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The Organization of the Atrial Nervous System of Amphioxus (Branchiostoma lanceolatum (Pallas))

Q. Bone

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THE ORGANIZATION OF THE ATRIAL NERVOUS SYSTEM OF AMPHIOXUS (*BRANCHIOSTOMA LANCEOLATUM* (PALLAS))

By Q. BONE

The Marine Biological Association's Laboratory, Plymouth

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CONTENTS

The Acrania possess an histologically complex peripheral nervous system, the atrial nervous system, lying just under the epithelium lining the atrium and covering the various organs within it. The system contains both sensory and motor components, and is especially rich in peripheral sensory cell bodies. It is in connexion with the central nervous system by way of the dorsal root nerves.

Most of the motor axons entering the system pass to the cross-striated pterygial muscle flooring the atrium, others pass to the cross-striated trapezius muscles, and there is also a large ciliary-motor component, which controls the action of the lateral ciliary tracts of the gill bars. Unipolar sensory neurons are abundant upon the surface of the pterygial muscle, and are also found upon the parietal walls of the atrium.

Multipolar sensory neurons are abundant upon the foregut and its diverticulum. The hindgut (outside the atrium) is more sparsely innervated, but occasional multipolar sensory neurons occur there. The multipolar neurons of the foregut and diverticulum appear to be in connexion one with another asynaptically, but their axons pass to the central nervous system. Similar sensory neurons of several types are found in the richly innervated atrio-coelomic funnels.

The function of the atrial nervous system is not yet entirely understood, but it is probable that it is mainly concerned with the regulation of the feeding process, and with spawning. It is concluded that the system is not evidently homologous with the 'sympathetic' systems of the craniates, and that it is unwise at present to attempt to homologize the visceral nervous systems of the two groups.

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Introduction

Many histologists have examined the peripheral nervous system of amphioxus (the works of Heymans & van der Stricht (1898), and Dogiel (1903) are the classical accounts); yet there remain several points of importance about which we know little. The present account describes the peripheral nervous system of the atrial region, whose interpretation has been a matter of dispute.

The early students of the system observed nerve fibres and occasional nerve cell bodies at various positions around the atrium, both upon its parietal walls, and upon the viscera lying within the atrium. These fibres and cells were later found to lie just under the thin sheet of ectodermal atrial epithelium that covers the organs within the atrium and lines its walls, and thus the *atrial nervous system* (Holmes 1953), may be defined conveniently as the nervous tissue overlain by atrial epithelium. Because this system is related to the viscera, it was early suggested that it was homologous to the autonomic system of the craniate.

Rohon (1882) believed that he had discovered the homologue of the visceralis branch of the craniate vagus, but other workers such as Fusari (1889) were more cautious, and felt that homologies of this kind were unwarranted until much stronger evidence was provided. As Holmes remarked, it was not until the work of Boeke (1935 *a*, *b*), that the homology of the amphioxus atrial nervous system and the craniate autonomic gained general acceptance. Boeke described the neurons of the gut of amphioxus in some detail (even going so far as to recognize homologues of the plexuses of Auerbach and Meissner!), and concluded that the enteric nervous system in amphioxus was directly comparable to the enteric systems of the craniates. His conclusions have been accepted, and in recent texts (such as Drach, in Grassé 1948) it is stated that amphioxus possesses a sympathetic nervous system. It was Holmes who first questioned Boeke's interpretation of the amphioxus atrial system, and who pointed out that in several respects, the system differed greatly from the craniate autonomic system. Among other points, Holmes showed that the neurons of the enteric plexus were almost certainly sensory elements, sending their axons to the central nervous system, and were not, as Boeke had supposed, motor cells similar to those of the craniate enteric plexus. My own observations confirm those made by Holmes, and suggest that it is unsafe to make a direct comparison between the acraniate atrial nervous system and the craniate autonomic system; the two differ in their components, in their anatomical relations, and in their functions.

