the lobula. The perikarya of these latter cells are situated behind the inner (posterior) face of the lobula plate. These send cell-body fibres directly through the lobula plate neuropil, across the intra-complex tract to the outer face of the lobula where each gives rise to the initial component in its outer stratum. A recurrent fibre from each of these projects back across the tract to terminate in the lobula plate. Both bundles of processes have oval fields with a horizontal lateral extent of about 15 μm (*Pieris*). The Y_1 and Y_2 cell components in the lobula plate have processes that both invade its striate portion and partly compose the inner non-striate neuropil.

Observations on class I endings in the lobula derived from the medulla and lobula plate

The lobulae of *Pieris* and *Sphinx* have three strata. The transmedullary cell endings, lobula component of the T_5 cells and the outer unilateral branches of the Y_1 cells are all confined to the outer stratum of this region. The endings of the deep T-cells (T_2) and the terminal processes of both types of Y-cells are situated within stratum 3. The shallow T-cell, (T_3) end in stratum 2 and the outermost quarter of 3.

The lobula of *Pieris* has a further class I connexion to the lobula plate via the translobula plate cells. These neurons have perikarya situated behind the plate which give rise to initial processes in this region. Each group of processes sends between one and four parallel branches across the intra-complex tract which terminate as narrow-field endings in lobula strata 1 and 2 (figure 65, plate 10). These cells have not been seen in the nocturnal Lepidoptera but are, however, a common feature of Diptera lobula complexes (see part II). The lateral extents of their fields, in *Pieris*, is approximately double those of the T_4 and T_5 components.

Class II elements in the lobula

As in the medulla the class II components of the lobula and the lobula plate are arranged at characteristic levels in these regions and have very precise orientations with respect to the vertical and horizontal planes. Some lobula class II components are easily identifiable in *Pieris*, and have been seen at corresponding levels in *Sphinx*. The same forms of most of these elements have been seen in the lobula complexes of the Diptera and are described in detail in the subsequent account (part II). These include two horizontal strip-field elements in the lobula plate, another in the lobula, wide-field elements over the surfaces of both these regions and others in deeper strata of the lobula. In addition, the giant optic lobe tangential invades both these regions as well as the medulla. Other strip-field elements, disposed in stata 2 and 3 of the lobula have similar forms, orientations, and field sizes in both these orders. However, we do not know if their corresponding mid-brain projections are the same. But it is convenient to use the same system of classification for them in the lobula complexes of both species. Thus the strip-field element at the surface of the lobula plate of both the Lepidoptera and Diptera is called LP.sf.1. The wide-field elements of the surface and in stratum 3 of the lobula are called, in both species, L.wf. 1 and L.wf. 2, respectively.

The Diptera have many forms of small-field elements in the lobula which project centrally. These have been grouped together as a separate subclass (IIS cells; see part II). Only two forms of these small-field components have been seen in *Pieris* and one in *Sphinx*. The form common to both species links the lobula to the optic tubercle. The other type of element, in *Pieris*, consists

of two overlapping fields in stratum 2 (figure 66, plate 10) derived from a perpendicular axis-fibre which extends, as a linking-fibre, to a region near the lobula in the ipsilateral proto-cerebral lobe. Its cell-body fibre arises from the axis-fibre at the inner face of the lobula. Its location and the central projection of this cell make it clearly analogous to the IIS : 1 cell of Diptera.

The lobula complex elements are shown schematically in figures 53 and 74. Many of them are only detectable in well impregnated brains amongst many other fibres. Thus they cannot be photographed. Some components are illustrated by camera lucida drawings (figures 69 to 73). These include a fragment of a vertical strip-field element which has only been seen in *Pieris* and not in the Diptera. However, it has only been seen in part, and it must be listed, at present, as

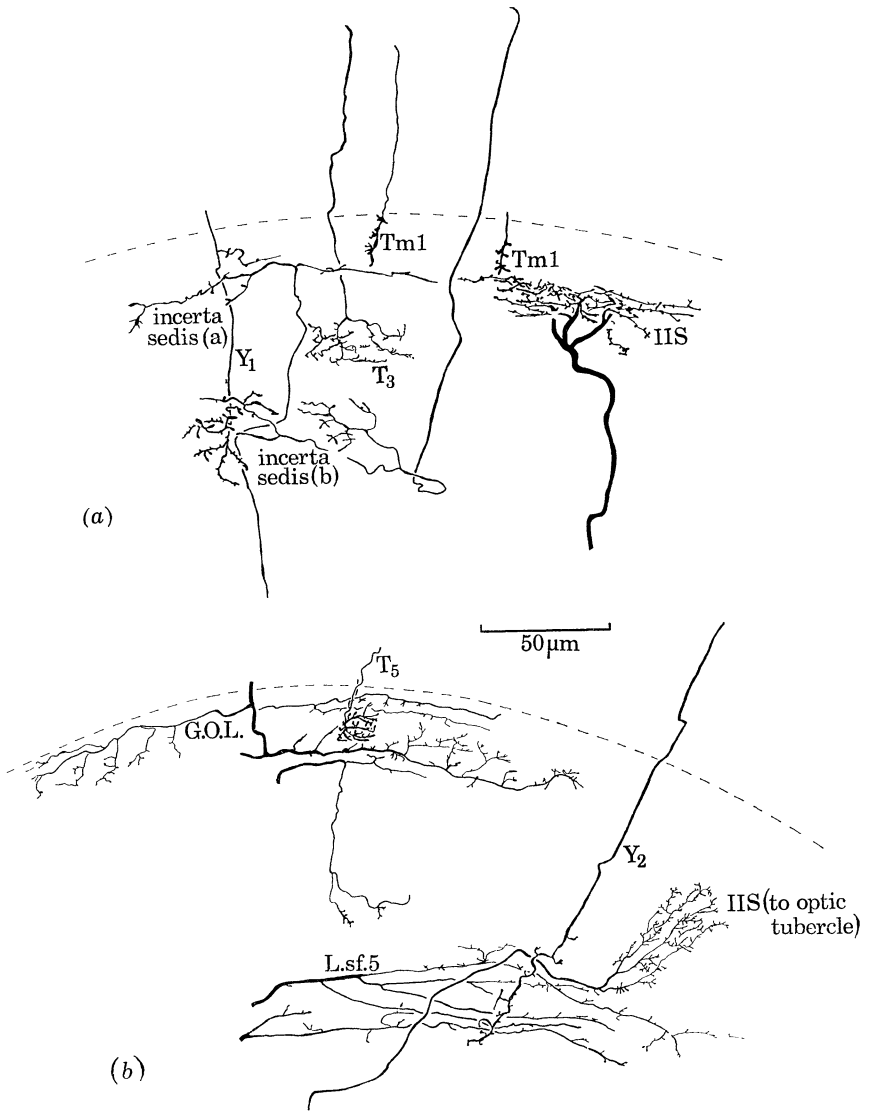


FIGURE 69(*a*) and (*b*). *Pieris brassicae.* Two camera lucida drawings of some lobula components. (*a*) (horizontal section): Tm1 = endings of transmedullary cells; Y_1 = lobula endings of deep Y-cells; IIS = small field lobula element (subclass IIS); *incerta sedis* = elements possibly intrinsic to the lobula region (lobula amacrine cells). (*b*) (vertical section): G.O.L. = a vertically oriented strip sub-field component of the giant optic lobe element; T_5 = the initial component of a lobula-lobula plate T-cell; Y_2 = the lobula component of a tangential Y-cell; Lsf 5 = some processes of the type 5 lobula strip-field tangential; IIS = small component linking this region to the optic tubercle.

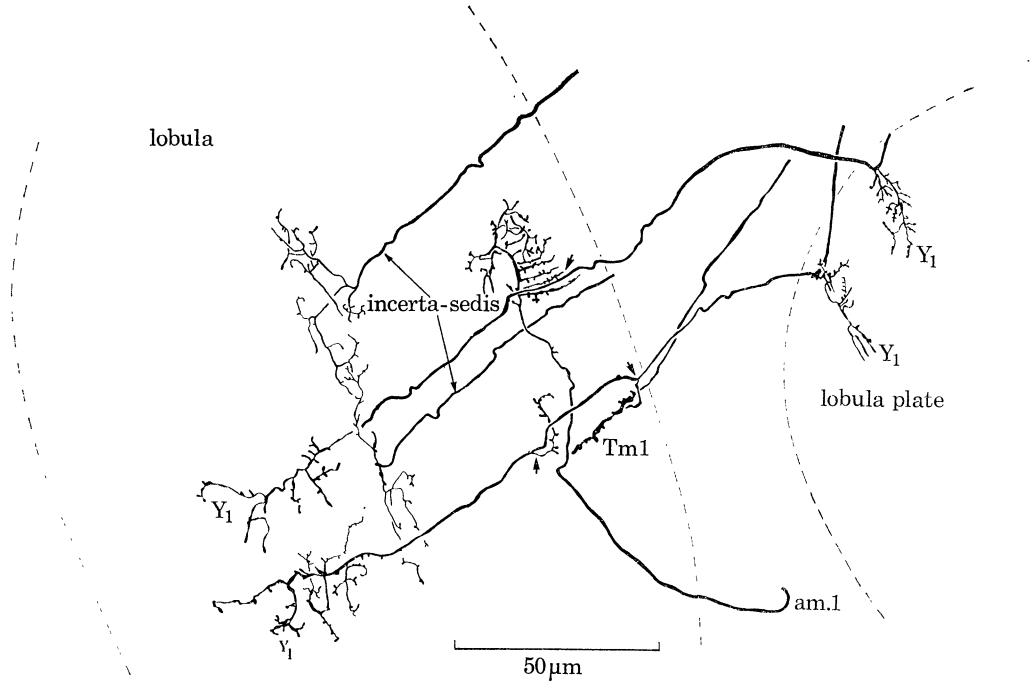


FIGURE 70. *Pieris brassicae.* Camera lucida drawing of some lobula complex elements. am. 1 = lobula amacrine cell; *incerta sedis* = elements possibly intrinsic to the lobula (lobula amacrine cells); Y_1 = the lobula complex components of deep Y-cells.

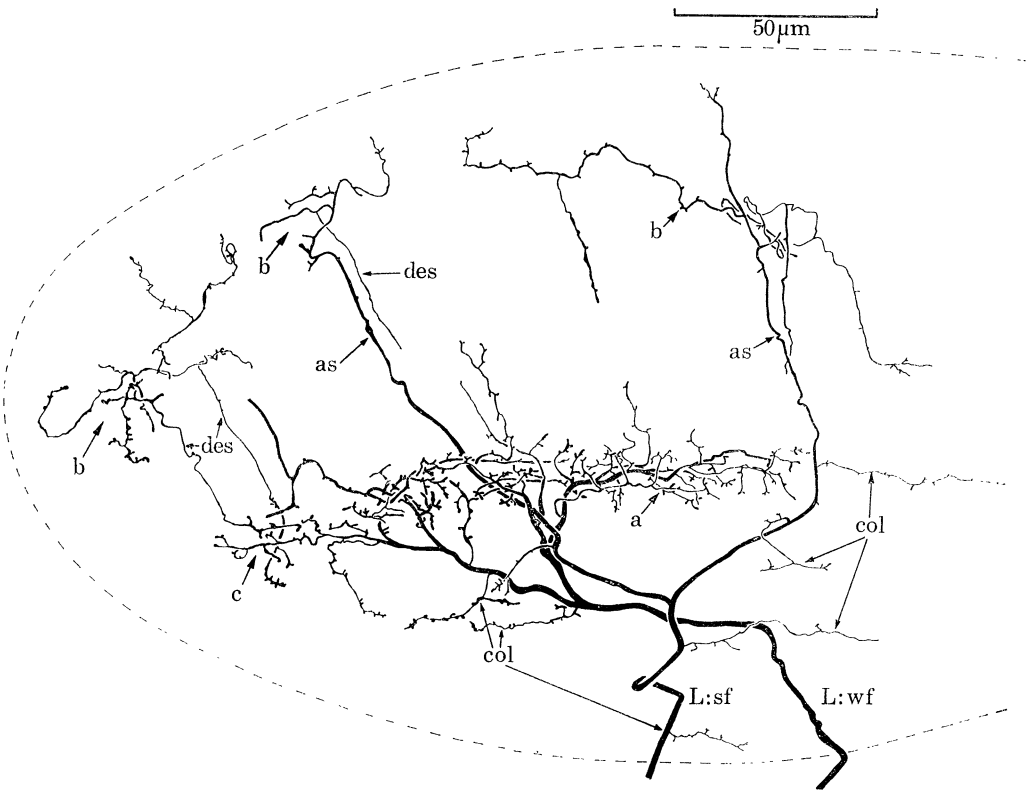


FIGURE 71. *Pieris brassicae.* Camera lucida drawing of the type 2 wide-field tangential (L: wf) in lobula stratum 3 and some ascendent (as) and descendent (des) branches of the bistratified vertically oriented, type 4 lobula strip-field tangential element (L: sf). The outer processes (*b*) of the strip-field element are restricted to stratum 2. The inner processes are in stratum 3, at the same level as the wide-field element. Both elements have thin collaterals (col) deep in stratum 3.

incerta sedis (figure 74). The giant optic lobe tangential has vertical strip-field components in the lobula of *Pieris*. In *Sphinx* and in the Diptera this anterior component is invariably oriented anteroposteriorly.

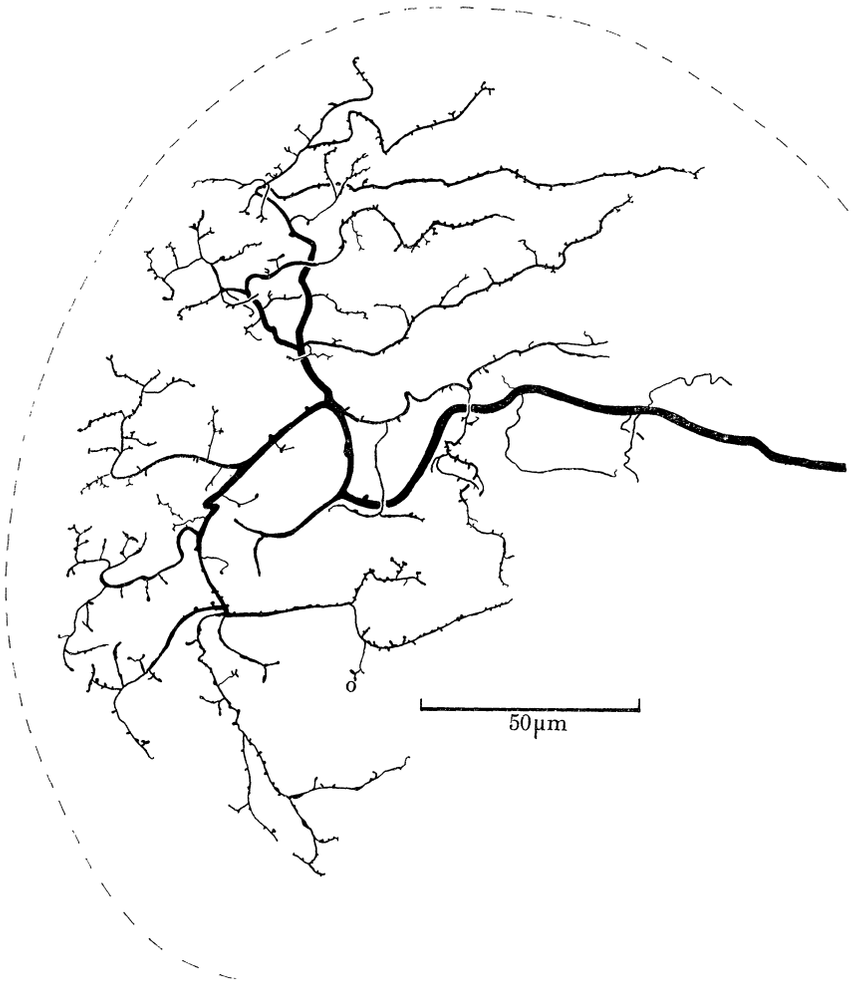


FIGURE 72. *P. brassicae*. Camera lucida drawing of part of the wide-field tangential element over the lobula plate surface.

The optic tubercle

This region (figure 67, plate 10) is comprised of only three detectable cell-types. Two are derived from the optic lobe, the third is derived from the mid-brain. The former components stem from the line tangential in the medulla, and a small-field element in the lobula. These both send linking fibres to the tubercle via the dorsal branch of the anterior optic tract. Other elements also share this tract, but by-pass the tubercle ventrally (figure 68, plate 10). The core of the tubercle consists of a tangle of blebbed and spiny processes derived from the central element and from the line tangentials. Processes from the lobula element extend over the tubercle surface and side branches ramify into it. The central component is derived from a stout linking-fibre of undetermined origin which also sends collaterals into other regions of the ipsilateral proto-, deutero- and tritocerebrum. The region centrally adjacent to the optic tubercle is also invested by this element (figure 73) and is, in addition, connected to its contralateral counterpart by a bundle of comissural fibres.

Observations on other endings in the lobula complex

There are no endings in the lobula plate which cannot be placed in either the class I or class II categories. But the lobula contains some components whose classification is still doubtful and which cannot be related to previously described class I and II cells. Three components in the lobula of *Pieris* may possibly be amacrine cells (figures 69*a* and 70). They have long

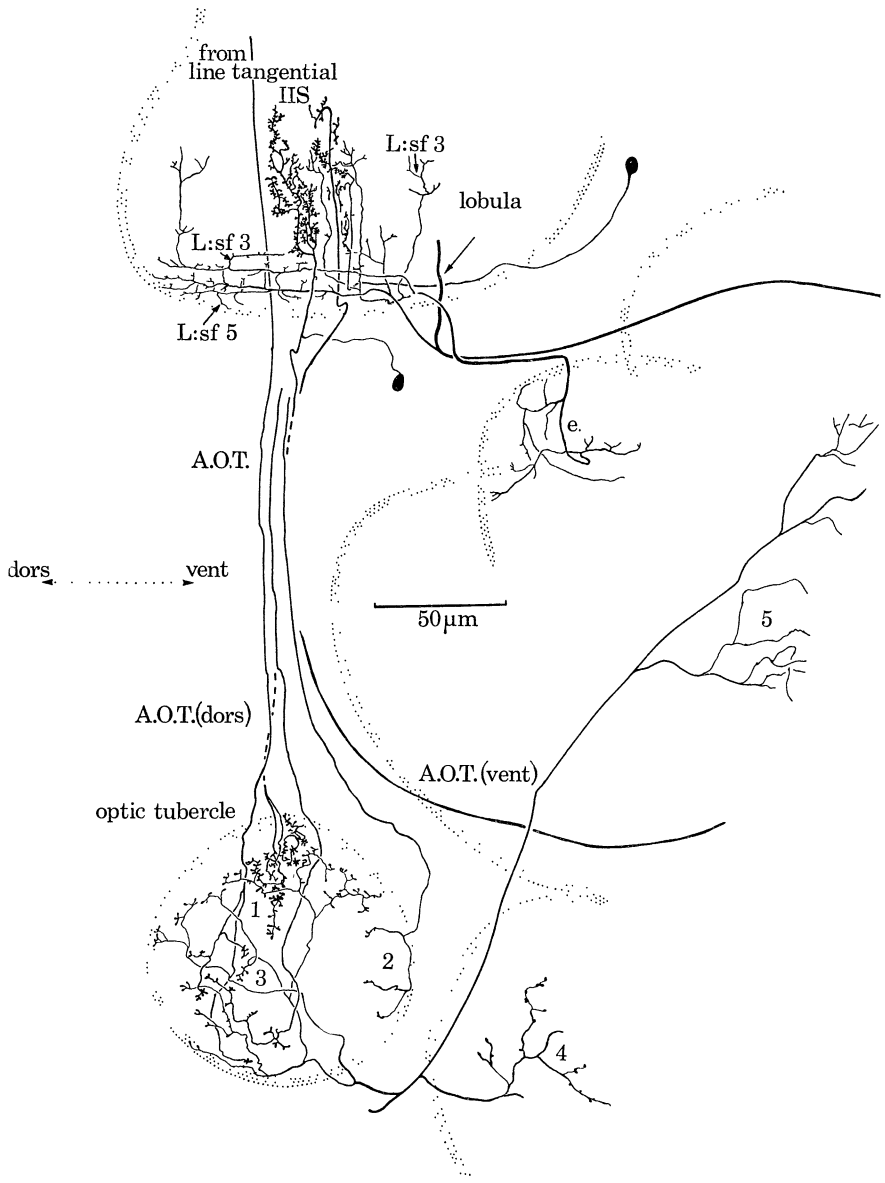


FIGURE 73. *P. brassicae* (vertical section, late pupa). Camera lucida drawing of some elements in the lobula and the three elements in the optic tubercle. Elements in lobula: Lsf 3 = type 3 lobula strip-field tangential to the ipsilateral protocerebrum. Lsf 5 = type 5 lobula strip-field tangential to the contralateral median deutero-cerebrum. IIS = small-field element to the optic tubercle. Elements in the optic tubercle: 1 = diffuse processes derived from the line tangential in the medulla (via the anterior optic tract). 2 = processes from the small-field element (IIS) in the lobula (via the anterior optic tract). 3 = processes derived from a mid-brain element (this also gives rise to branches in the ipsilateral deutero and protocerebrum; 4 and 5). A.O.T. (vent), ventral branch of anterior optic tract (A.O.T.); its components are not known.

narrow field-spreads arranged dorsoventrally at the interface between strata 1 and 2 and a single slender process which can be traced from each towards the cell-body cortex above the second optic chiasma. However, it has only been possible to trace these fibres through serial sections. By chance, whenever a complete ending has been impregnated the fibres have always been obscured, dorsally, by crystalline deposits.

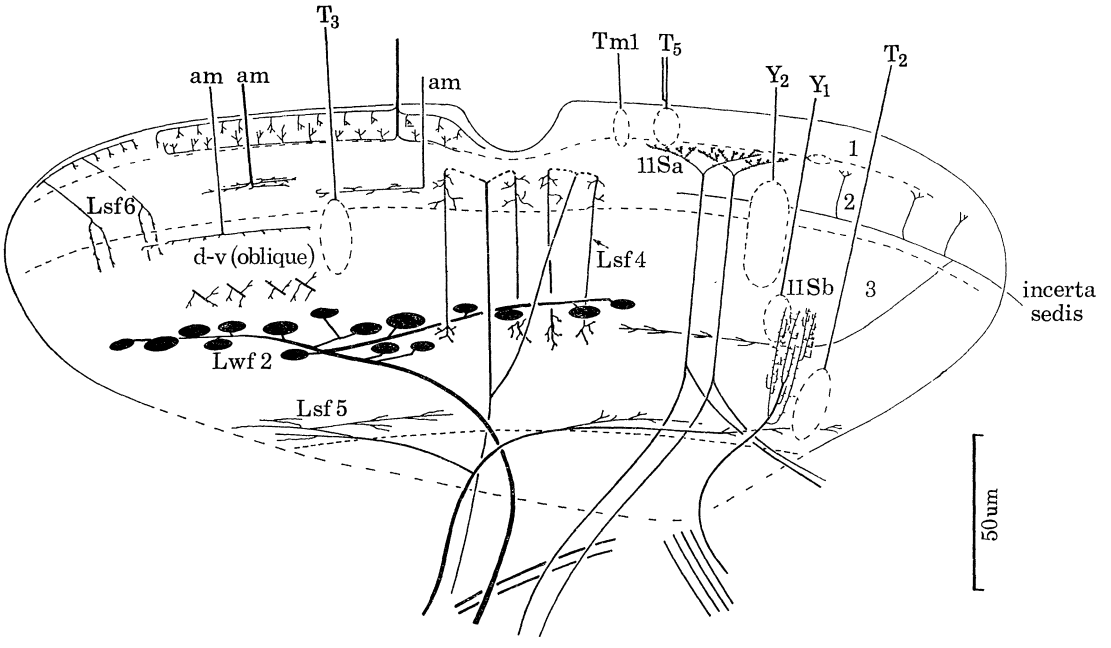


FIGURE 74. *P. brassicae.* Diagram of the lobula (vertical section). Stratum 1 contains the vertically oriented strip subfields of the lobula component of the giant optic lobe element. These are derived from its lobula plate subfield. Fragments of another strip-field are also oriented vertically (Lsf 6). This component has only been seen in *Pieris. incerta sedis* = a seventh form of strip-field element. Its two layers of processes are derived from a slender (0.3 μm diameter) fibre at stratum 2. L wf 2 = deep wide-field element. L sf 4 = ascendent-descendent processes of the type 4 strip-field tangential element. L sf 5 = deep strip-field processes of the type 5 lobula strip-field tangential.

Class 1 endings and components. These end at characteristic levels (1, 2, 3) in this region. Tm 1 = trans-medullary cell ending. T_2 = deep T-cell ending. T_3 = shallow T-cell ending. T_5 = the initial component of the lobula-lobula plate T-cell. Y_1 = lobula bistratified component of the deep Y-cell. Y_2 = lobula component of the tangential Y-cell. am = elements probably intrinsic to the lobula (amacrine cells).

Another tangential component, in the outermost lobula stratum, also presents difficulty of interpretation. It has only been stained in pupal animals and although its orientation and outermost location is similar to that of the lobula component of the giant optic lobe cell it has additional collateral branches which extend deeply, as far as the middle of stratum 2. Possibly this fragment may represent the pupal structure of the giant optic lobe cell in this region and the descendant branches may be unreceptive to Golgi staining in the adult. Conversely, it might represent a further form of tangential which has never been impregnated in adult tissue.

DISCUSSION

In the Diptera, groups of retinula cell endings in the lamina are associated with first-order interneurons to form discrete groups of components called optic cartridges (Cajal & Sanchez 1915; Trujillo-Cenoz & Melamed 1965; Braitenberg 1966, 1967; Pedler & Goodland 1965).

Each of the groups of receptor endings is commonly composed of a ring of six terminals. In *Lucilia* and *Phormia* these apparently establish synaptic contact with both axial monopolar cells in the outer plexiform layer (Trujillo-Cenoz & Melamed 1965). These authors have also demonstrated a tight junction between the pair of monopolars. It has also been suggested that there are two sets of presynaptic centrifugal fibres which are intimate with the six retinula cells and the two monopolar cells (Trujillo-Cenoz 1965). However, Golgi studies on the Diptera suggest that only one centrifugal component is associated with each first order interneuron and the retinula cell endings, although two other types of ending in the lamina, from the medulla, may have a special relationship with a long visual fibre or one of the monopolar cells (see part II).

Most ommatidia of *Sphinx* and *Pieris* apparently give rise to eight photoreceptor processes. This number of elements has also been reported in a number of other Lepidoptera (see, for example; Fernandez-Moran 1958; Yagi & Koyama 1963). However, exceptions to this number have been reported in some other species (Johnas 1911), and Novikoff (1931) reported *Pieris* as having nine receptor elements in an ommatidium. Methylene-blue preparations show up eight receptor cell-bodies in some ommatidia of *Pieris brassicae* and nine in others; the ninth lies just beneath the level of the crystalline cone and corresponds to minute hair receptors that are scattered sparsely over the corneal surface (Strausfeld 1968).

The visual receptor processes penetrate through the basement membrane to enter the fenestration zone, and in avoiding the extensive tracheation may appear to form local pseudo-chiasmata with their neighbours. However, in *Pieris* it seems that at least four retinula cells, terminating in the same optic cartridge are derived from the same ommatidium. Two of the four are simple type 1 plug endings. The others are a unilateral type 2 ending and a bilateral type 3 ending. There is evidence, from Golgi preparations, that two of the remaining receptor terminals in a cartridge are derived from nearby ommatidia; one is a bilateral ending, the other is a type 1 ending.

It must be pointed out that the complete patterns of projections of the retinula cells have been derived from reconstructions of the retinula cells over some distance between the retina and plexiform layer. It is rare to find more than four completely impregnated retinula cells together in an optic cartridge or four from an ommatidium. However, it has been possible to substantiate, by the Golgi method, the observations of Braitenberg (1967) and Trujillo-Cenoz & Melamed (1966) on the retinula cell projections in species of Diptera (part II). In this order the distance between the plexiform layer and retina is much shorter and the visual cells impregnate frequently and well.

There are three major variants of monopolar cells in *Pieris*. These are the midget, radial and bilateral small-field monopolar cells. Nocturnal Lepidoptera, such as *Sphinx* and *Automeris* have only giant monopolar cells which have fields that overlap one another. In *Sphinx* there are only three sizes of these elements; however, each size variant has the same radial arrangement of processes from its axis-fibre. The laminae of *Calliphora*, *Pieris* and *Sphinx* are compared in figure 75. *Apis*, *Aeschna* larvae and the locust have mixed populations of monopolar cells. They are either radial diffuse in form or are multi-stratified with two or three strata of distinctly different forms of lateral processes (Strausfeld; in preparation).

The lateral spreads of monopolar cells in open rhabdomer eyes of the Diptera extend through only one optic cartridge in the external plexiform layer. However, the closed rhabdomere eyes of the Lepidoptera, Hymenoptera and Orthoptera (Goldsmith 1962; v. Frisch 1965; Horridge 1966*b*) have at least one type of monopolar cell whose processes extend through more than one

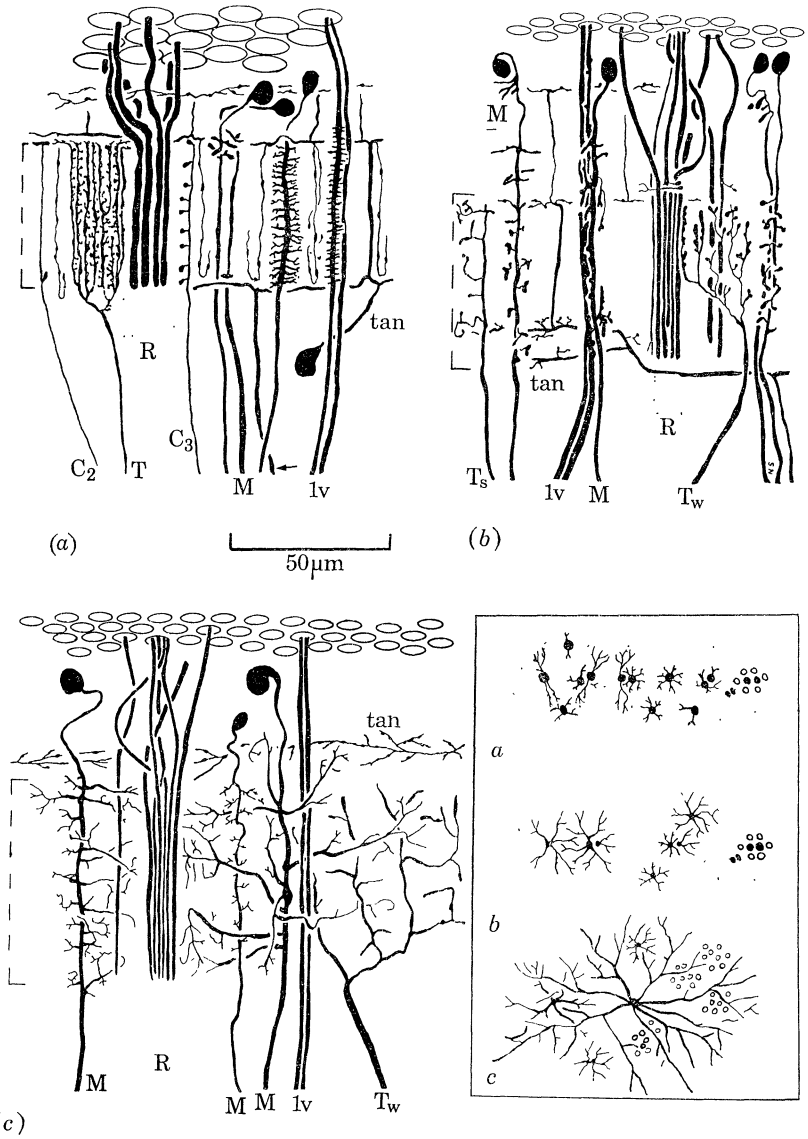


FIGURE 75. Diagram showing the comparison between the laminae of *Calliphora vomitoria* (*a*), *Pieris brassicae* (*b*) and *Sphinx ligustri* (*c*). Note the paired arrangement of long visual fibres in each species (lv) and the differences between the projection patterns of the retinula cells and their grouping (R) in *Calliphora* and the two species of Lepidoptera. (See also Braitenberg 1967; Strausfeld; part II.)

(*a*) T = basket ending of a 'centrifugal' T_1 cell from the medulla. C_2 = ending of a type 2 centrifugal element from the medulla. C_3 = ending of a type 3 centrifugal element from the medulla. M = monopolar cells (from left to right: small unistratified radial monopolar, a midget monopolar cell and a bilateral monopolar cell). tan = bistratified tangential element.

(*b*) T_s = small-field ending of a T-cell from the medulla. T_w = large-field ending of a T-cell from the medulla. M = monopolar cells (from left to right: wide-field radial monopolar cell, small-field radial monopolar, midget monopolar, bilateral monopolar). Tan = bistratified component of the tangential cell linking the lamina to the medulla.

(*c*) T_w = wide-field ending of a T-cell from the medulla. M = giant radial monopolar cells. tan = unistratified tangential element.

Inset: tangential plans of optic cartridges and their monopolar cells (the groups of retinula cell endings in *Sphinx* are not definable as discrete optic cartridges). Note the differences of monopolar extents between *Calliphora* (*a*) *Pieris* (*b*) and *Sphinx* (*c*). In the former two species each cartridge contains two monopolar cells surrounded by about six retinula cell endings (plan shown in extreme right-hand cartridge).

group of retinula cell endings, even if this extent is limited to the adjacent cartridge. In the nocturnal Lepidoptera this is particularly obvious: some cells have a total lateral extent which exceeds 50 μm (*Sphinx* and *Automeris*) and thus spread through projections from over 100 ommatidia. It is clear that unless each cell is receiving a narrow-field input from only a few ommatidia it will be susceptible to excitation from a large portion of the retinal mosaic. Possibly these large over-lapping fields provide a system of lateral inhibition between the first-order interneurons much in the same way as the so-called eccentric cells of *Limulus* provide lateral inhibition in its receptor layer (Ratliff, Hartline & Lange 1966; Lange, Hartline & Ratliff 1966). The spines and knobs of neighbouring monopolar cell processes are often detected closely applied against one another; however, it is necessary to determine whether these do, in fact, represent synaptic intimacy. But it is clear that if these lateral processes serve only to excite one another, 'focused' information could not be transmitted to the medulla by these cells.

The projection patterns of the fibres between the lamina and the medulla are described in a subsequent account (part II). It suffices to say here that there is little permutation of the lateral relationships of monopolar cells and long visual fibres between these two regions. Two types of centrifugal endings invade the lamina of *Pieris* from the medulla. Both endings are derived from T-cells whose initial processes invade the outermost strata of the medulla. These cells are clearly analogous to the T-cells between the medulla and lamina in the Diptera (part II). In the Lepidoptera, at least, they cross the first optic chiasma in the same way as do the monopolar cells: that is to say a linear horizontal array of initial components in the anterior of the medulla is simply reversed in the posterior lamina, and vice versa.

The wide-field basket endings in *Sphinx* have lateral spreads which are equivalent to the range of sizes of the giant monopolar cells. However the lateral extents of their medullary (initial) components do not reflect the peripheral size variations. Each has a lateral spread through about 30 μm. Although the lateral spreads of monopolar cells also vary enormously in the lamina they too have the same sizes of endings in the outer layer of the medulla.

The essential plan of organization below the level of the lamina is much the same in both species of Lepidoptera and in the Diptera. However, in the latter order there are more types of perpendicular cells which link the medulla to the lobula complex and also there are more forms of small-field elements in the lobula which project to, or are derived from the mid-brain. The stratifications of the diurnal *Pieris* medulla and that of the Diptera are extremely clear. Similar forms of tangential cells are disposed at similar levels of the medulla and lobulae of these insects. However, in *Sphinx* the stratification is difficult to determine from Golgi preparations. The tangential cells in particular are more diffusely arranged in the medulla. But the same forms of class II elements are present in both the present species of Lepidoptera; the lateral processes of the type 1 cells also lie at equivalent levels, in both species, so that they could interact with analogous tangential elements.

The number of ommatidia of *Pieris* have been estimated by counts from whole mounted retinal surfaces, and serial sections through the eye; this has been estimated as varying between 8500 and 8600 units. Similarly, the lamina has these numbers of optic cartridges. The medulla is also organized into distinct columns, each of which has an axis, in the outer layer, composed of the four fibre terminals from the lamina ('Quads'; see p. 106). Holmes–Blest silver preparations show the surface of this region as a vertically elongated disk which is regularly stipple throughout its area by groups of four, or occasionally five, fibres (see plate 4). Fraser-Rowell preparations stain these groups of four, selectively, to reveal their characteristic double paired

arrangement. It has been estimated, from serial sections of these reduced silver preparations, that there are as many columns in this region as there are ommatidia. However they are difficult to count at the curved perimeter of the region and some columns appear to have only two or three lamina-input components. Nevertheless it seems clear that the retinal mosaic is at least closely, if not precisely, preserved numerically in the medulla. Golgi preparations indicate that the pairs of long visual fibres preserve the spatial relationship of the mosiac in these two geographical regions. The pairs of monopolar cells may both preserve and permutate the lamina mosaic in the medulla (see part II).

There is good evidence that the optic cartridges of the Diptera are formed by groups of retinula cells where the receptor elements of a group share the same optical axis in the retina (Kirschfeld 1967; Braitenberg 1967; Kirschfeld & Franceschini 1968). One monopolar cell type, axial to the optic cartridge, may be receiving summed information from the receptor endings (Trujillo-Cenoz & Melamed 1966; Scholes 1969). Thus the functional retinal mosaic is probably carried by some components in all the cartridges in the lamina. Monopolar cells (radial forms that may contact all six retinula cell endings in a cartridge) and also the long visual cell fibres could project a point to point representation of the retina-lamina mosaic onto the surface of the medulla. However, we do not know how the receptor elements in *Sphinx* and *Pieris* are oriented with respect to one another and to the total visual field. The long visual fibres are the most obvious candidates for relaying the topographical and numerical configuration of the retinal mosaic to the deeper regions. The cartridge organization of *Pieris* is similar to that of the Diptera even though the retinula cell projection is not (see part II). Monopolar cells have been followed alongside the fibres of the long visual cells in the optic chiasma and there is good evidence, from Golgi preparations, that in *Pieris* these first-order interneurons also represent the topographical map of the lamina in the medulla. In *Sphinx* the situation is less clear; the columnar arrangement of the medulla suggests a topographical representation of the retinal mosaic. However, the arrangement of monopolar cells in the lamina, although columnar, is not immediately suggestive of a morphological point to point representation of the receptor mosaic by these cells.

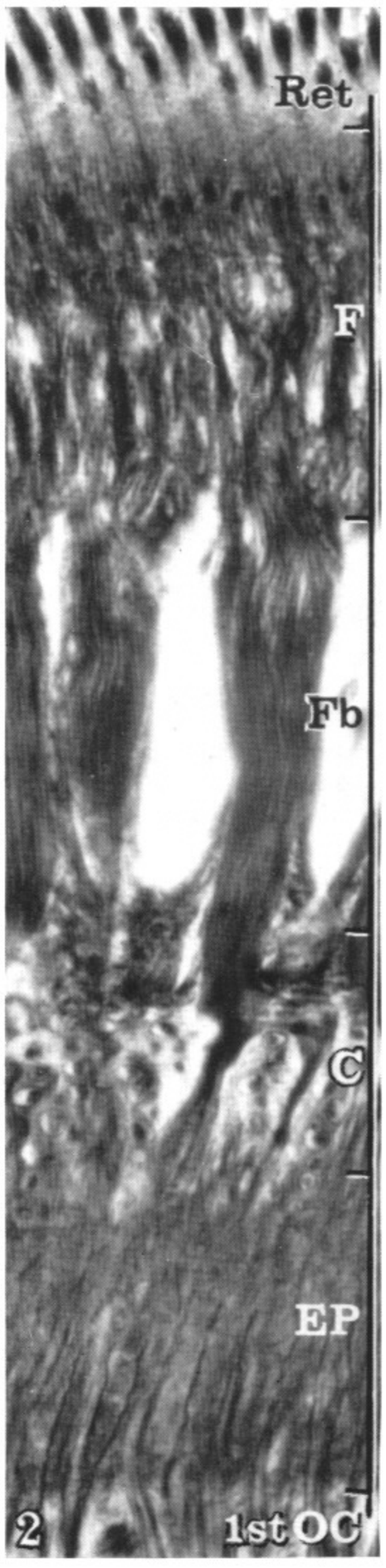
At all levels of the system, fields of tangential elements interact with restricted groups of perpendicular processes that morphologically relay the mosaic configuration. These tangential systems vary from those like the broad-field outer tangentials in the medulla, some of whose fields are sufficiently extensive to interact with two-thirds of the mosaic units at the medulla surface, down to the small multistratified elements in strata 3 to 6 and the strip-field elements in strata 7 and 8. These latter elements must interact with quite restricted oval or linear arrays of mosaic elements at these levels.

The most significant difference between this account and those of previous authors, however, lies in the number of mosaic relays which can be demonstrated between the outer surface of the medulla and the lobula even in the relatively simple neuroarchitecture of the Lepidoptera (cf. part II). In essence the early accounts suggested that the retina was represented in the lobula through two or three channels: (i) transmedullary neurons (nos. 11 and 12, in Bullock & Horridge's classification), and (ii) a T-cell of one type (Bullock & Horridge 21). It is now clear that there is a minimum of at least six channels: (1) the transmedullary cell Tm 1 (in the Diptera there are many forms of these cells in each species which end in at least three different levels in the lobula); (2) to (4) three T-cells, T_2, T_3 to the lobula, and T_4 to the lobula plate where there is a potential relay between the lobula and lobula plate via T_5; and (5) to (6) the

Y-cells. There is a translobula plate cell which also links the two lobula complex regions. Also in the Diptera there are several distinct forms of Y-cells ending in the lobula plate and at different levels in the lobula. All these forms of units have processes at characteristic levels in the medulla and lobula complex. Their fields are of varying lateral extents and the Y_2 cell processes in stratum 8 of the medulla have branches disposed along a narrow strip, amounting to almost a fifth of the arc of this region, giving it the morphological status of a tangential component.

Most of the class II linking-fibres project towards the mid-brain. A few of their endings have been seen but their description is out of the scope of the present account (N. J. Strausfeld, in preparation). The strip-field tangentials of the lobula plate and stratum 1 of the lobula characteristically project to regions in the ipsilateral proto- and deutero-cerebrum. Those in the medulla seem to project to both ipsilateral and contralateral mid-brain regions. The line tangential projects to the optic tubercle. Holmes–Blest silver preparations show a small tract of linking-fibres derived from the anterior edge of the medulla which project to the inner face of the lobula. However, their corresponding endings have not been impregnated by the Golgi stain.