Material and methods

Chief reliance has been placed upon silver-impregnated whole mounts of adult animals fixed in sea-water Bouin. Adult amphioxus (including some small specimens 2 cm long) were obtained from the Eddystone shell gravel, anaesthetized with magnesium chloride, and fixed for not more than 2 days. They were then washed out in 70 % ethanol for 24 h at 37 °C, and portions dissected out and further washed for 24 h in frequent changes of distilled water. These were impregnated by Holmes's technique (Holmes 1947). Other pieces were similarly treated and impregnated by Bodian's protargol method (Bodian 1936).

Gold-toning, followed by aniline-alcohol (Palmgren 1948) was sometimes used, but as a rule proved disadvantageous, as thick preparations were thereby rendered too opaque.

After examination in balsam, further dissection of the preparations was generally found necessary, the brittleness of the preparations after even some years in balsam assisting such further dissection. Other animals were fixed in the same way, embedded in polyester wax (Steedman 1957), and stained by Palmgren's silver on the slide technique. Polyester wax sections are markedly superior to normal paraffin wax sections. Yet other animals were stained supravitally with methylene blue solution, and fixed in Alexandrowicz's modification of Cole's fluid. Best results were obtained by pinning out portions of the animal on a wax plate, and staining in a damp chamber.

In addition to the adult amphioxus material mentioned above, I have also stained some adult *Asymmetron lucayanum* Andrews, and a number of very small post-metamorphic and larval specimens of amphioxus, from Professor E. S. Goodrich's collection of acraniate material. I am indebted to Professor Sir Alister Hardy, F.R.S. for his kindness in allowing me to examine this material.

Results

The atrial nervous system is in connexion with the central nervous system by dorsal root nerves. The dorsal root nerves of the atrial region send a ventral branch to a point about two thirds of the way down the sides of the body, where it divides into three rami. The first, the superficial sensory ramus, passes to the skin of the metapleural folds of the ventral region. The second, the descending visceral ramus, runs inwards, and ramifies over the surface of the pterygial muscle flooring the atrium. The third, the ascending visceral ramus, likewise passes inwards, but then runs upwards on the parietal wall of the atrium and passes finally to the organs within the atrium. This well-known arrangement is illustrated in figure 1. It should be emphasized that the ascending and descending rami have been named from their course in the body of the animal, and not from the course of the fibres of which they are composed; each contains both centripetal and centrifugal fibres.

In the following sections, the atrial nervous system will be considered under five headings; there is a certain amount of overlap between the elements of some of these sections, but in general, they form anatomically (and probably functionally) separate divisions. These sections are:

(i) The innervation of the pterygial muscles and atriopore.
(ii) The innervation of the parietal walls of the atrium and the gonad sacs.
(iii) The branchial and sub-endostylar plexuses.
(iv) The innervation of the atrio-coelomic funnels.
(v) The innervation of the foregut and diverticulum.

Together with the atrial system proper, as defined earlier, it is reasonable to consider the innervation of two other regions, both of which are related to the atrium, and to the atrial nervous system. The hindgut is not covered with atrial epithelium but its nerve supply is in some ways similar to that of the foregut. The innervation of the buccal and velar regions (likewise outside the atrium), is functionally closely connected with the atrial nervous system, for both sensory and motor responses of the region directly affect conditions within the atrium.

This account will therefore be divided into seven sections, including these two extra-atrial regions.