Some class II linking-fibres from the lobula project towards the calyces of the corpora pedunculata. This arrangement is particularly clear in *Apis* (N. J. Strausfeld, unpublished). Fibres from other sensory regions also project to these locations (Vowles 1955; Jawlowski 1960). The giant optic lobe tangential cell invests the lobula plate, lobula and medulla of both the ipsi-lateral and contralateral lobes. Non-visual stimulation can be recorded from the optic lobes of the locust (Horridge *et al.* 1965) and of noctuid moths (T. S. Collett, personal communications); whether the fibres responsible for these recordings directly link the optic lobes with other sensory regions is not known. Several of the class II elements are complex in form and collaterals project from their linking-fibres to several different locations in the mid-brain. Possibly the optic lobes may contain integrative systems other than those for visual analysis.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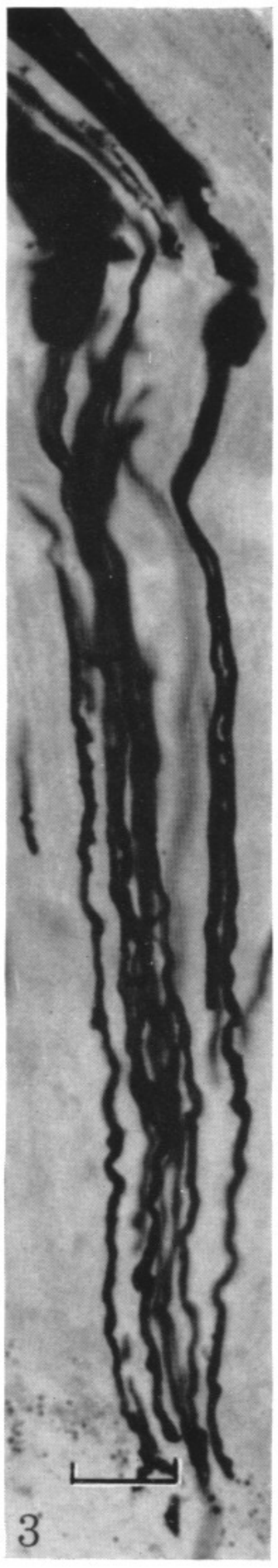
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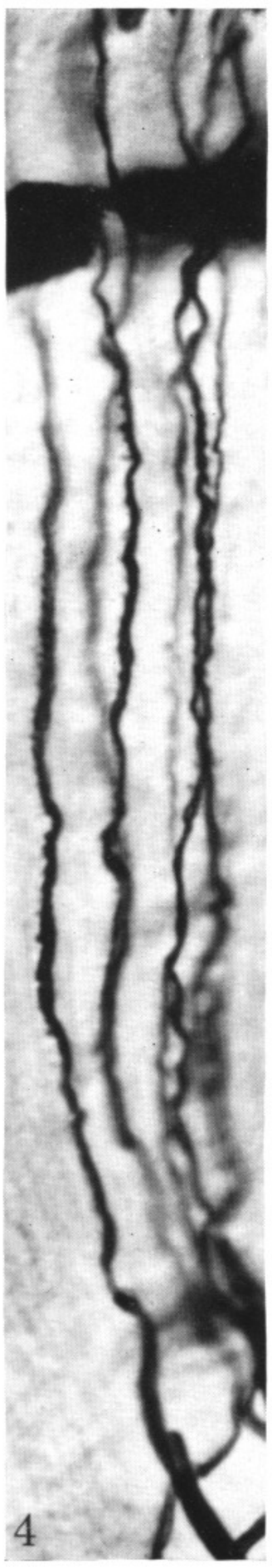
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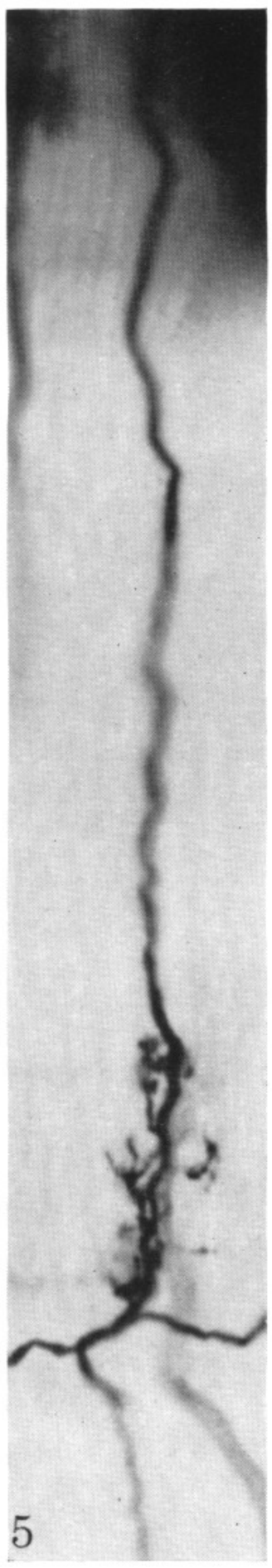
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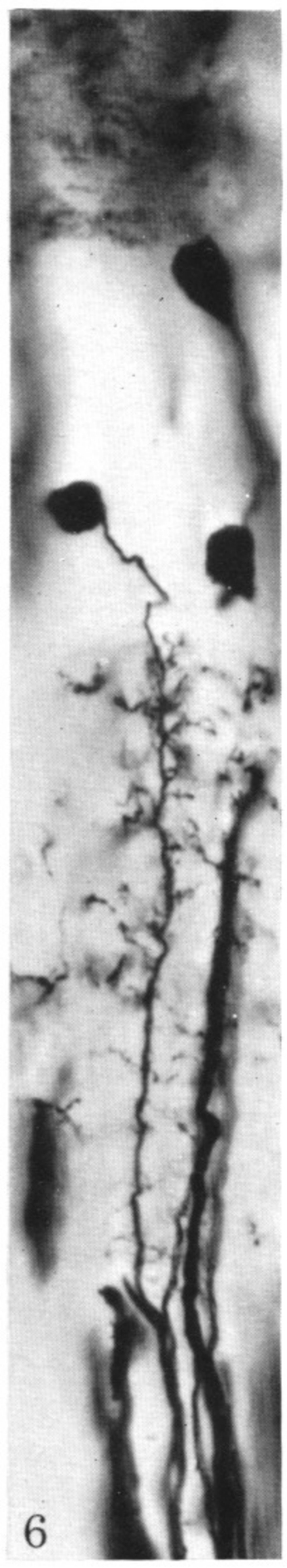
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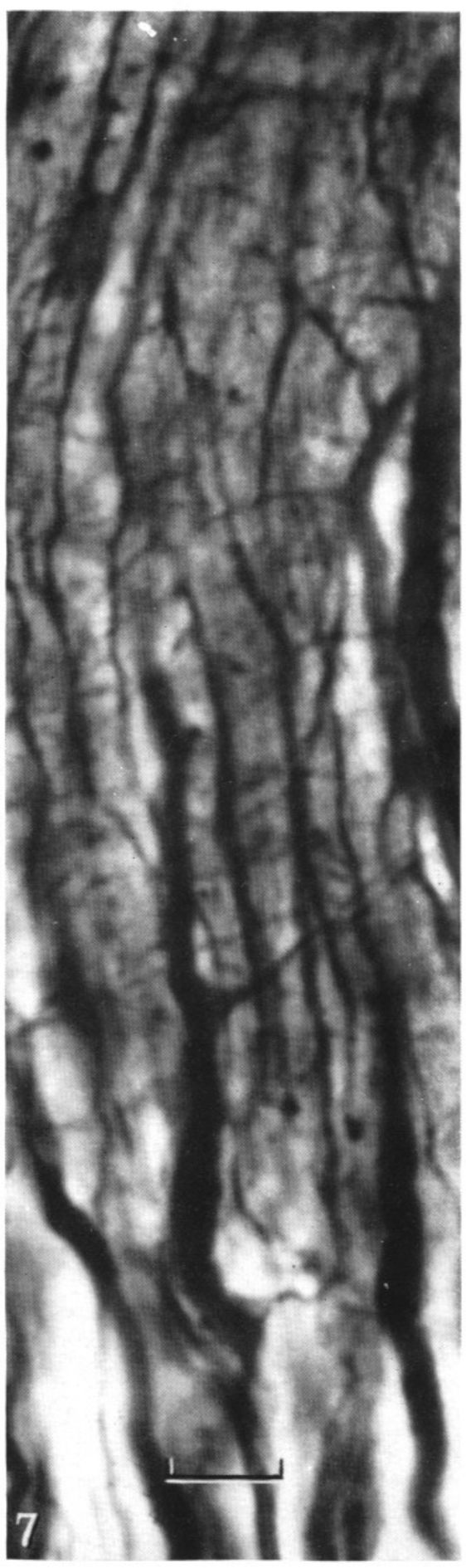
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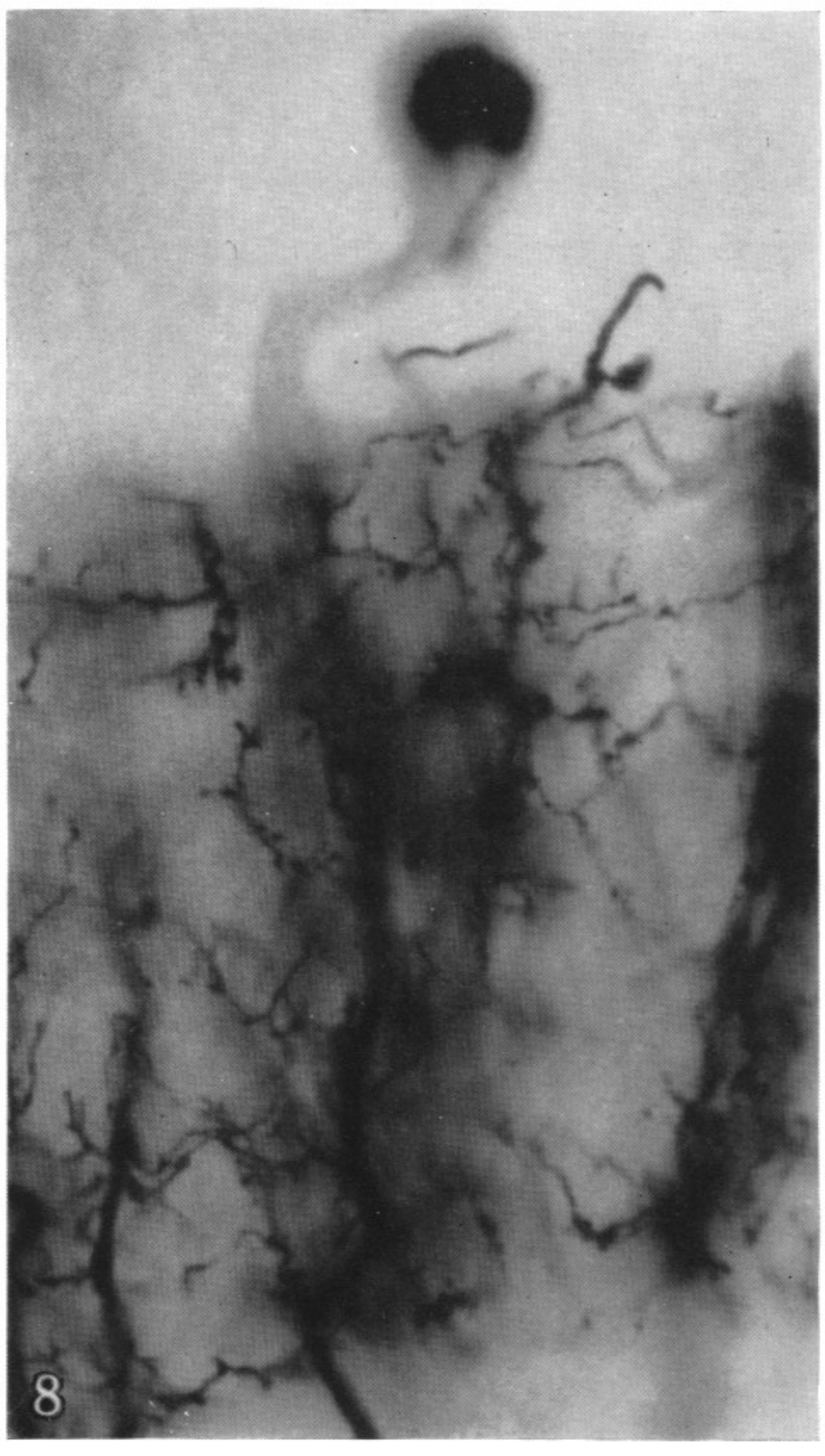
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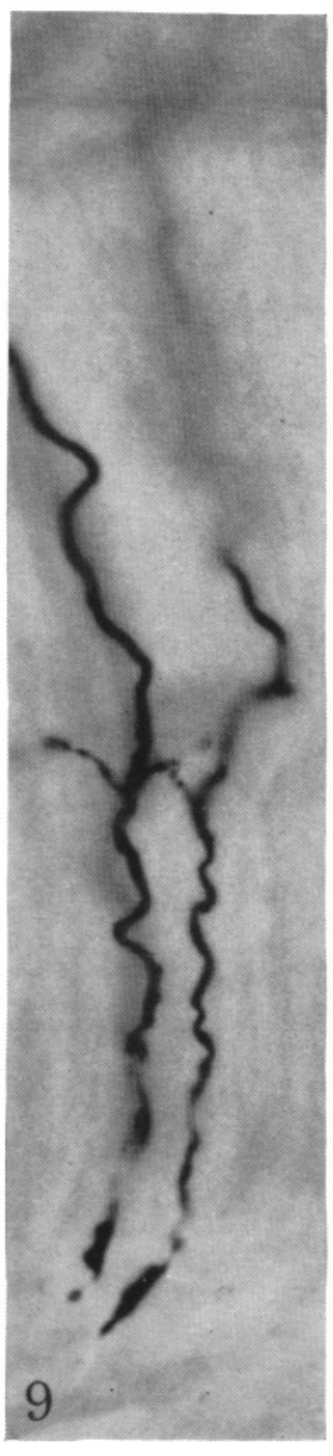
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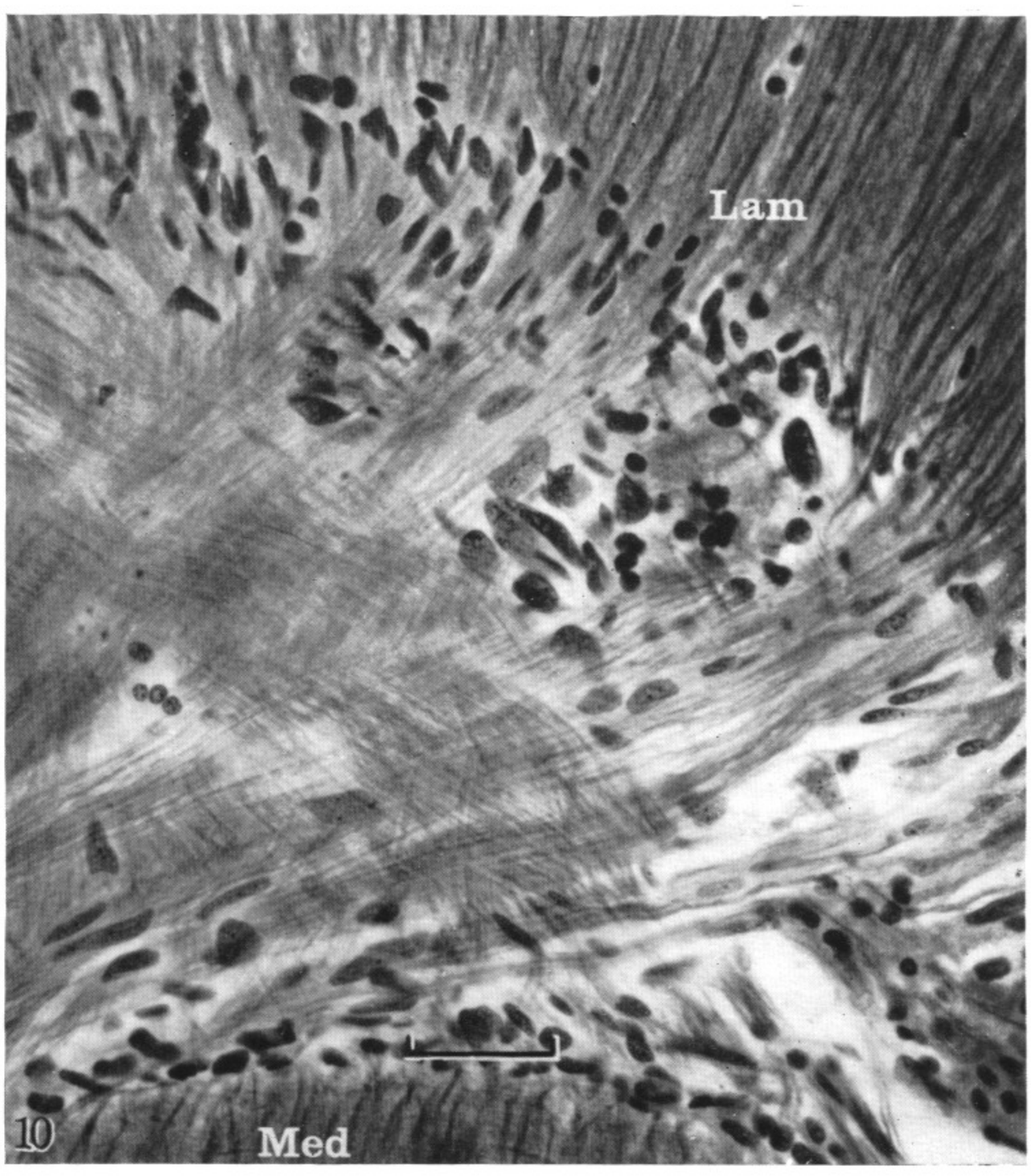
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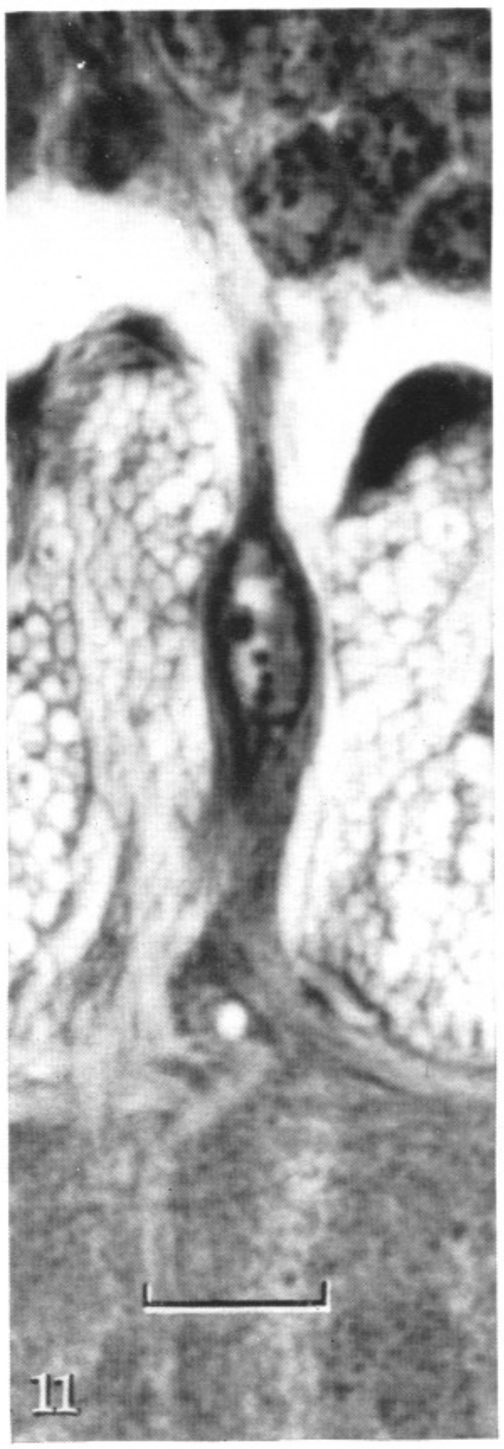

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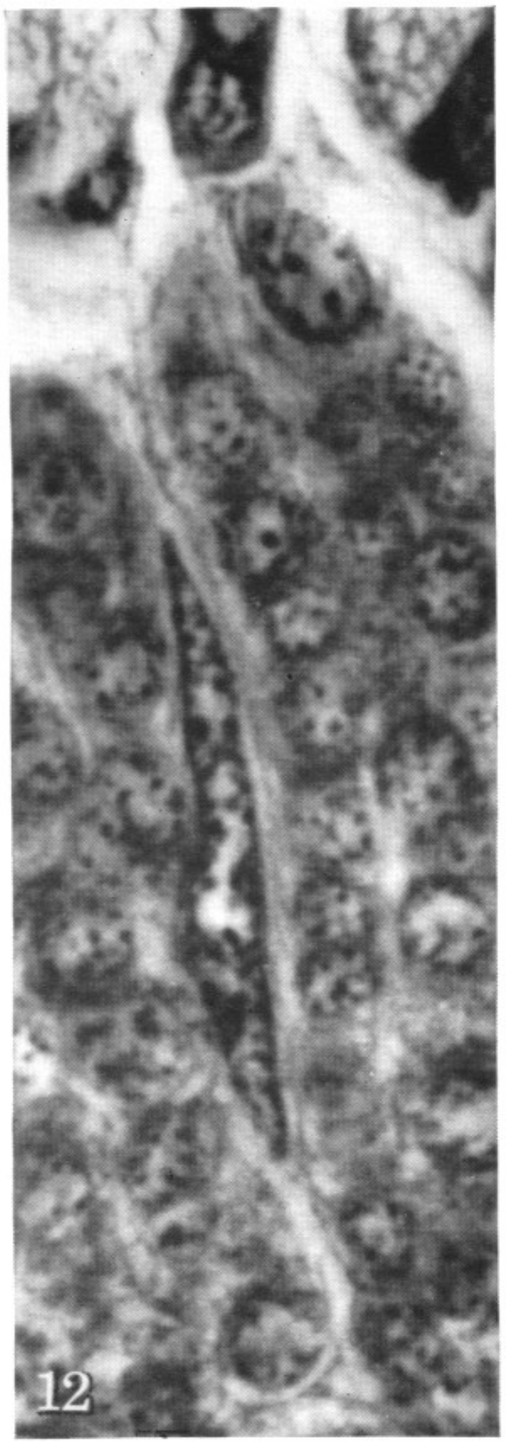

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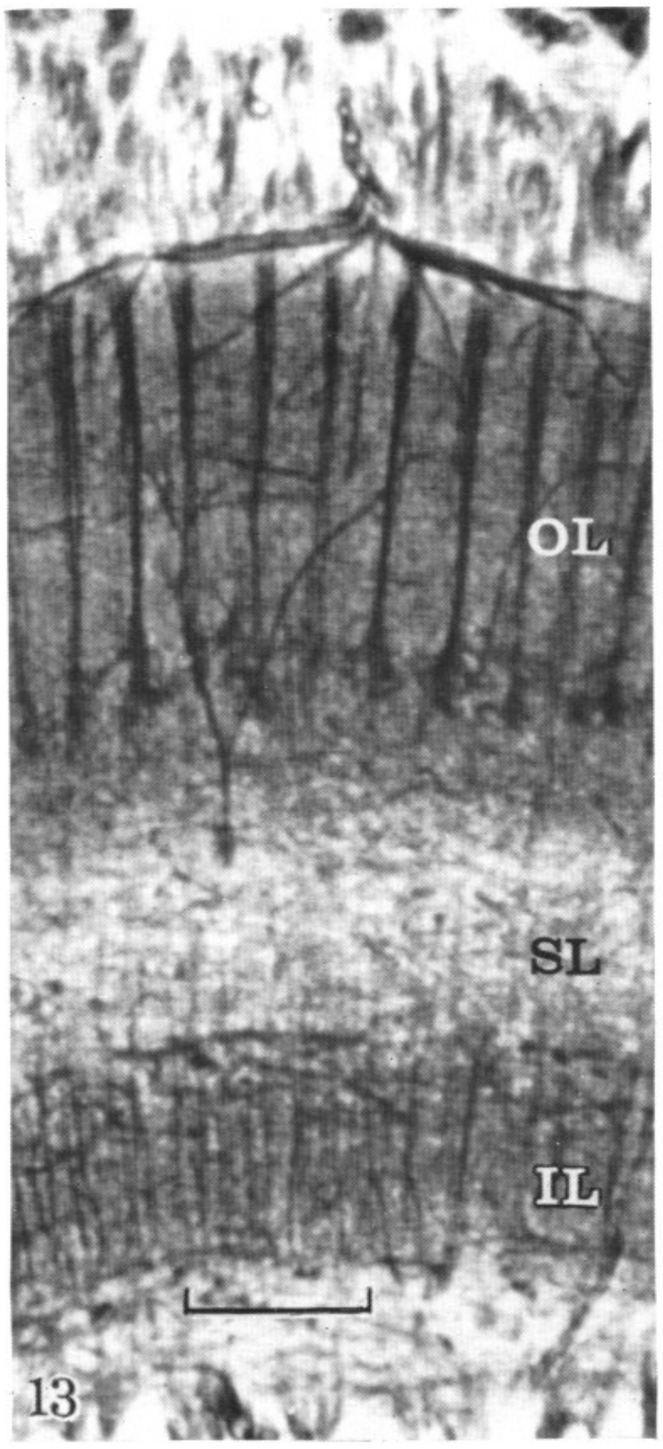

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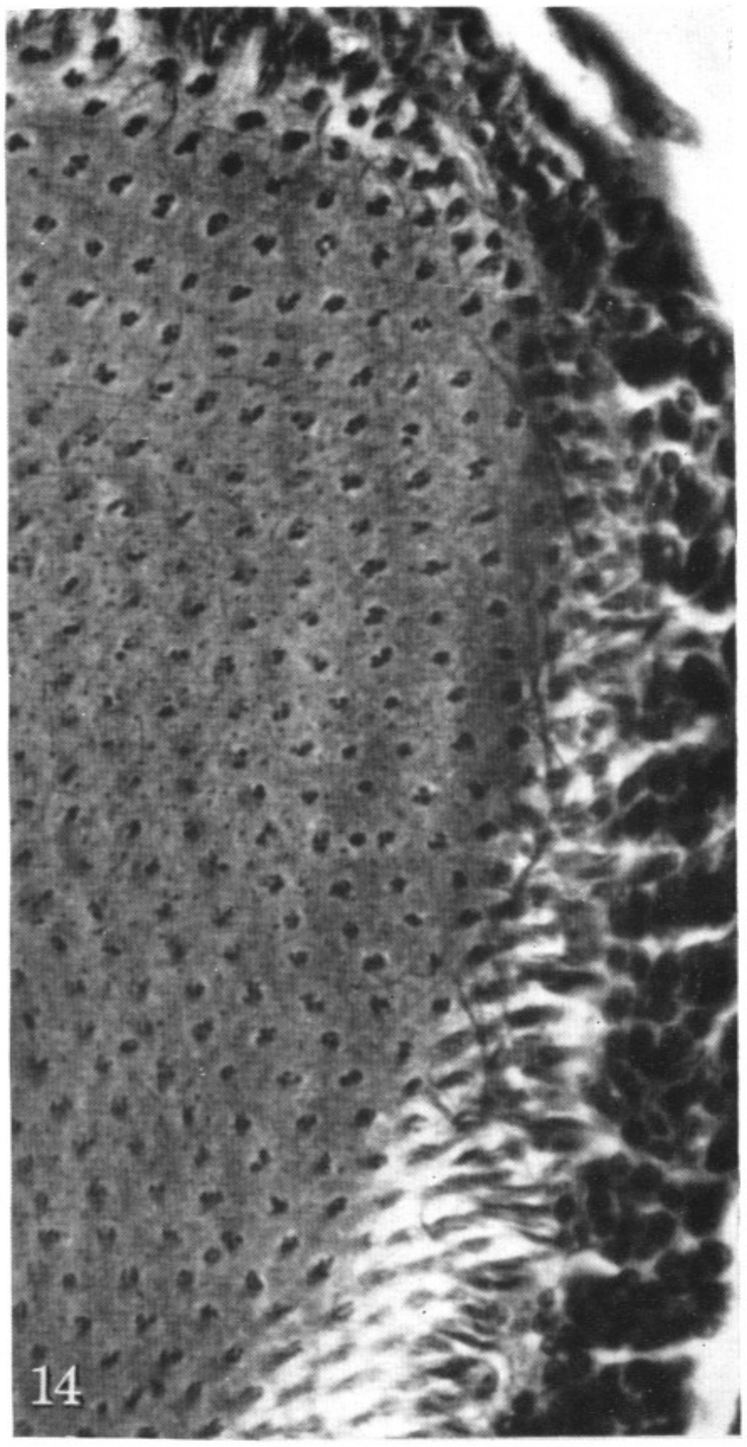

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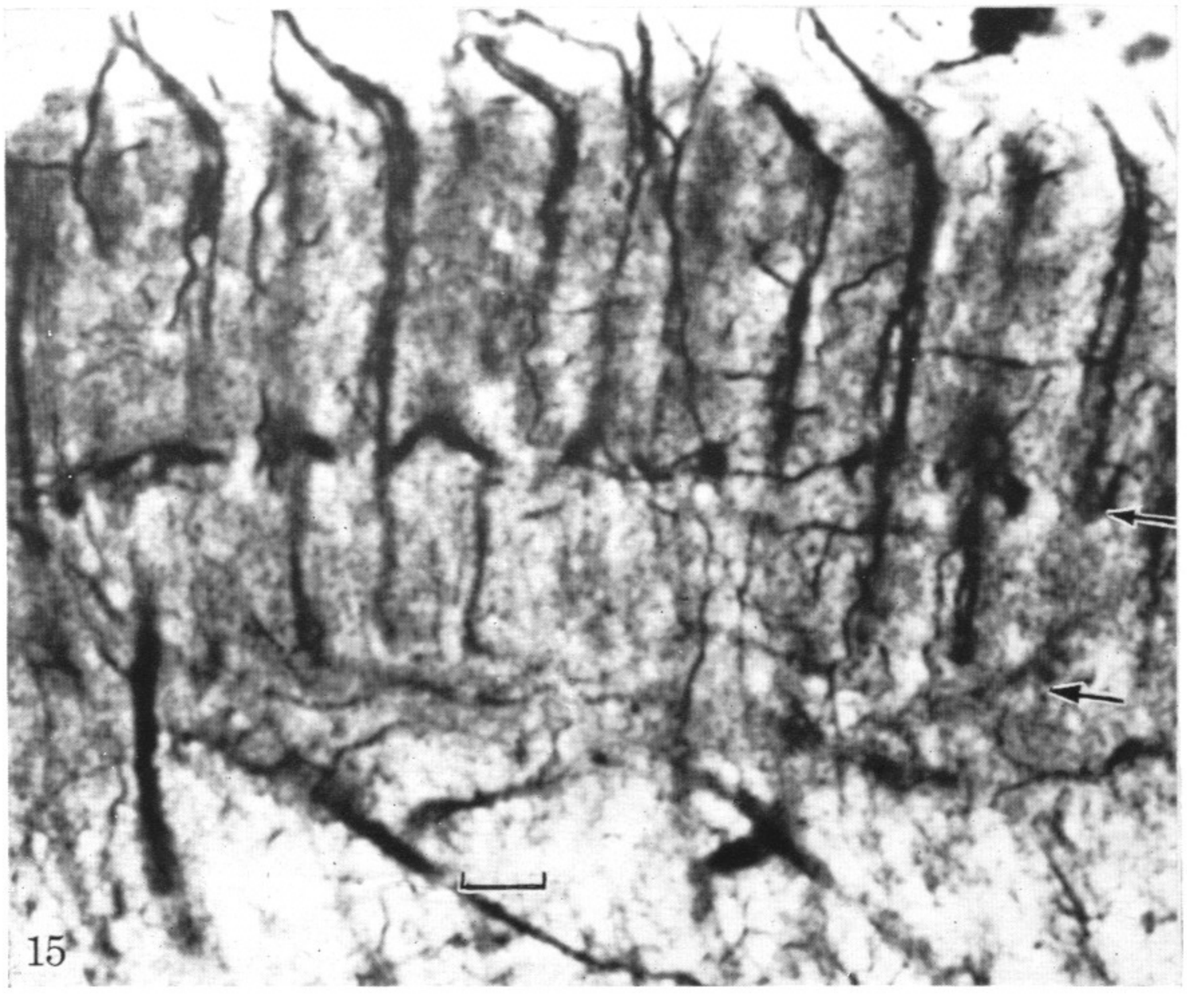

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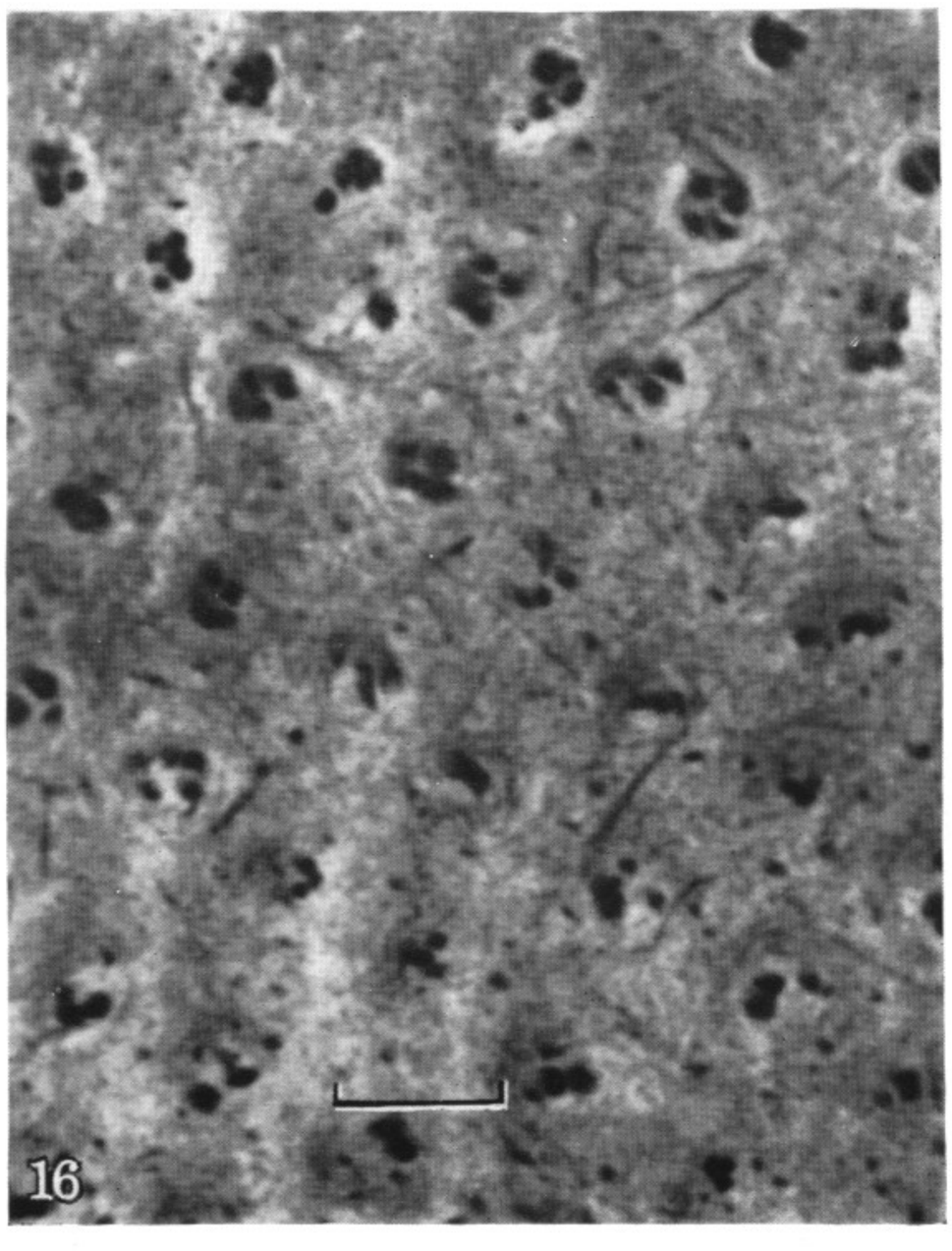

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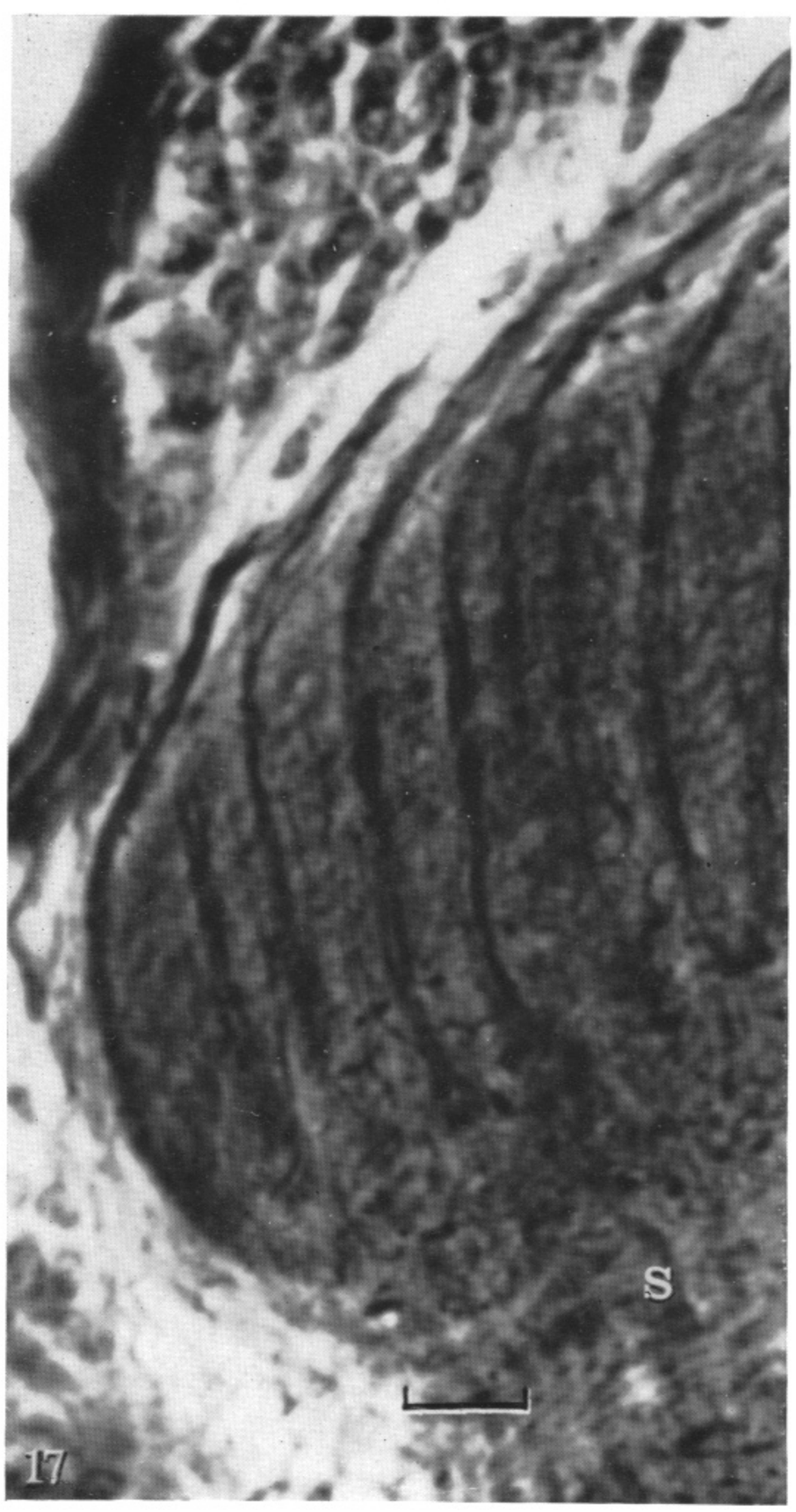

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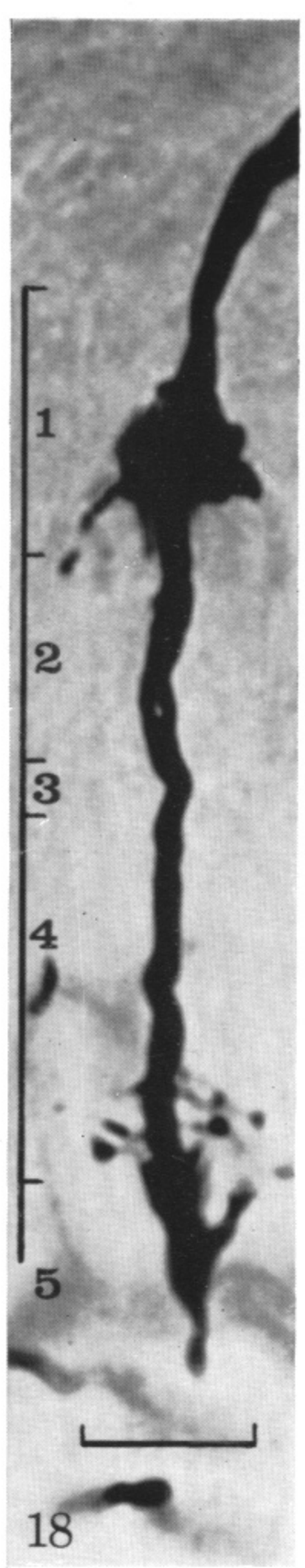

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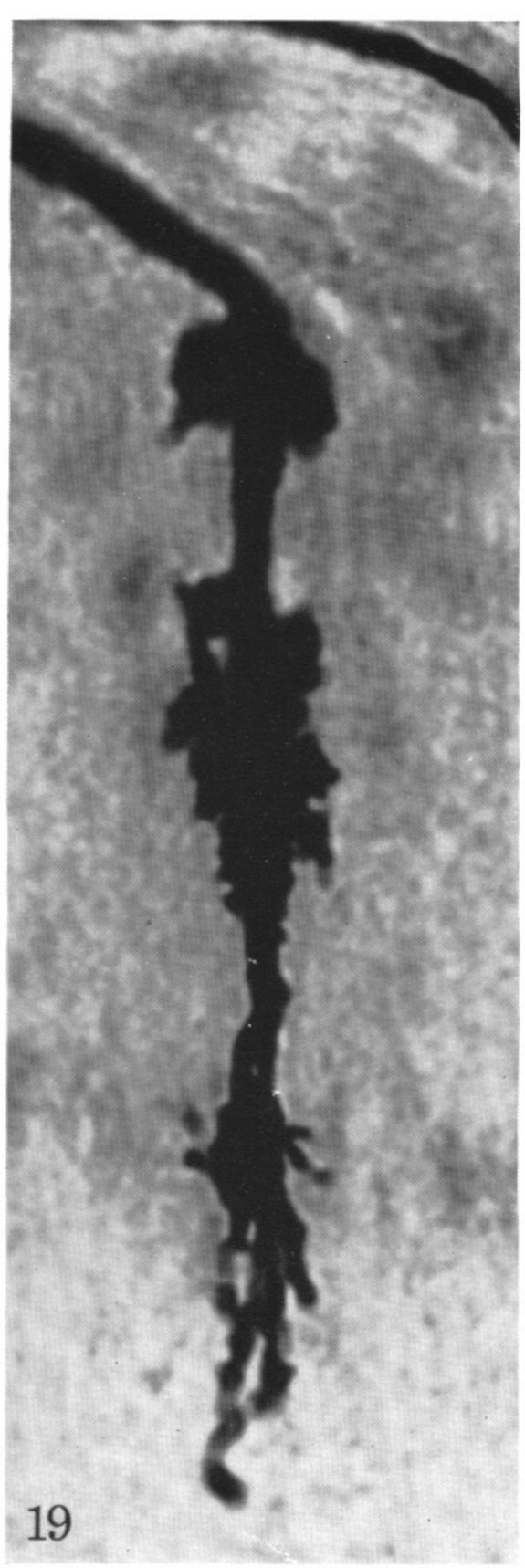

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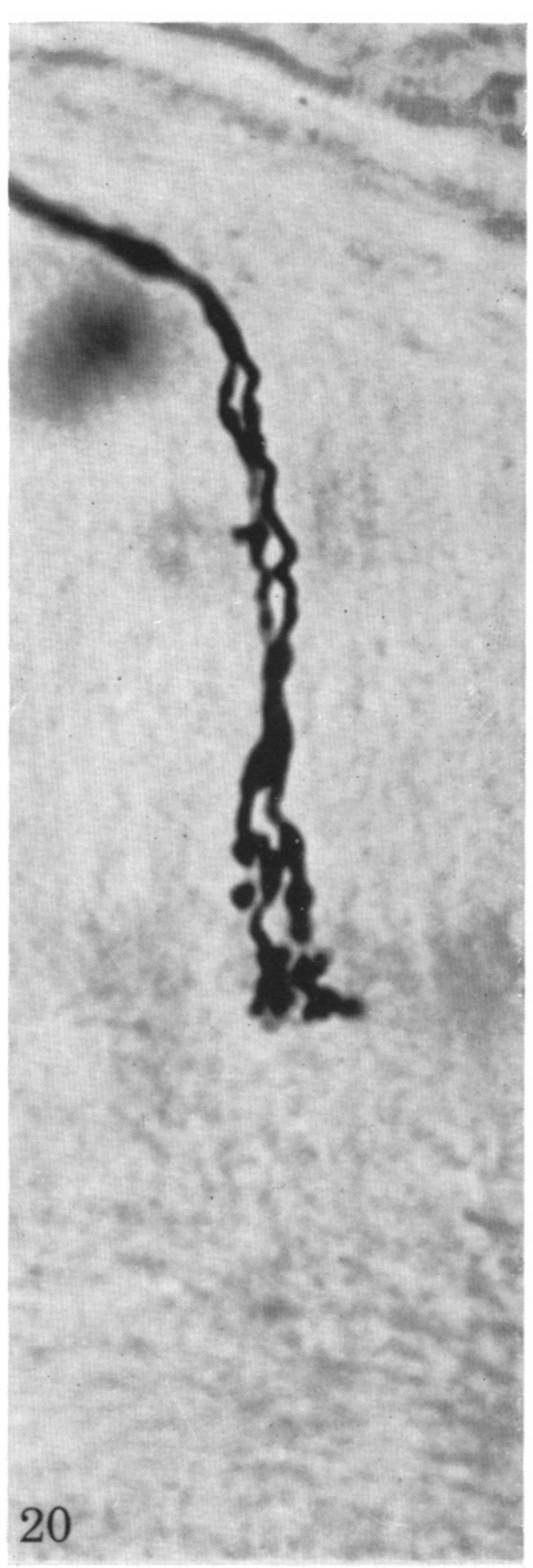