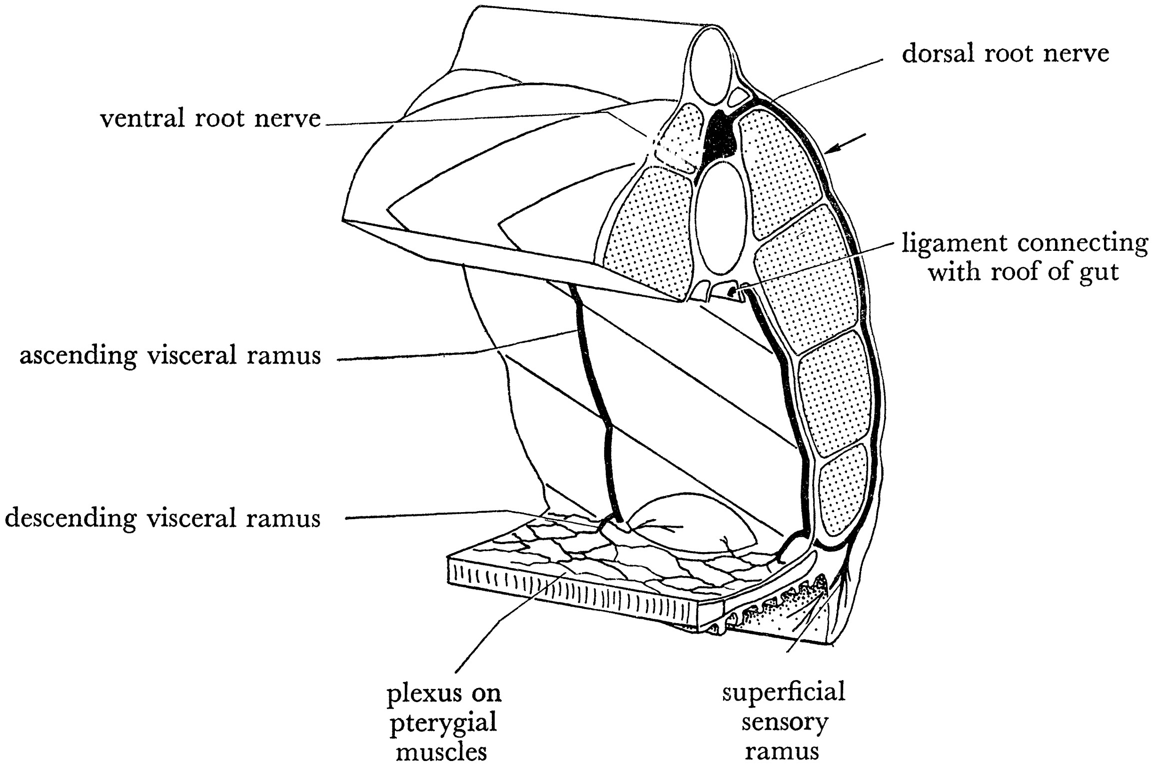

Figure 1. The relations of the rami of the dorsal root nerves. (The arrow indicates the position of the interruption of the nerve referred to on p. 248.) (From Bone, *Quart. J. micr. Sci.* **99**, 1958.)

Table 1. The components of the atrial nervous system

	peripheral cell bodies	location	assumed function
motor systems			
1. pterygial plexus	—	pterygial muscle	motor to cross-striated muscle fibres
2. atrio-coelomic plexus	—?	trapezius muscle	motor to cross-striated muscle fibres
3. vaso-motor system?	—?	blood vessels?	vaso-motor
4. sub-endostylar plexus	—?	endostyle	secreto-motor
5. branchial plexus	—?	gill bars	ciliary-motor and motor to smooth muscle fibres
6. gonad plexus	—	gonad sacs	motor to smooth muscle fibres
sensory systems			
7. Pterygial plexus	unipolar	pterygial muscle	sampling waterflow, or particle concentration of water stream
8. Parietal plexus	unipolar	atrial walls	as pterygial
9. branchial plexus	unipolar and multipolar	gill bars	?
10. buccal and velar system	unipolar	buccal cirrhi and velum	tactile and chemo-receptive
11. sub-endostylar plexus	multipolar	endostyle	?
12. atrio-coelomic plexus	multipolar and unipolar	atrio-coelomic funnels	water sampling
13. foregut system	multipolar	foregut	?
14. hindgut system	unipolar and multipolar	hindgut	?

(vi) The innervation of the hindgut.

(vii) The innervation of the buccal and velar regions.