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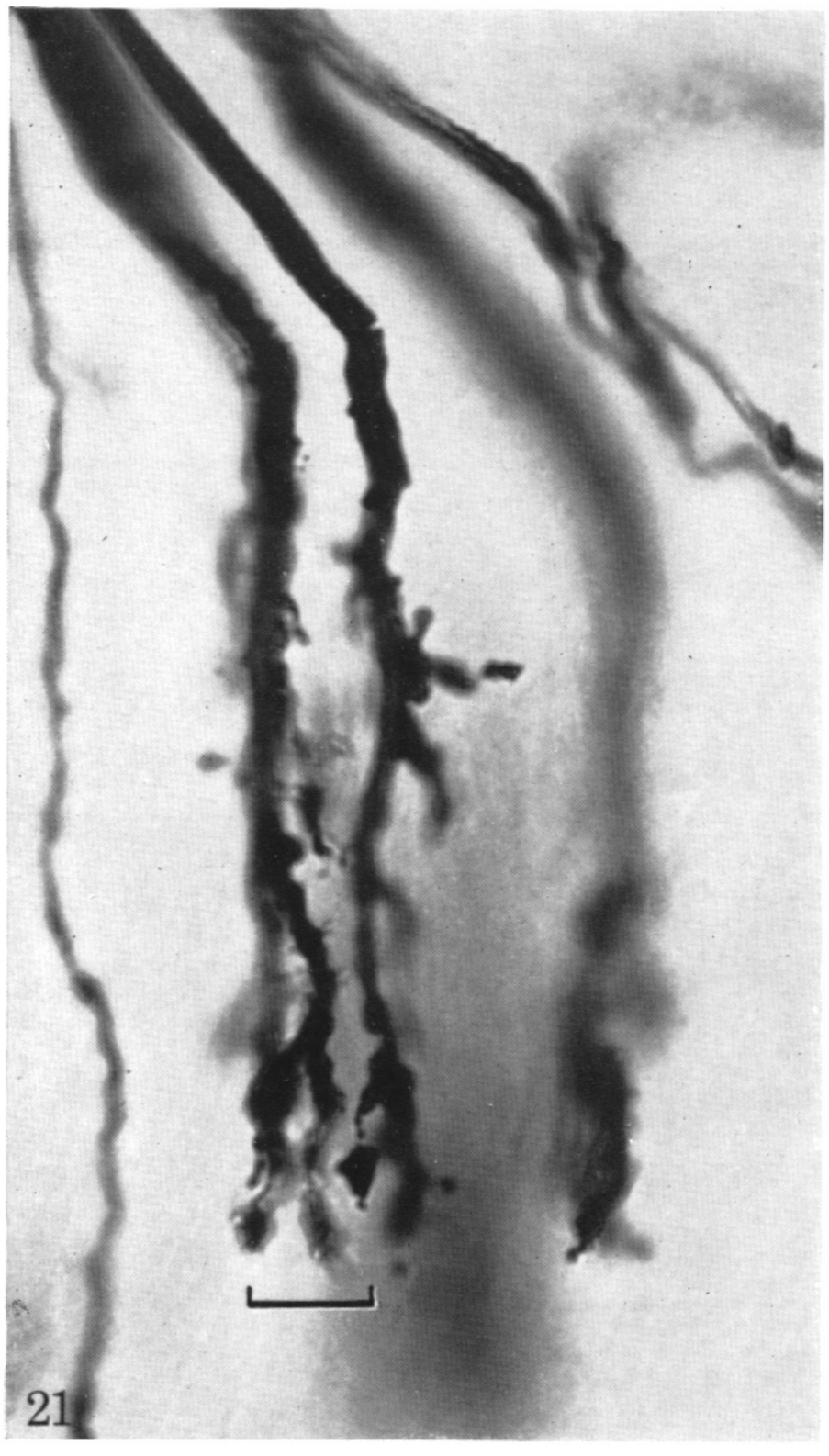

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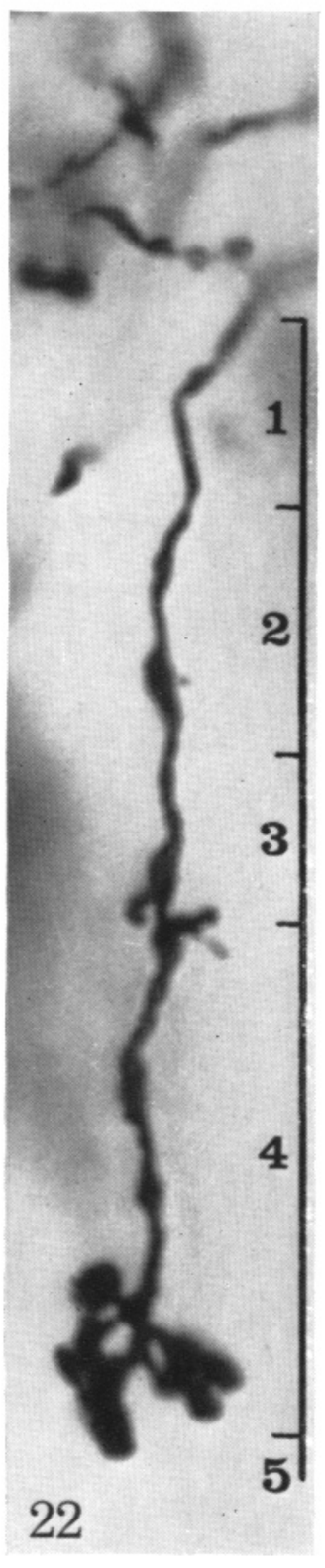

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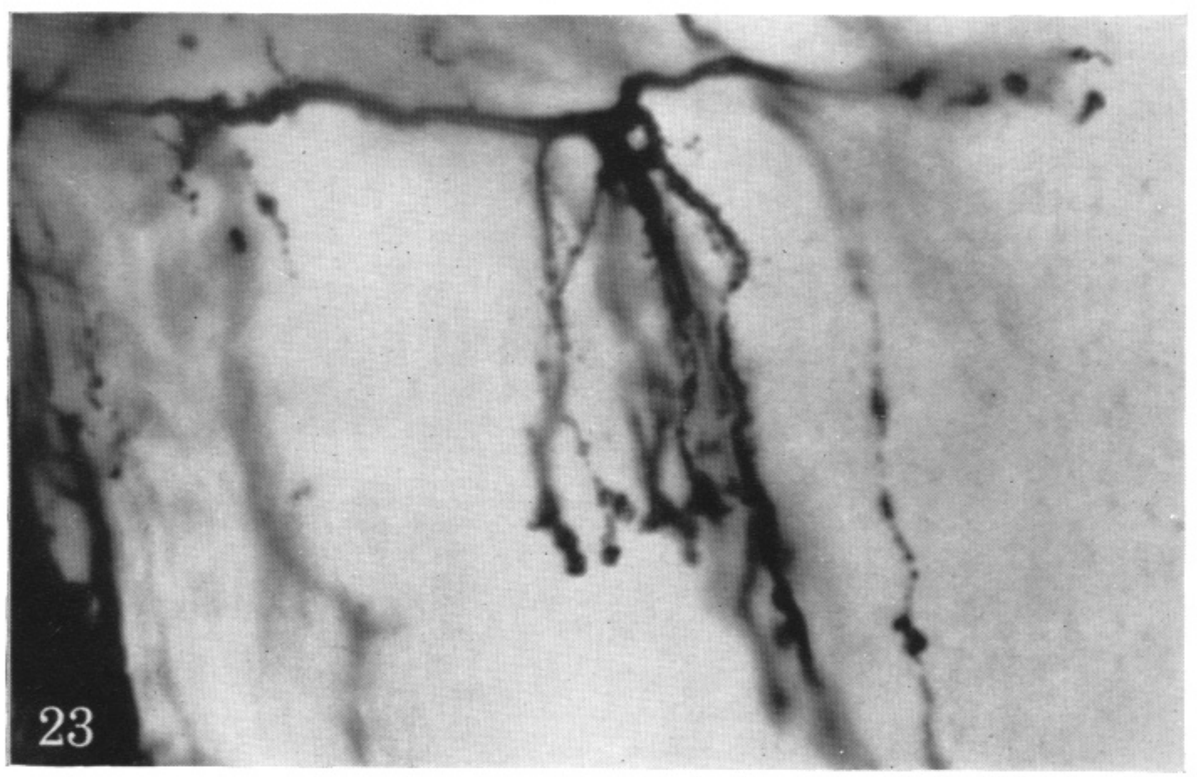

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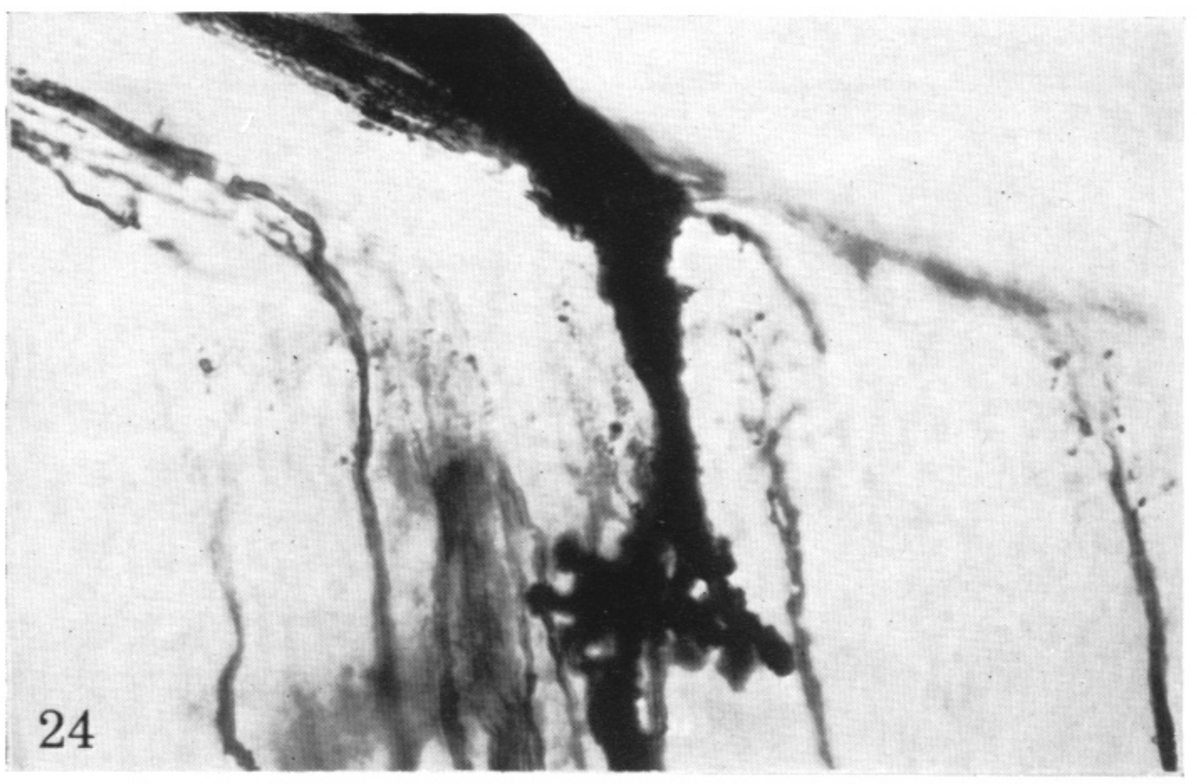

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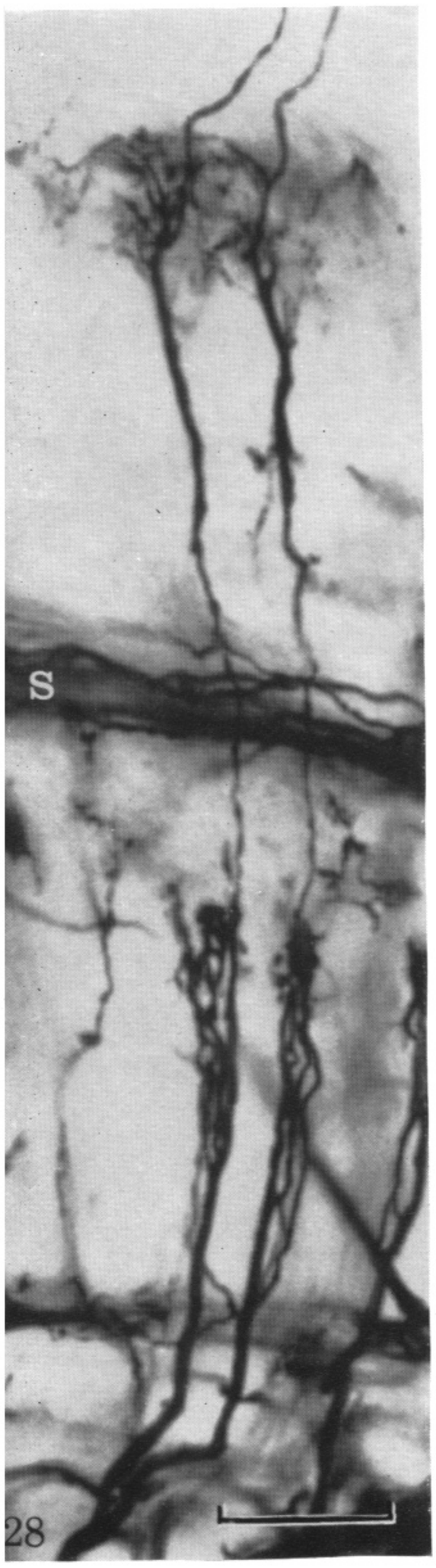

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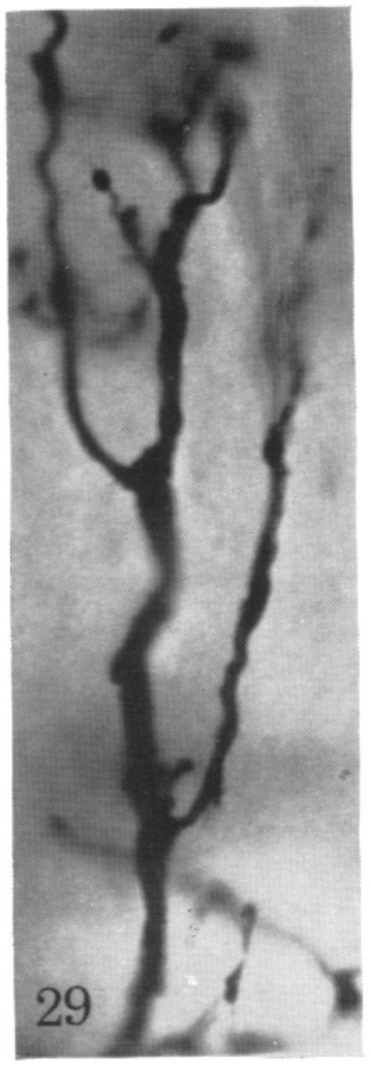

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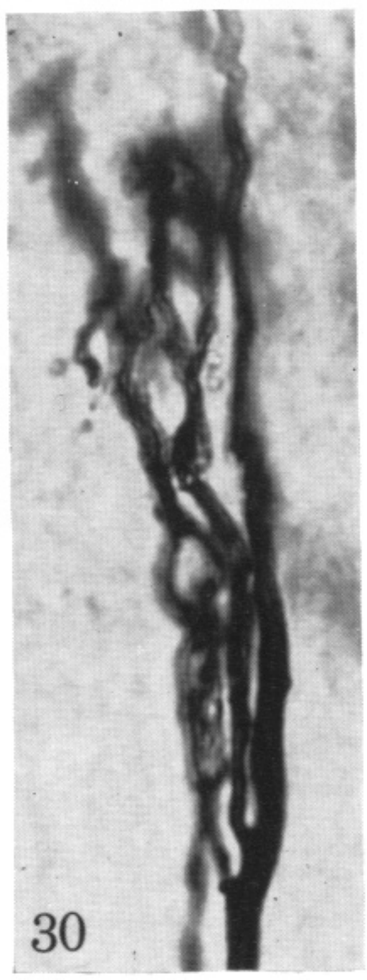

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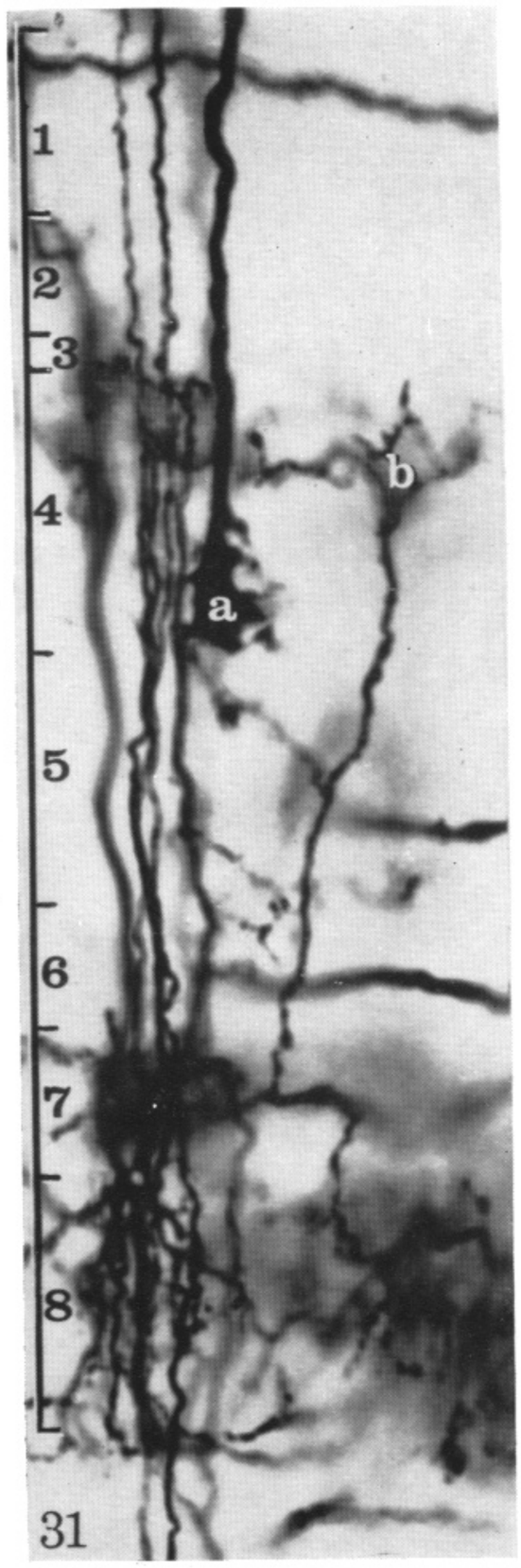

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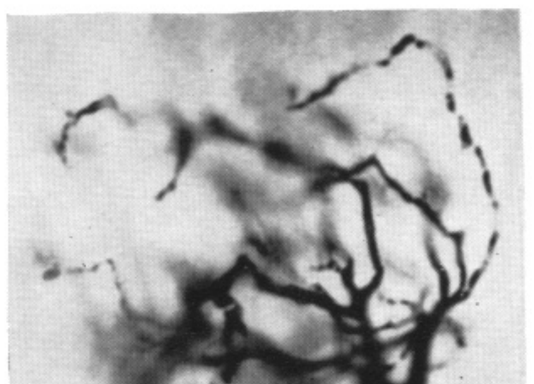

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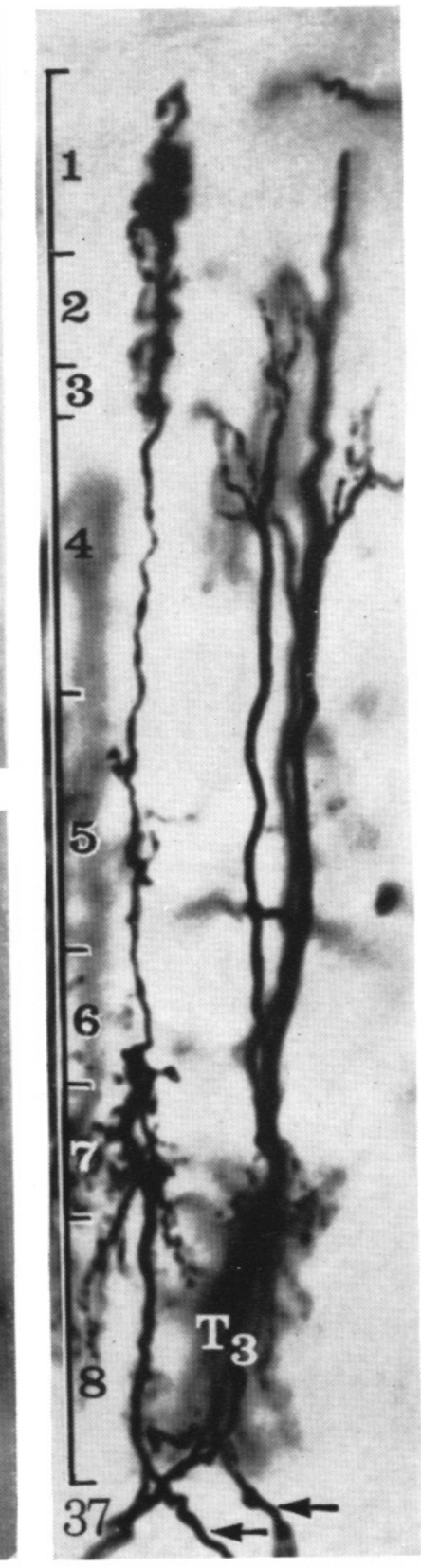

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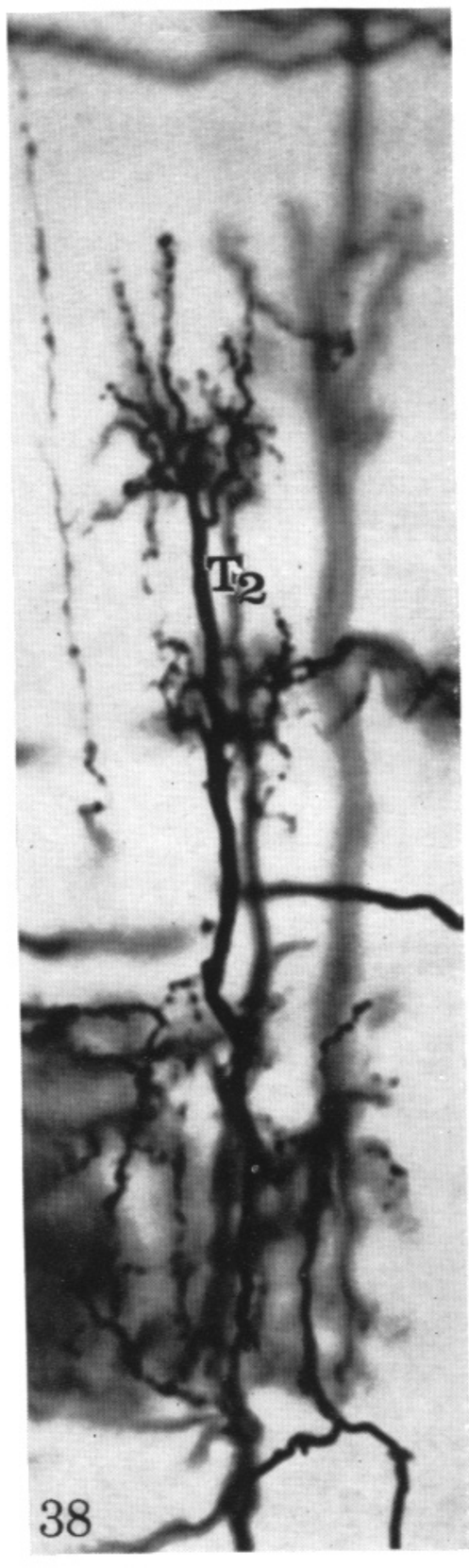