The innervation of some of the regions into which the system has been divided is well-known, and a good deal of accurate information can be found in the literature. For the sake of completeness, I have included a summary of the innervation of such regions, together with original observations. The whole system is one of some complexity, therefore, to give the reader some idea of the organization of the whole, before proceeding to the description of each section, I have summarized the conclusions reached in table 1 and in figure 2.

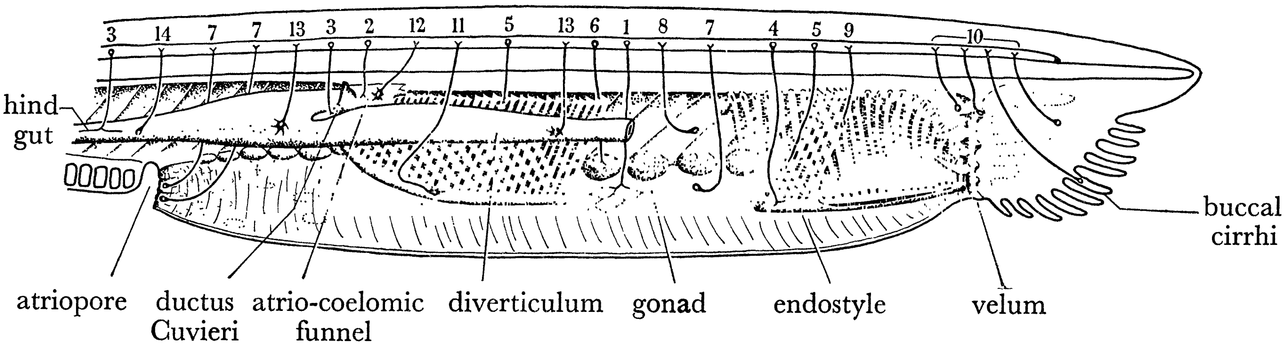

FIGURE 2. Scheme of the components of the atrial nervous system numbered as in table 1. No attempt has been made to indicate the actual course of the axons into and away from the atrial system; in fact they enter and pass out via the visceral rami.

(i) *The innervation of the pterygial muscle and atriopore*

The cross-striated pterygial muscle which floors the atrium arises at metamorphosis by the apposition of flanges from the metapleural fold of either side; this process results in the muscle being divided into symmetrical halves by a median longitudinal connective tissue septum. In addition to this median division, there are more or less regular transverse septa within the muscle sheet of either side, representing the limits of multiplication of the original muscle fibres, formed as the atrium increases in size. At the anterior end of the atrium, the pterygial muscle fibres are connected with those of the velar sphincter; at the posterior end they surround the opening of the atriopore.

Over the atrial surface of the muscle there is a rich plexus of motor fibres, in which axons from different descending rami overlap to form a complex meshwork. A brief account only will be given of the general arrangement of this motor system, for it has been well figured and described by several previous authors, notably Heymans & van der Stricht, and Dogiel.

The descending rami of the dorsal root nerves (figure 1) pass on to the pterygial muscle in a segmental manner, and then ramify irregularly to form a dense plexus, (figures 14 to 17, plate 62).

Some unilateral denervation experiments which were performed showed that no axons cross the median septum from the descending rami of one side to the muscle sheet of the other side, for only that side of the pterygial muscle still in connexion with the central nervous system will contract on stimulation by light touch. Holmes's earlier attempts to trace the paths of degenerating fibres in his operated animals, had suggested the strict bi-lateral innervation of the pterygial muscle. Axons do however, cross the less distinct transverse septa, and those from one descending ramus may pass to the territory chiefly innervated by those of an adjoining ramus, so that there is a considerable overlap between segments.

This pattern of innervation reflects the 'total' nature of response of the muscle to stimulation; either no contraction is observed after light touch, or the whole area of the muscle contracts. This contrasts with the situation at the atriopore and just beside it (where the pattern of innervation is rather different), where local contractions can be observed after light touch with a glass hair. Considering the functions of the pterygial and atrioporal musculature, this difference in response is to be expected, for the former acts to expel water from the atrium when it contracts, and thus contractions of small areas would be of little effect; whilst the latter acts as a sphincter, regulating the flow of water through the atrium, and hence a more delicate degree of control is requisite. In the normal animal, both sides of the pterygial muscle contract simultaneously (whether the stimulus is light touch on the buccal cirrhi, or light touch at the atriopore), hence, since there is no overlap of axons across the median septum, there evidently must be a commissural system linking the viscero-motor tracts of either side in the cord.

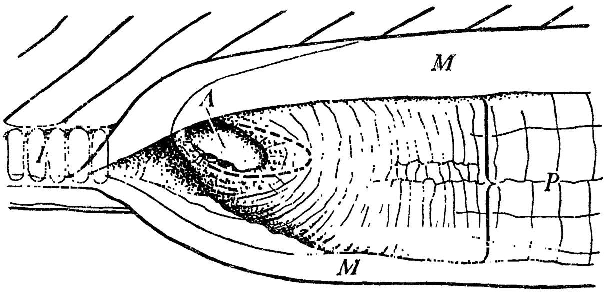

FIGURE 3. The external appearance of the atriopore, as seen in the living animal. The dotted line indicates the fully relaxed position of the atriopore. The muscle fibres to the left of the bracket show local contractions (see text). *A*, atriopore; *F*, ventral fin-ray boxes; *M*, metapleural fold; *P*, pterygial muscle.

Dennell (1950) and Holmes have given reconstructions showing the form of the atriopore in their fixed specimens, figure 3 illustrates the form of the atriopore as seen in the living animal.

The muscles of the atriopore regulate the flow of water through the atrium; in conjunction with the variations in activity of the gill bar cilia (Bone 1958*a*). The atriopore musculature is therefore directly involved in the mechanism of feeding of the animal, and in the reflexes of the feeding pattern. These reflexes appear to be of two kinds, differing in degree; they are here briefly described in order to indicate something of the mode of action of the pterygial musculature. The atrial floor contracts when different stimuli (touch, chemical) are applied to the sensory systems of the buccal and velar regions at the front end of the atrium, or to the sensory system of the atriopore lips. A single contraction, which gradually relaxes (after periods of a minute or more) results in the expulsion of water from the atrium, via the mouth if the anterior sensory cells are stimulated, via the atriopore if those of the atrial lips are stimulated. Clogging of the buccal cirrhi with detritus, or the contact of a large particle in the inhalant stream with the velar tentacles results in closure of the atriopore and a rapid pterygial contraction starting at the atriopore, forcing water out of the mouth. This 'cough' reaction, familiar to all observers of the living animal, has been described by Dennell in the Bermuda amphioxus. He suggested that it takes place at more or less regular intervals, but my own observations indicate that it is not in any way regular; it is simply evoked whenever the anterior sensory systems are stimulated sufficiently, resulting in a 'cleansing' of the buccal region of the animal.

More or less regular contractions of the pterygial muscle which have a similar result to the 'cough' reaction, can be observed in the apparent absence of special stimulation of the anterior end of the animal; they appear to form part of the normal feeding pattern, and to have rather a different basis from the 'cough' reaction.

When the animal is feeding in an algal suspension, the rate of flow of water through the atrium slows down at more or less regular intervals (in experiments at Plymouth, this was usually about every 10 to 16 minutes); the flow stops for a short period, the atriopore lips twitch slightly, and a contraction of the pterygial muscle takes place. The flow through the atrium then rapidly resumes its original rate. The basis for this type of contraction is clearly different from that resulting in the 'cough' reaction (there seems to be an analogy with the pattern of feeding seen in ascidians (Hoyle 1953); it is possible that sensory systems other than those of the buccal and velar regions may be involved.