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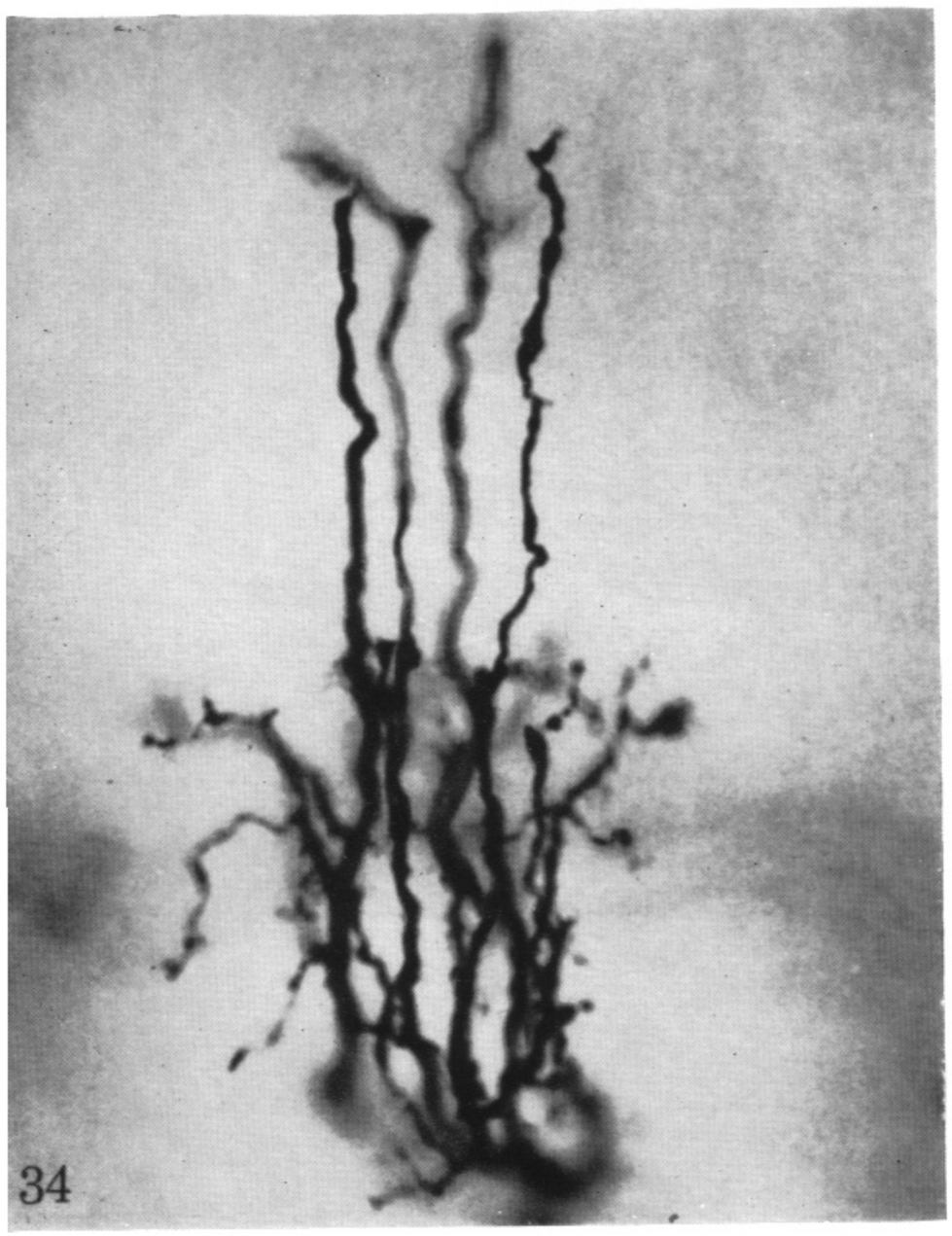

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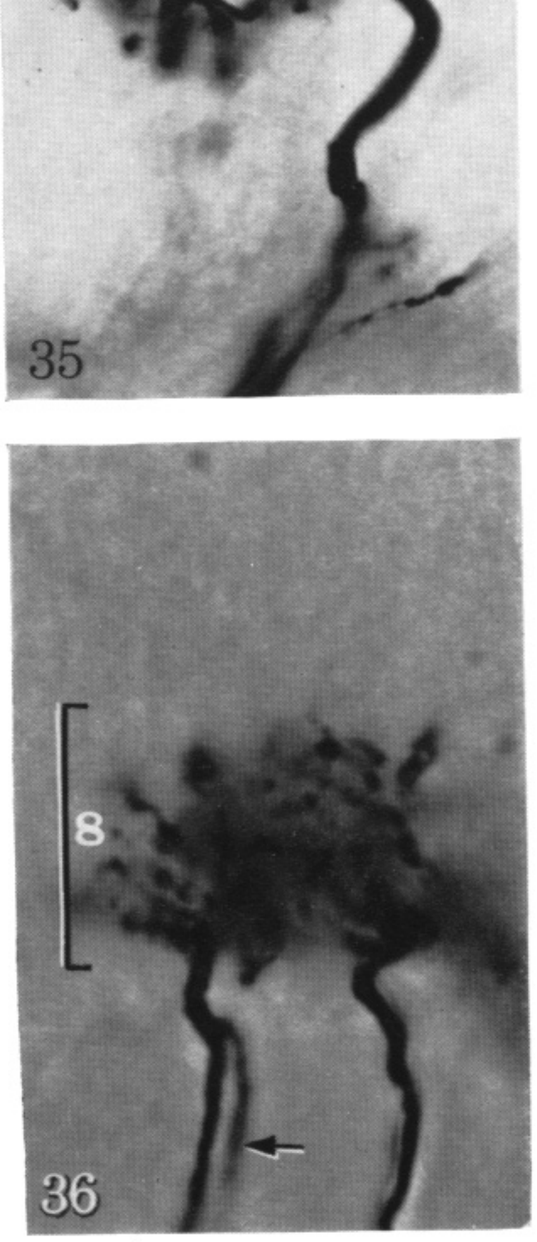

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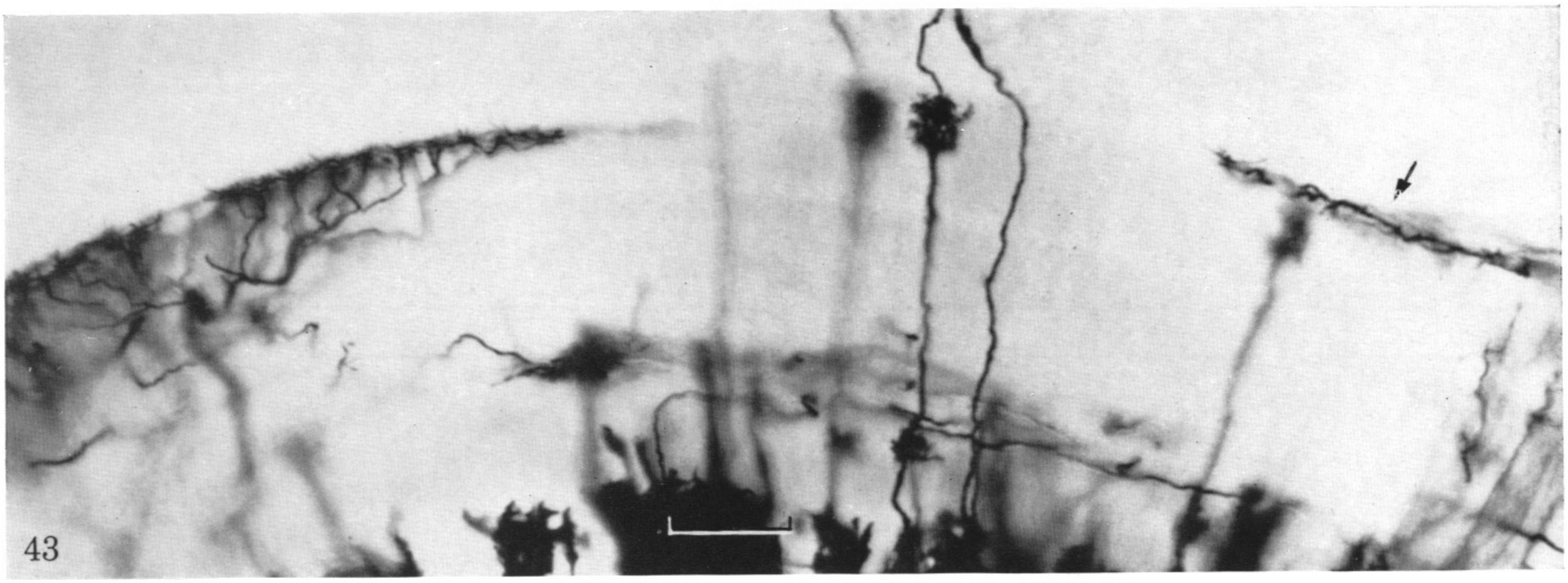

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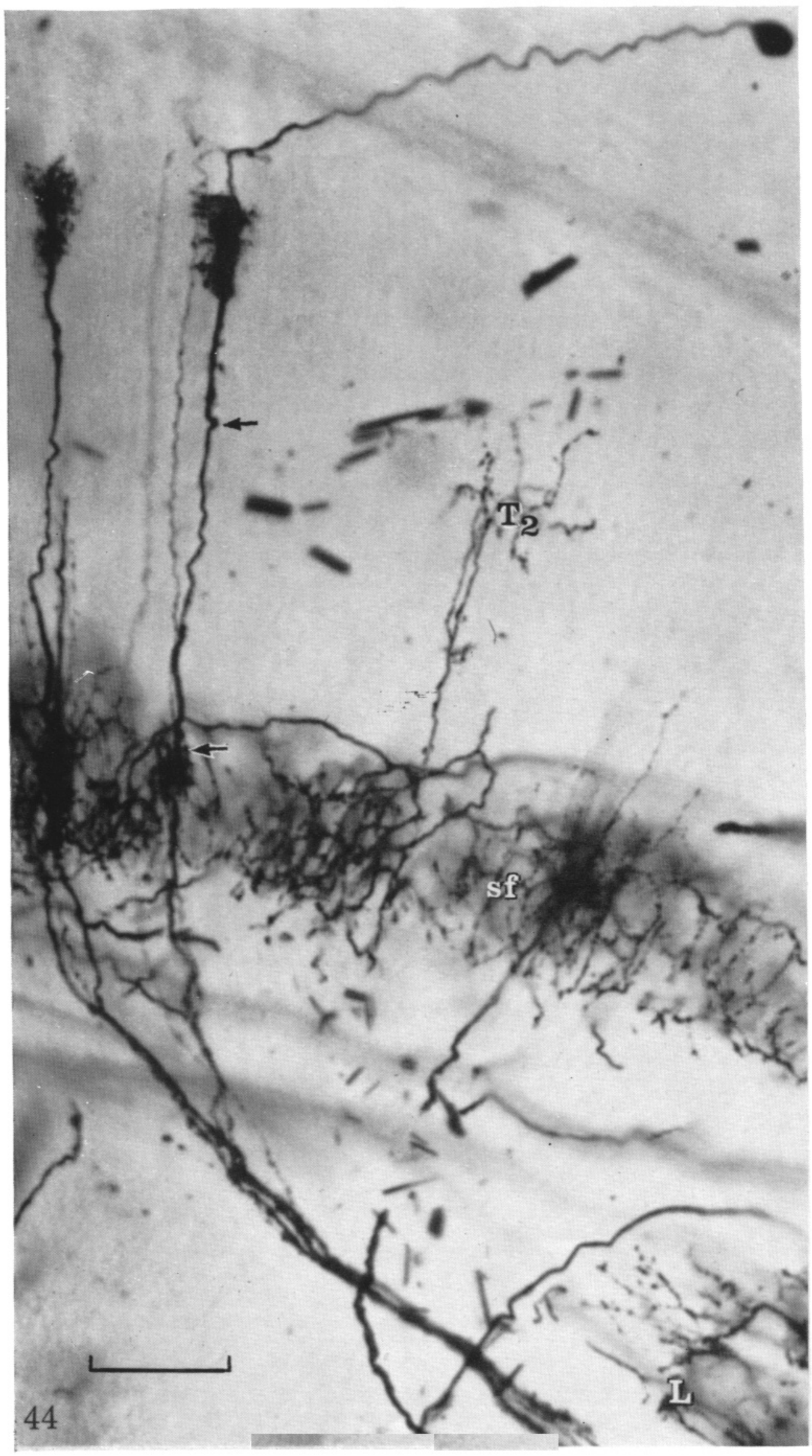

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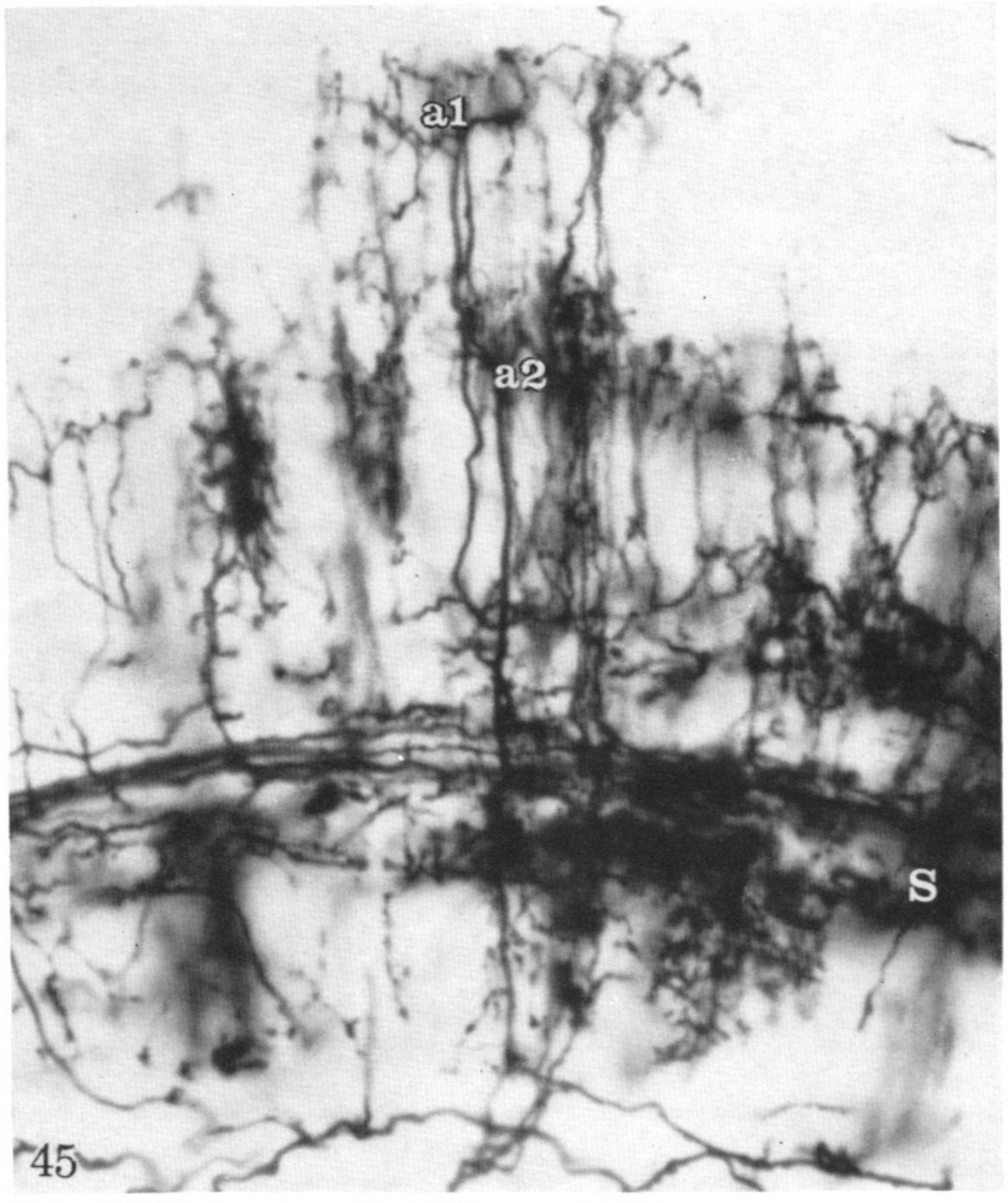

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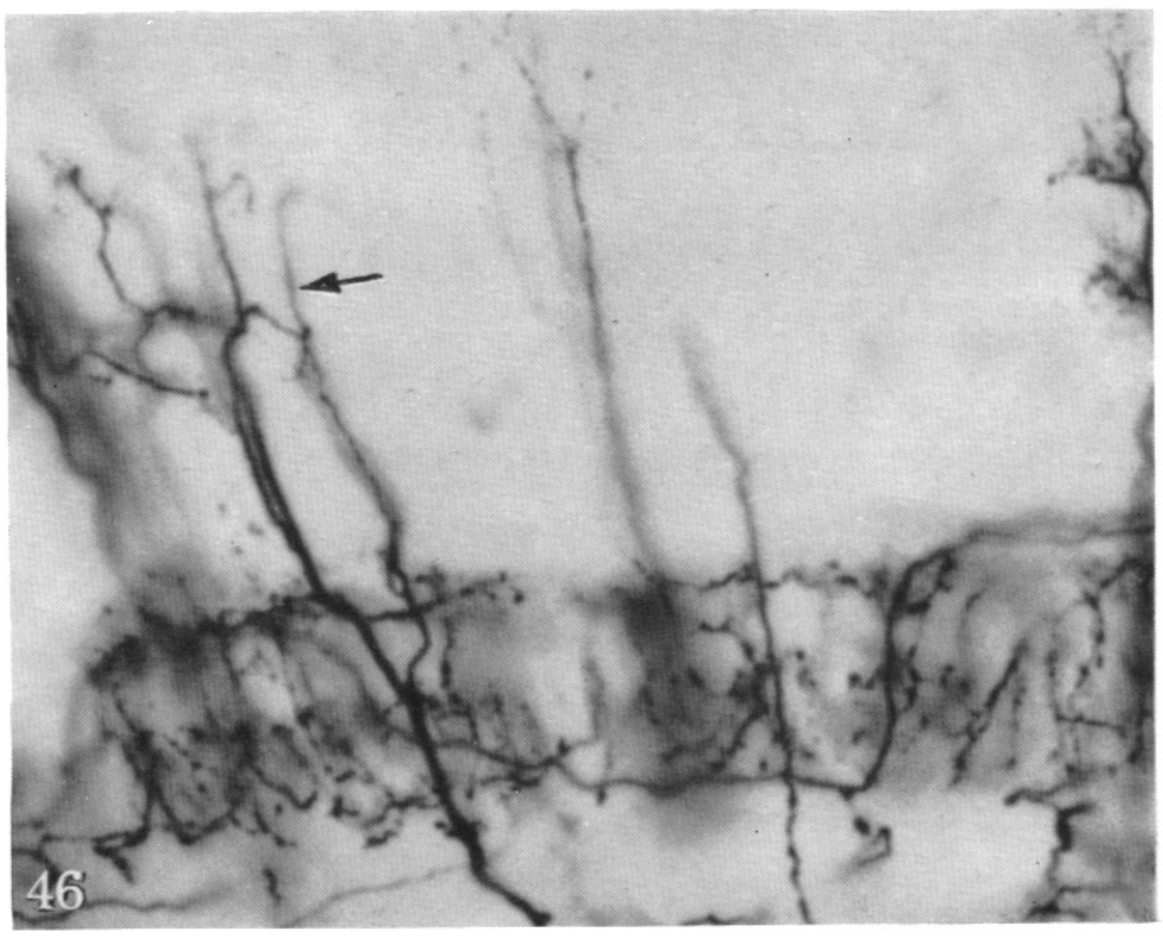

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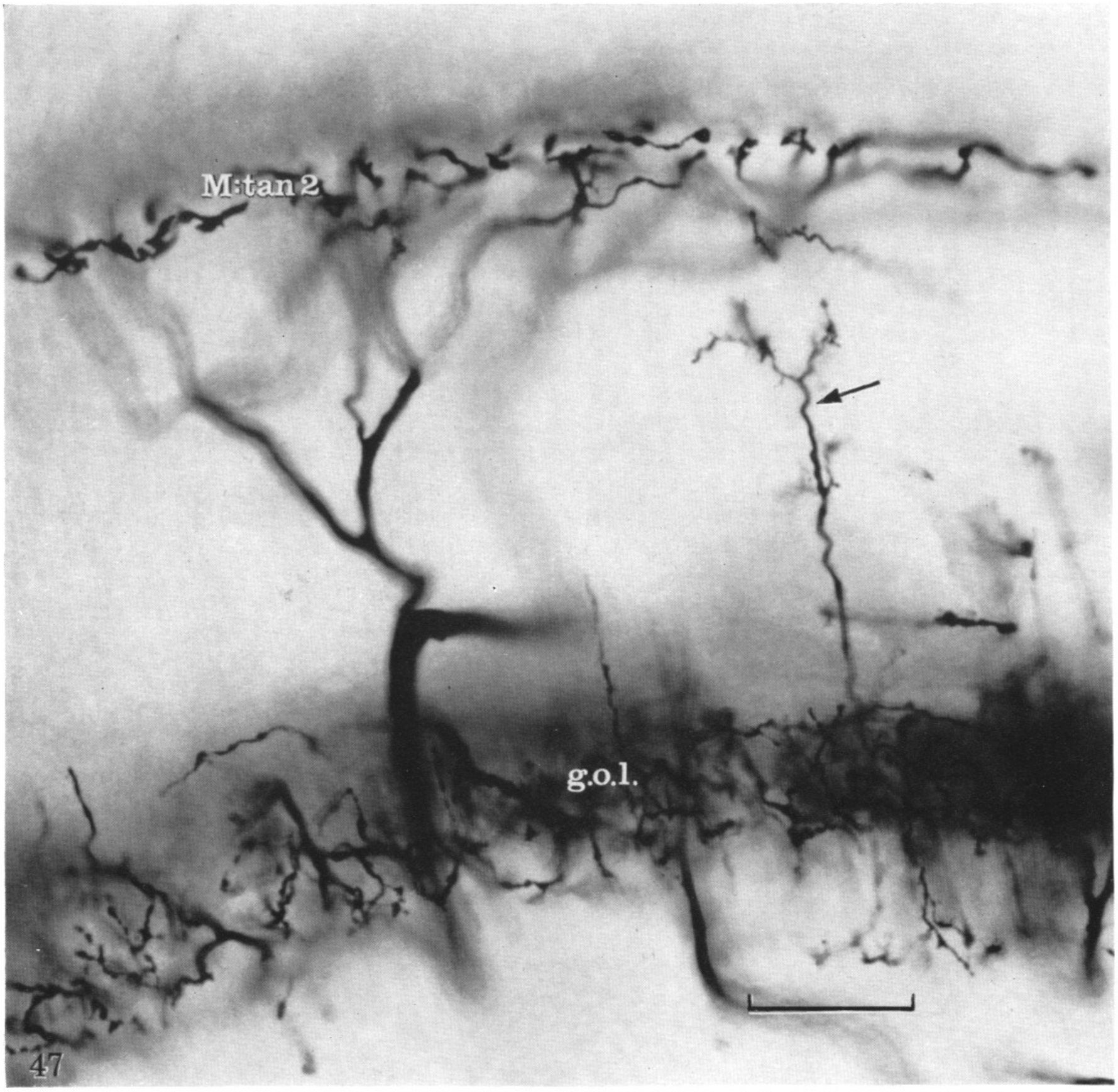

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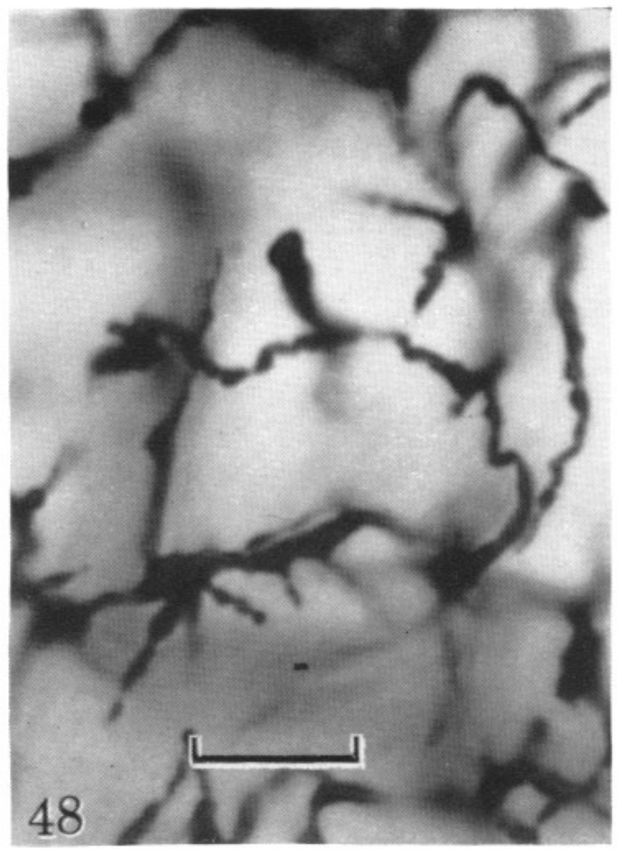

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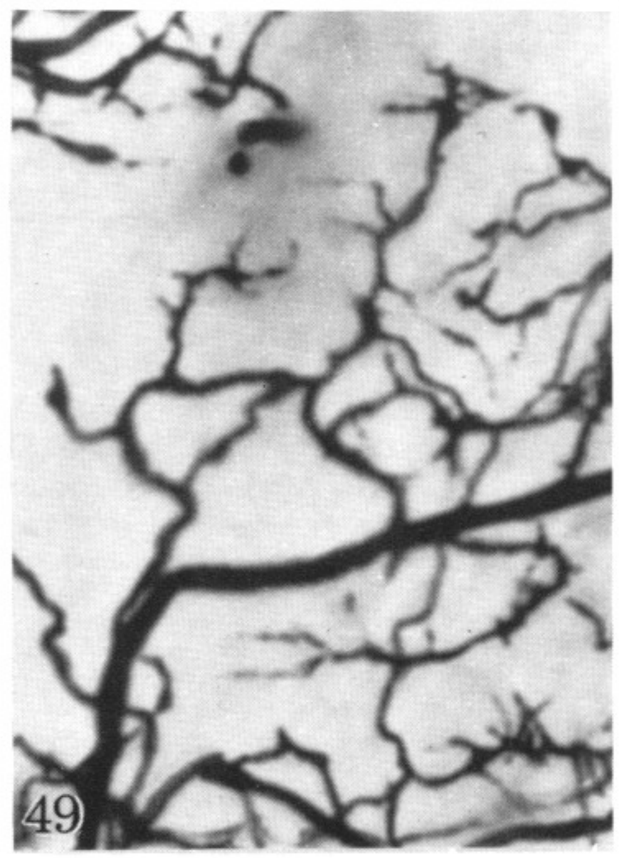

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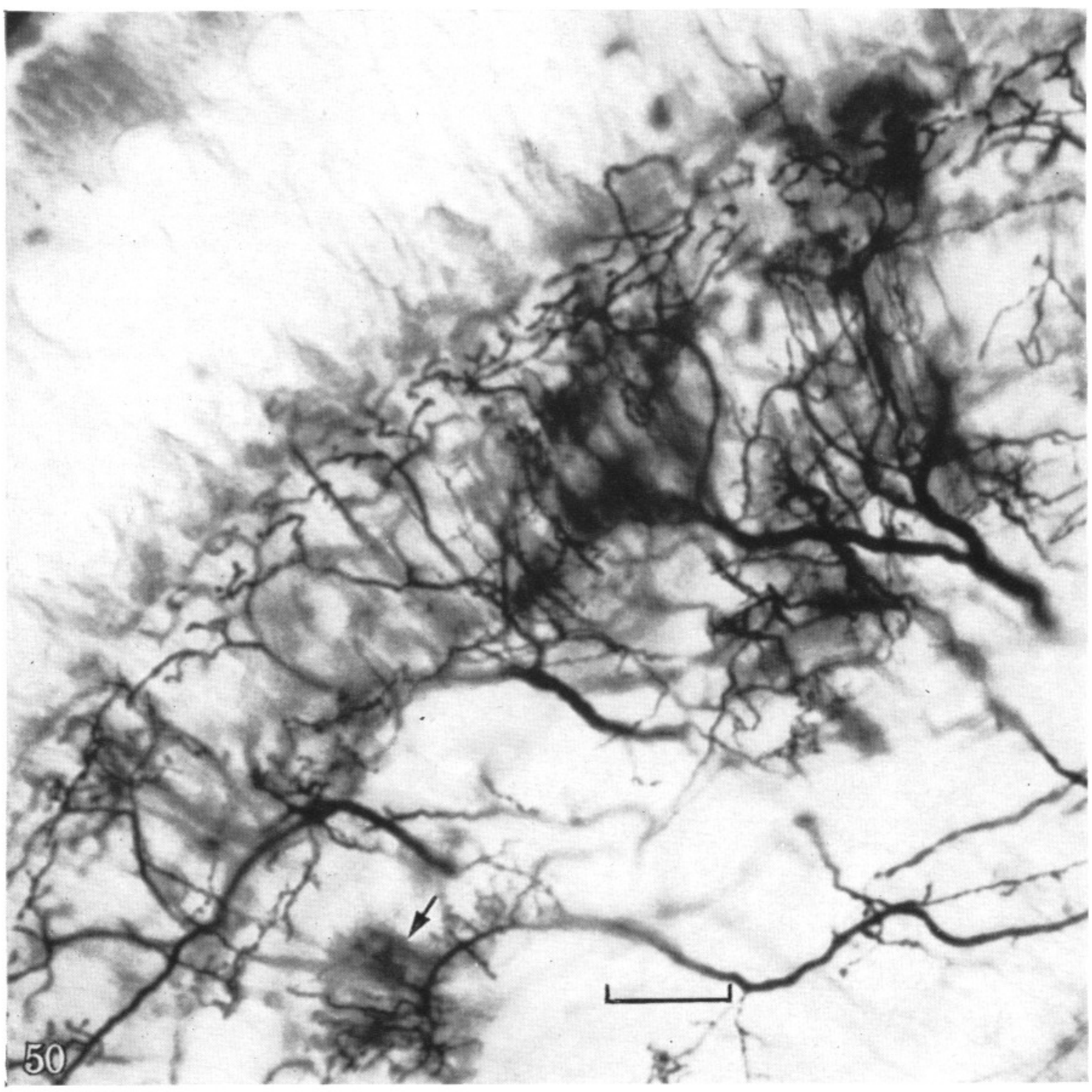

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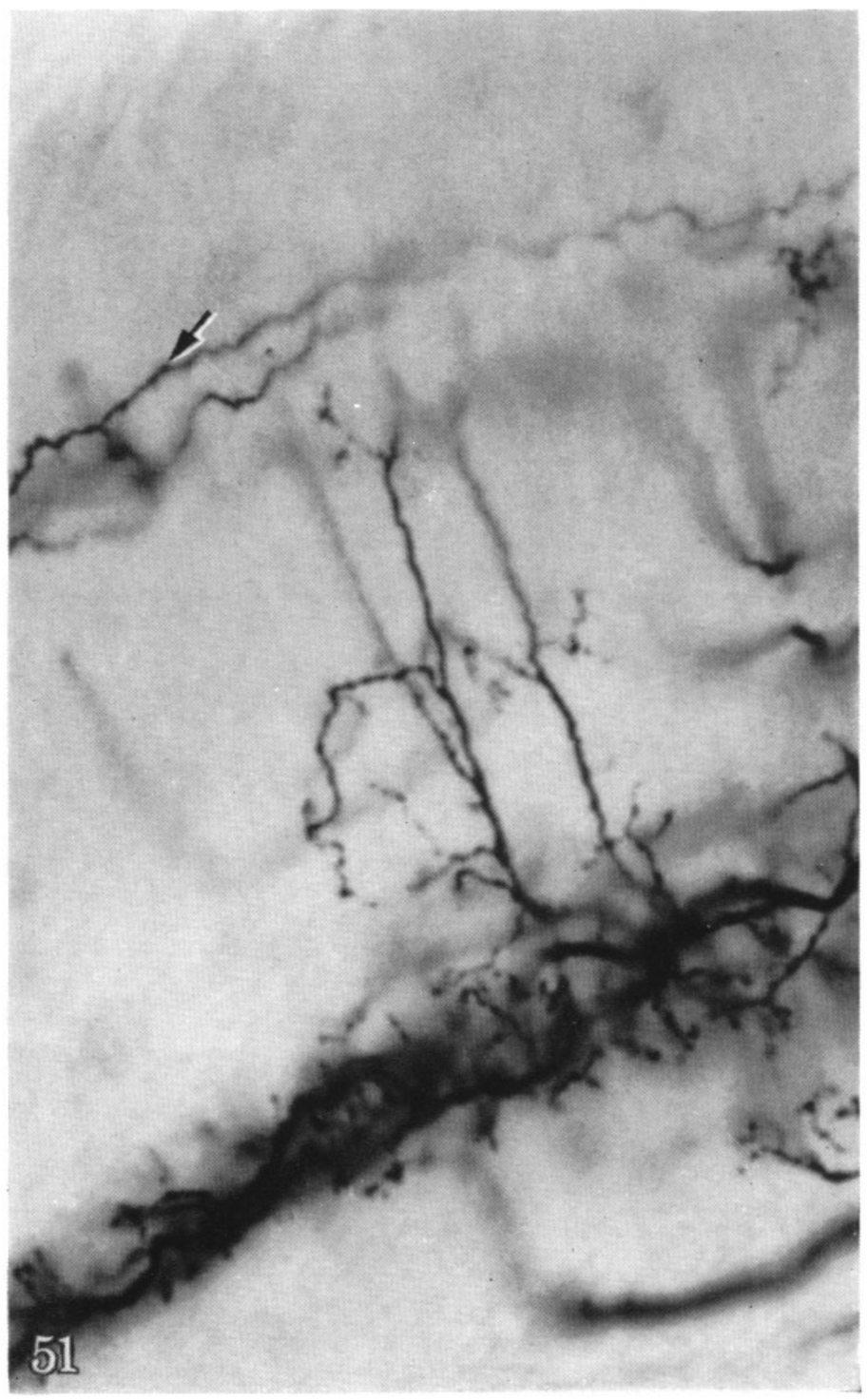

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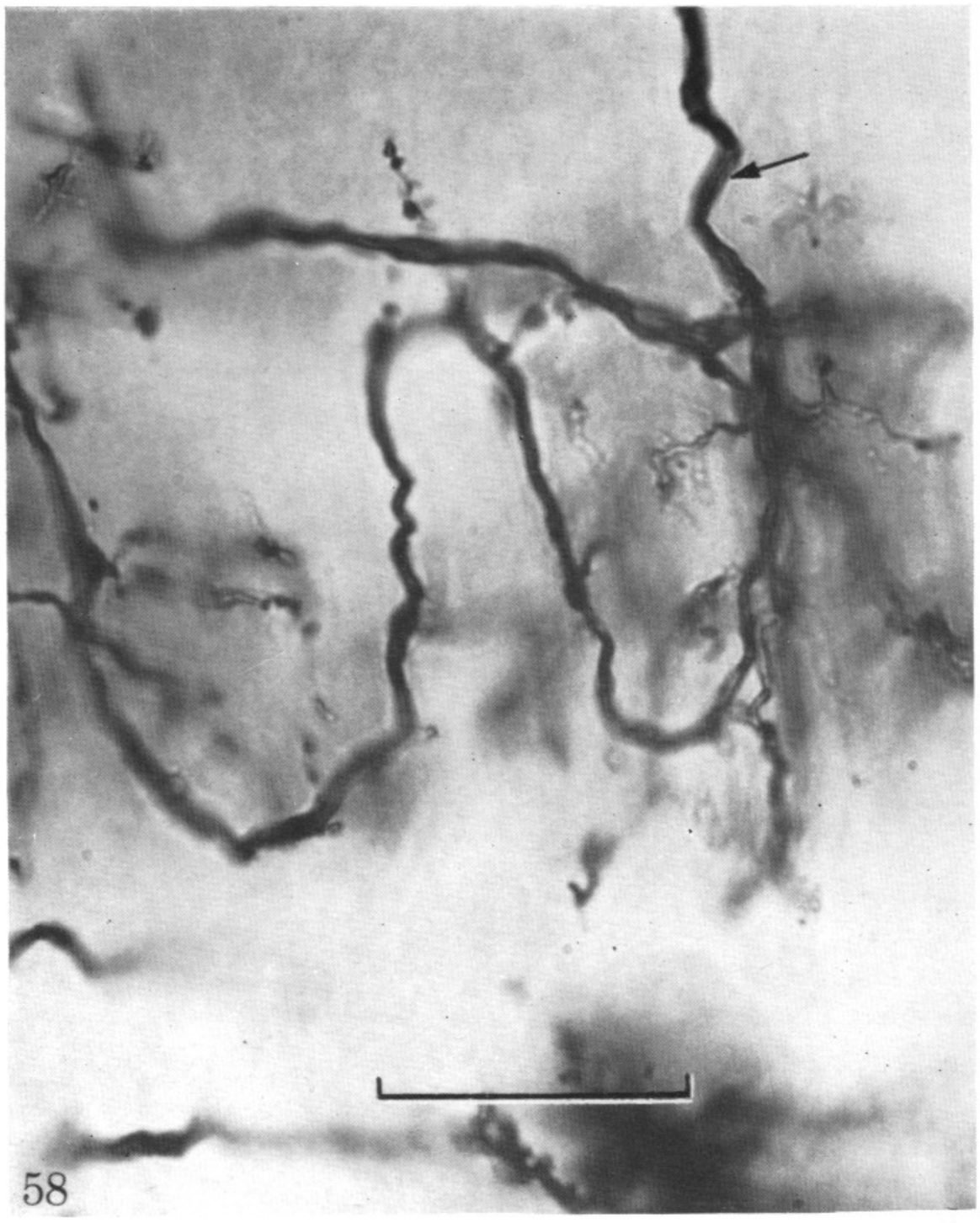

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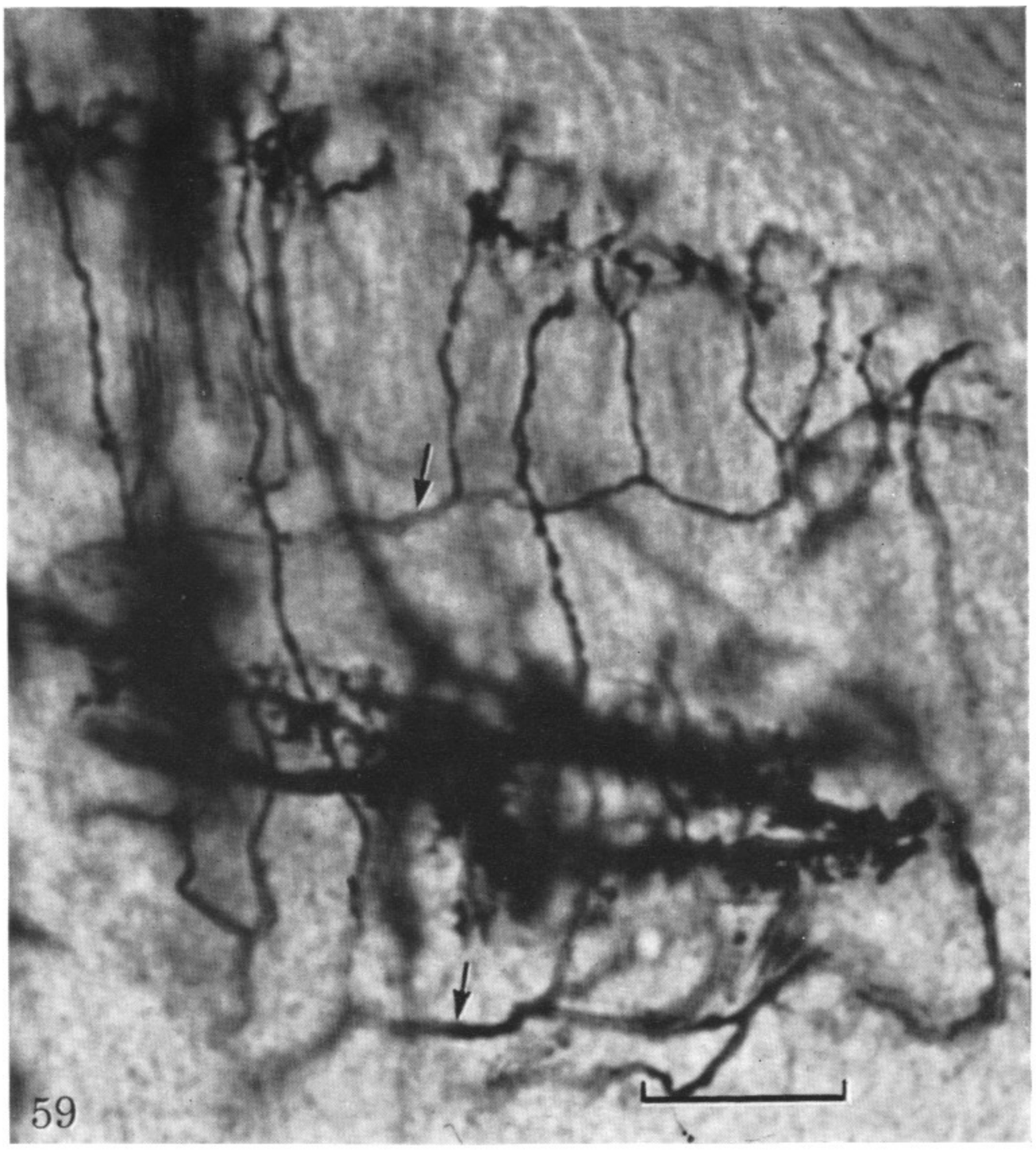

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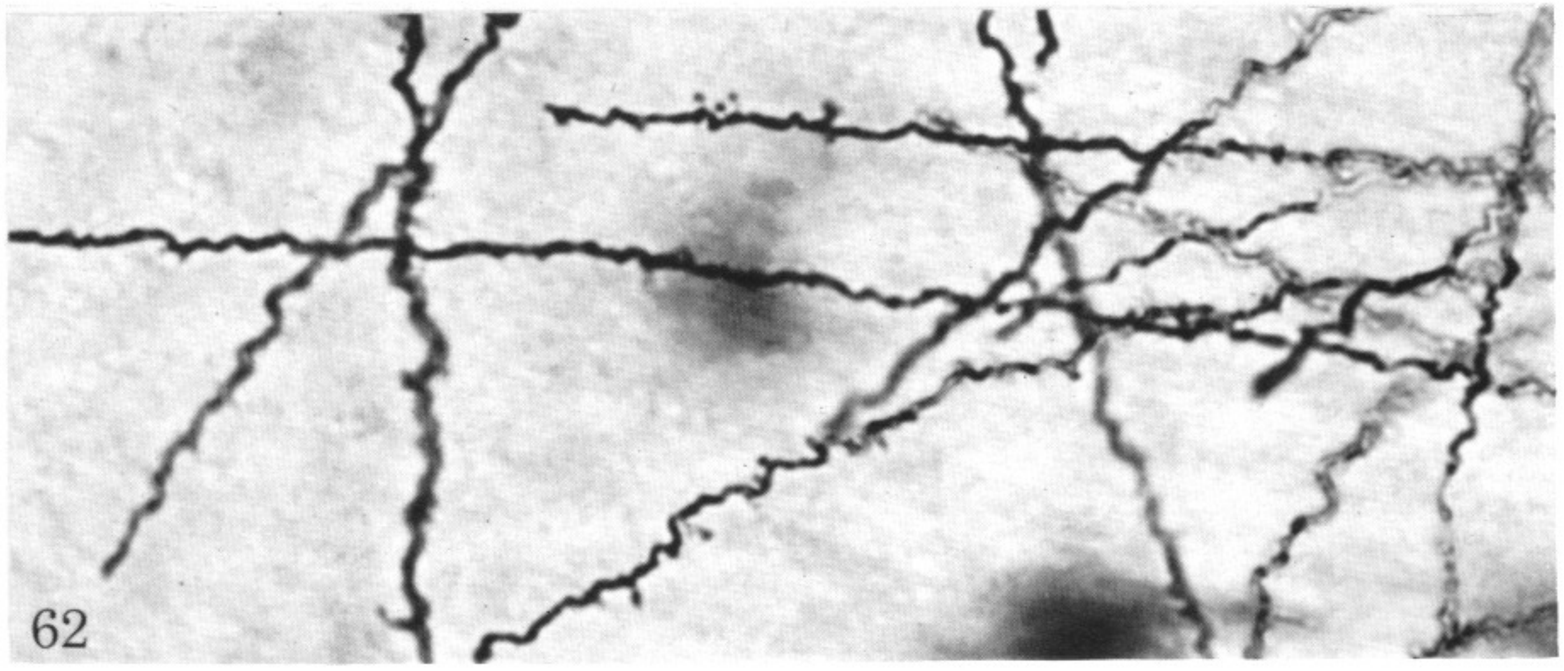

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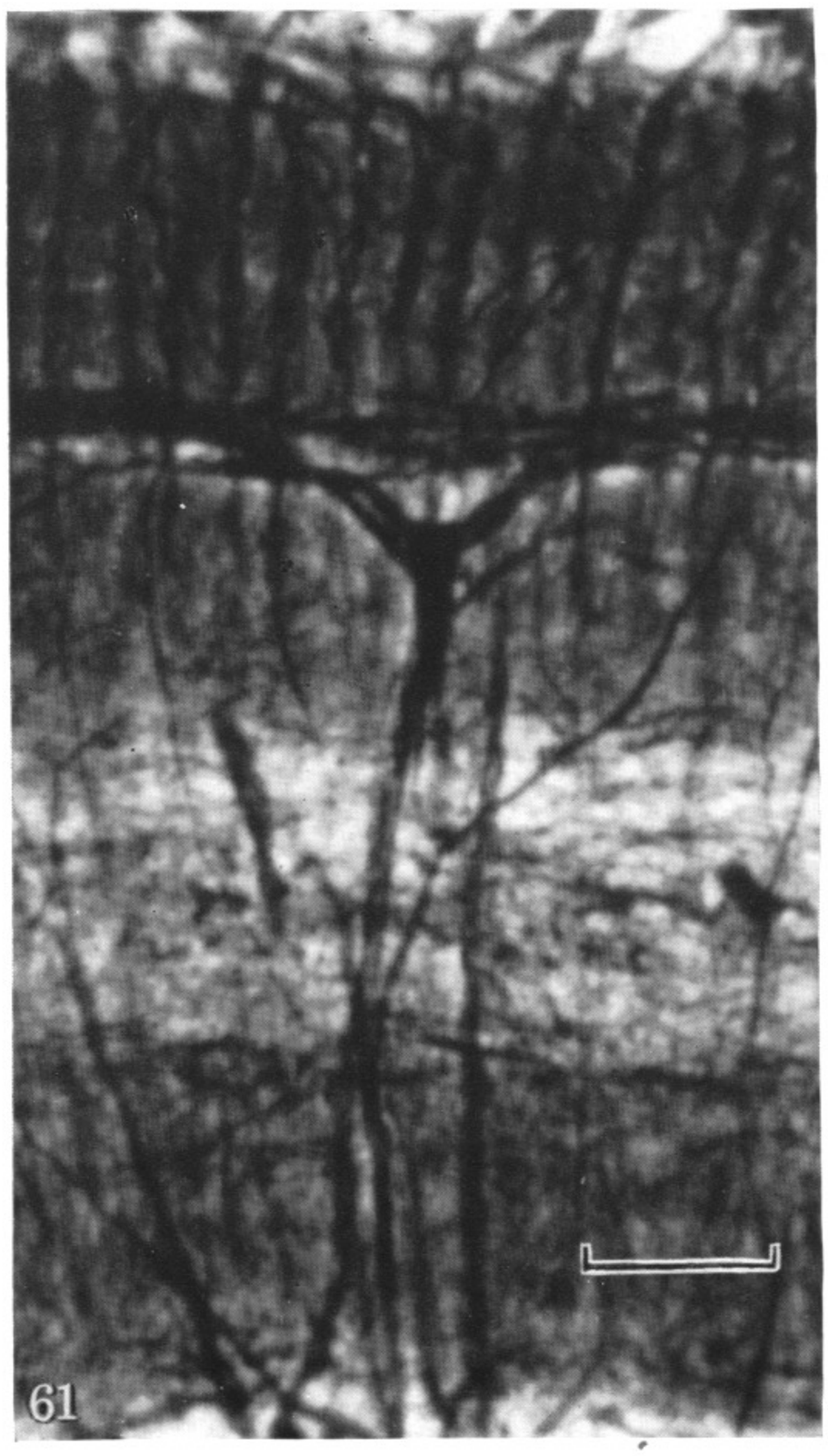

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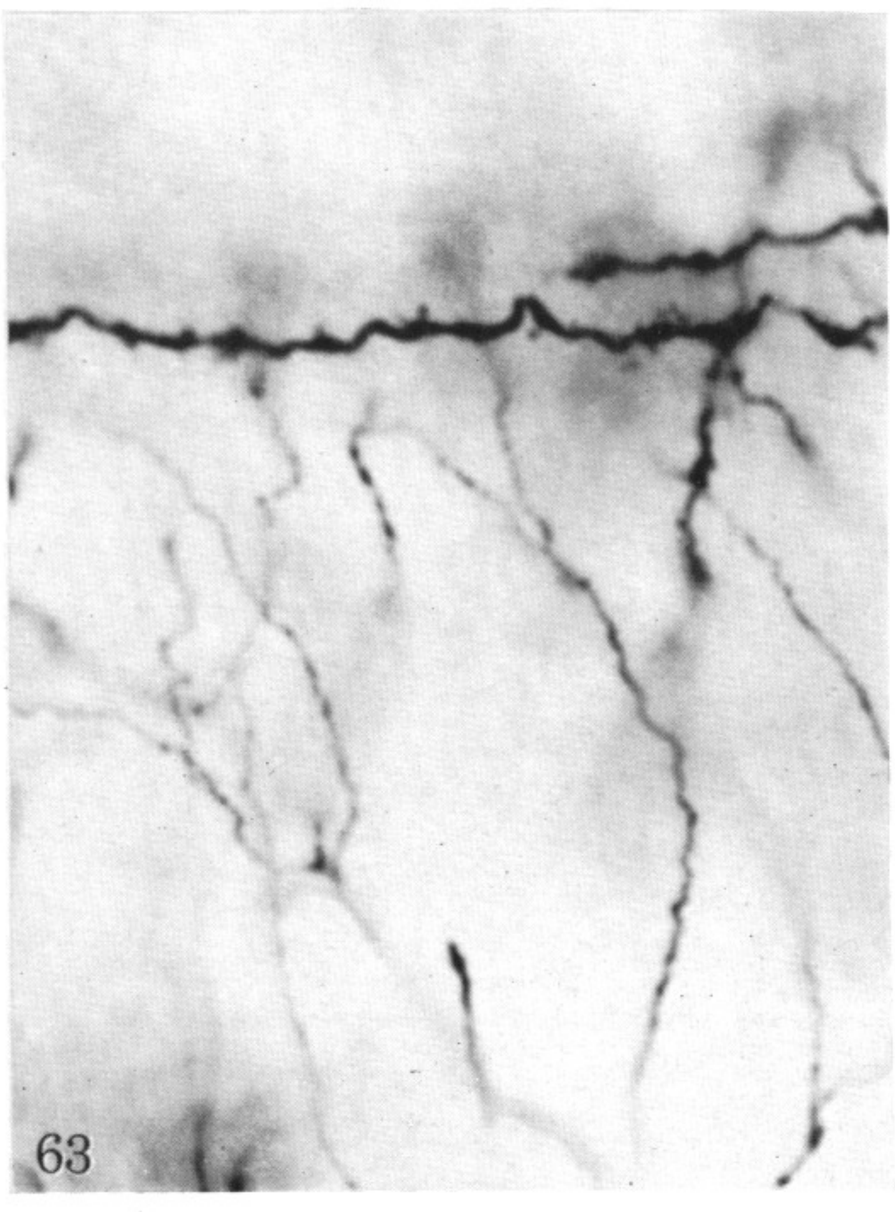

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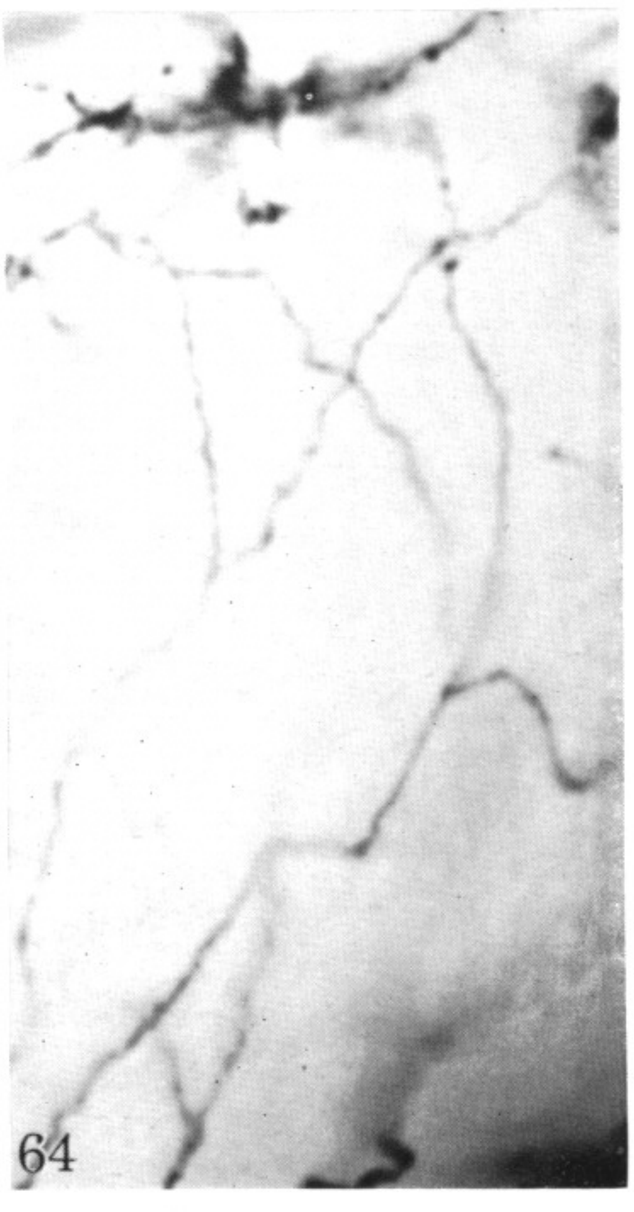

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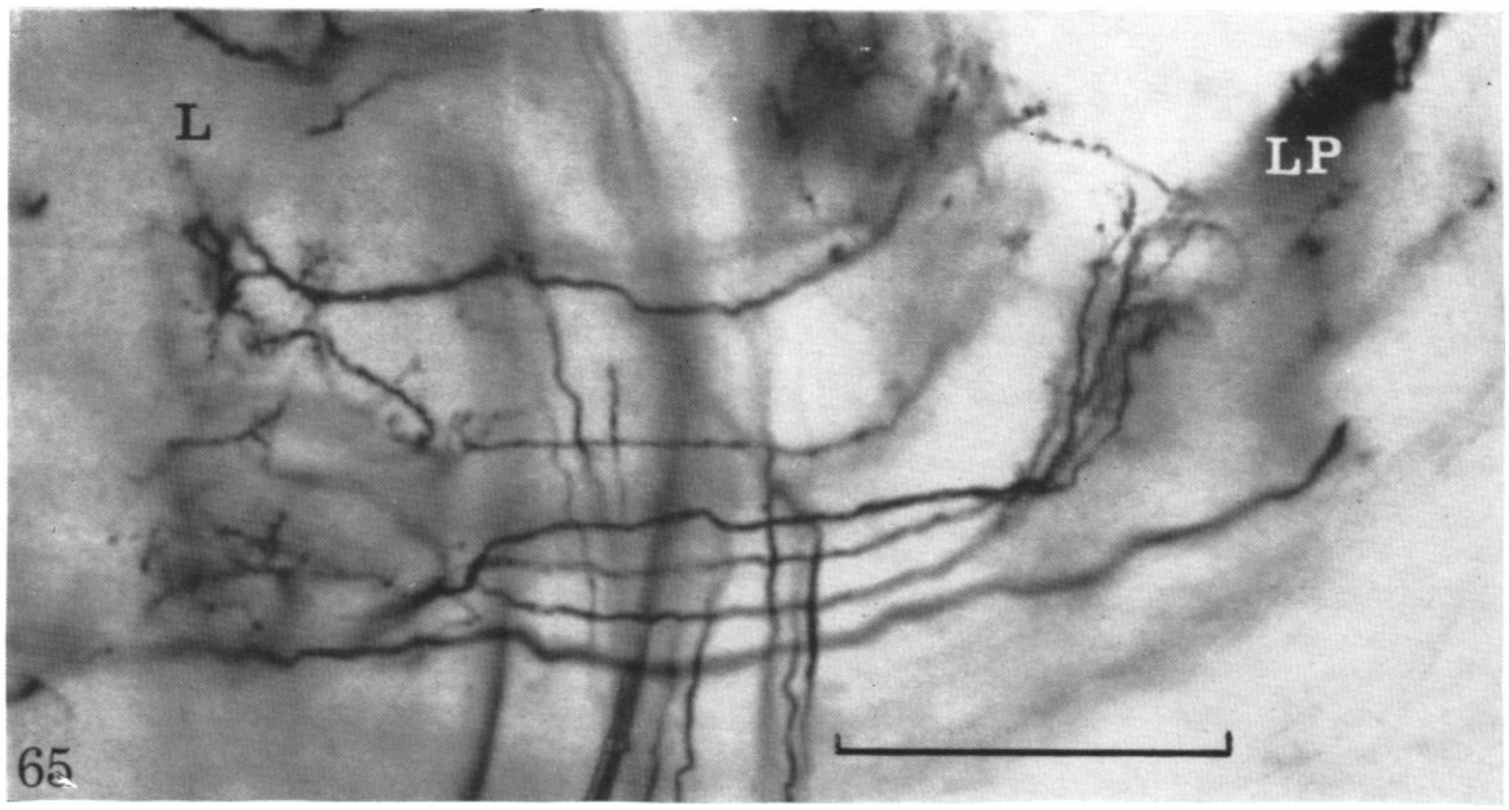

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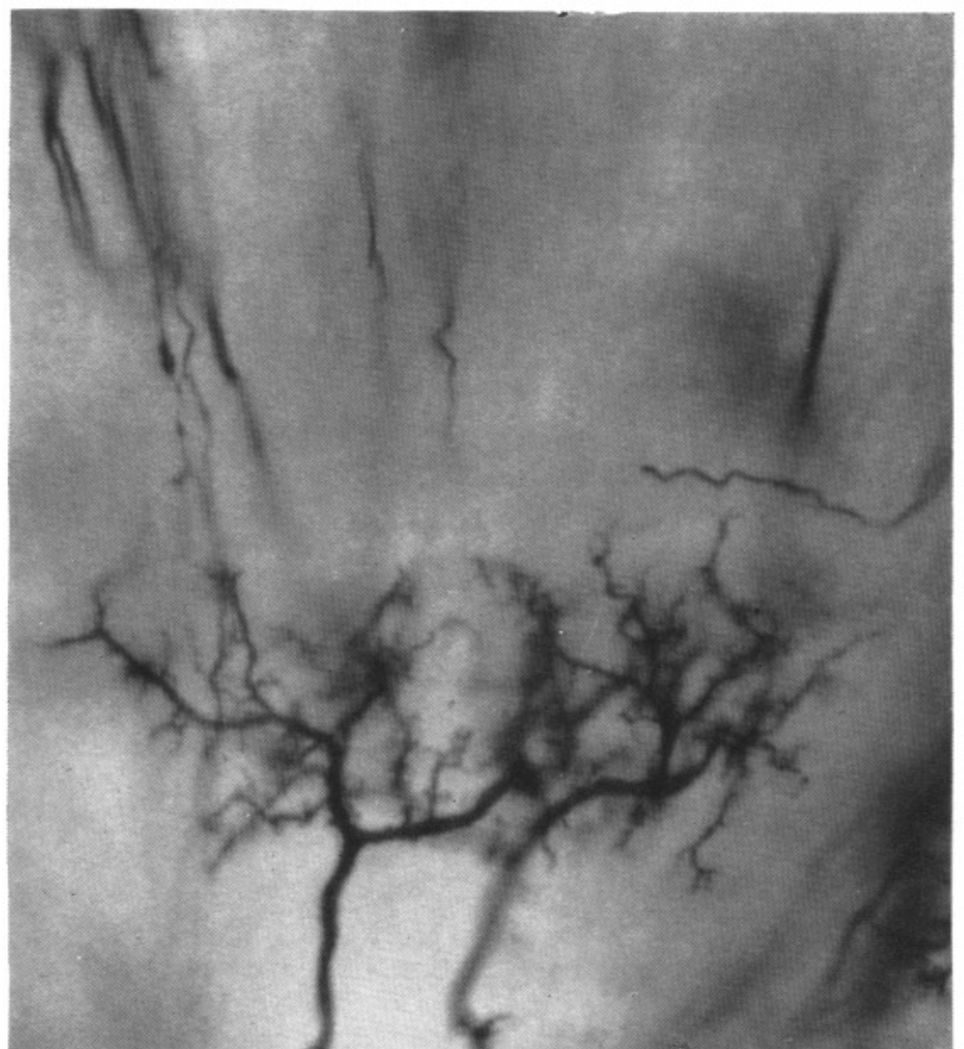

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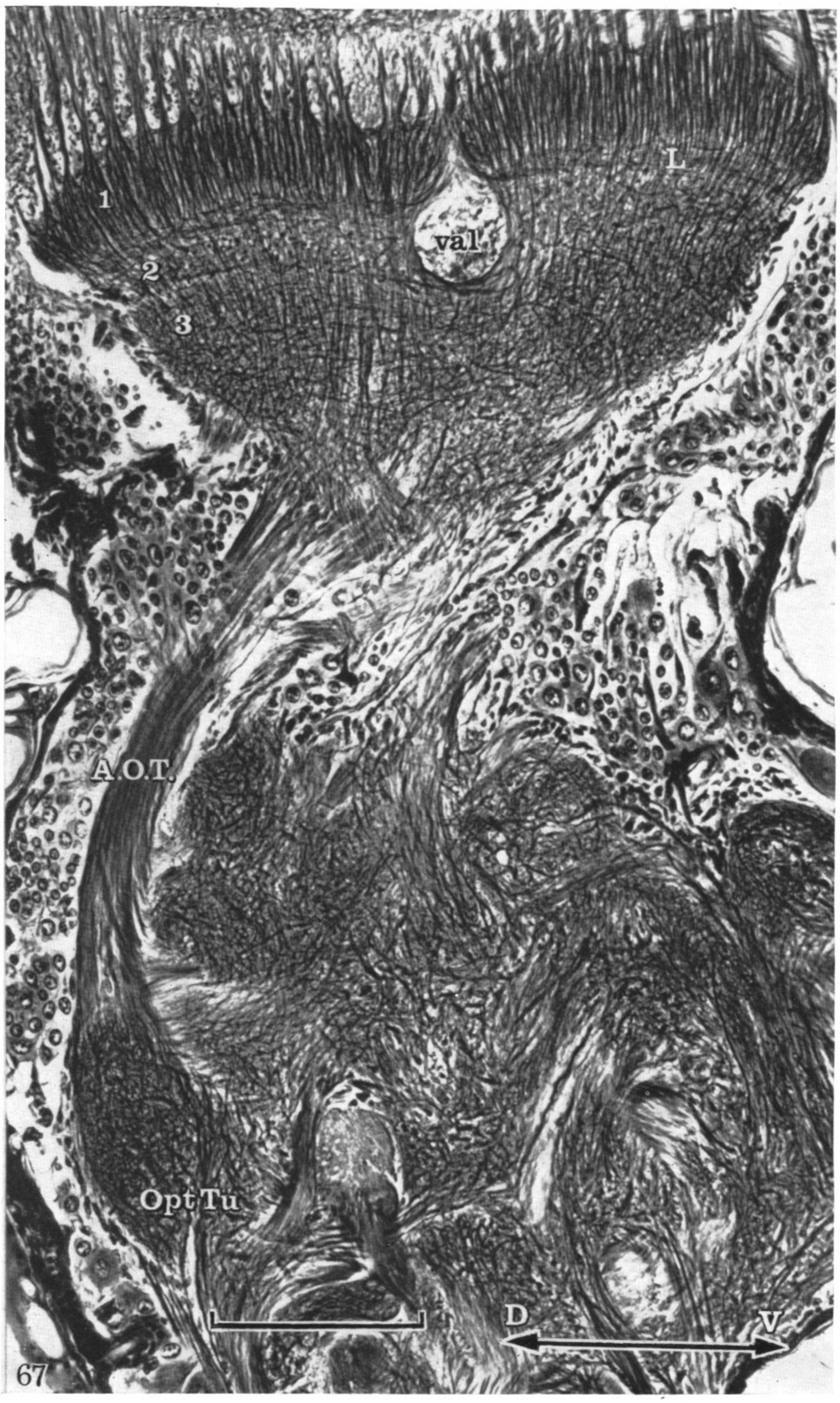

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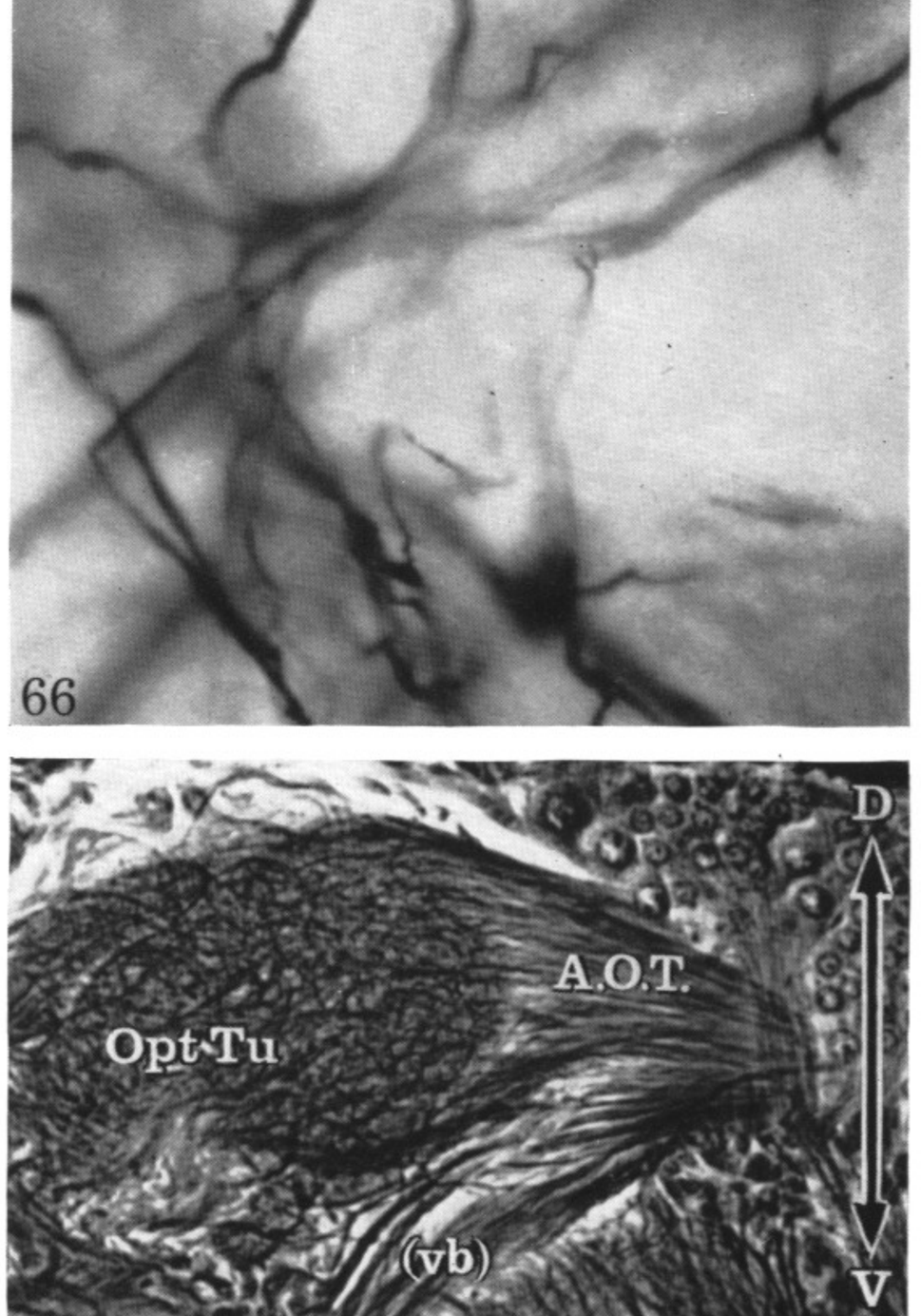

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