Upon the whole of the atrial surface of the pterygial muscle itself there are many unipolar sensory cells (figures 16, 18, plate 62), which probably bear short processes projecting into the atrial cavity. These cells are very rarely seen in silver preparations, and stain after methylene blue some time before optimum staining of the pterygial motor plexus (which may explain why they have not previously been observed). In silver preparations where they are visible, they appear more or less oval, with a large vacuole lying just above the nucleus; the axon passes into a plexus of fine sensory fibres lying in a plane slightly above that of the motor bundles to the muscle, and then finally joins with a motor bundle to ascend to the dorsal root via the descending ramus. Silver preparations do not give an idea of the abundance of these cells except at the atriopore (where they more often are visible than elsewhere). In methylene blue preparations (figure 16, plate 62) a concentration of $100/\text{mm}^2$ is usual in the midregion of the pterygial muscle. This concentration is denser at the atriopore and at the extreme front end of the atrium. Probably this figure is too low, for it is likely that only a proportion of the cells stain after methylene blue. This is indeed suggested by the observations of Holmes, who found 'darkly stained masses within the epithelium and at the end of the axons, looking more like blobs of axoplasm than cells...'.

These he described from the region of the atriopore, and within the atrial papillae of that region; there can be little doubt that these masses were in fact sensory cells, and that in methylene-blue preparations they are difficult to stain within the atrial papillae. Holmes has suggested that these elements might form part of a local system, influencing effector organs by local paths not involving the central nervous system. So far as the atrioporal region is concerned, and almost certainly for the rest of the pterygial musculature, this is not the case. When the nerves to the atrial region are interrupted at the point shown in figure 1, the atriopore loses its sensitivity to light touch (which immediately provokes a contraction in an unoperated animal). The spinal cord must therefore mediate the reflex initiated by the sensory cells of the pterygial plexus; local reflexes are not found. In life, these sensory cells probably respond either to water flow through the atrium, or to light touch by suspended particles in the water stream.

To sum up, it appears that two distinct processes can result in the contraction of the pterygial muscle, and it is probable that different sensory systems are involved in the two responses. Further, the pterygial muscle normally contracts as a whole, contractions of small areas of the muscle being rarely observed; thus contrasting with the atrioporal musculature, where local contractions are often observed.

(ii) *The innervation of the parietal walls of the atrium and the gonad sacs*

The parietal wall of the atrium, formed by the inner side of the somite muscles, is more sparsely innervated than are the other parts of the atrium. There are many large nerve bundles and nerve fibres there, but only a few of these fibres are concerned with the innervation of the parietal wall itself, for most are simply crossing the walls of the atrium on their way to such destinations as the gut, gill-bars, and atrio-coelomic funnels. There is, however, a special sensory component associated with the parietal wall, consisting of unipolar sensory cells and their axons.

The segmental ascending rami (figure 1) ascend the parietal walls of the atrium and pass to the viscera, anteriorly via the denticulate ligament, more posteriorly, via the ligaments attaching the gut to the atrial roof. As a rule, these ascending rami lie under the atrial epithelium attached to the parietal wall until they reach these ligaments, but at times, as Legros (1902) remarked, at the top of their course they may cross the roof of the atrium in an incomplete septal fold, or even almost freely, so that they do not run right up to the top of the parietal wall of the atrium.

DESCRIPTION OF PLATE 62

The pterygial plexus.

FIGURE 14. General view of the pterygial plexus, showing the major part of two descending rami; they enter at the top of the figure and ramify towards the mid-line at the bottom. The darkly stained patches forming a row near the top of the figure are atrial papillae.

FIGURE 15. A part of the atrioporal innervation at the same scale to show greater density of nerve plexus. Atrioporal lips to right.

FIGURE 16. Pterygial plexus showing density of sensory cells (dark spots). The fibres of the plexus are very incompletely stained.

FIGURE 17. Detail of pterygial plexus. Note passage of fine fibres across muscle fibres (horizontal in figure); absence of end-formations.

FIGURE 18. Sensory cell from pterygial plexus.

FIGURE 19. Nerve plexus on walls of gonad sac. Note sensory cell (S), and cilia of atrial epithelial cells in focus at *C*.

16 and 18 from fixed methylene blue whole mounts, remainder from Holmes's silver whole mounts. 500 μ scale refers to 14 to 16.


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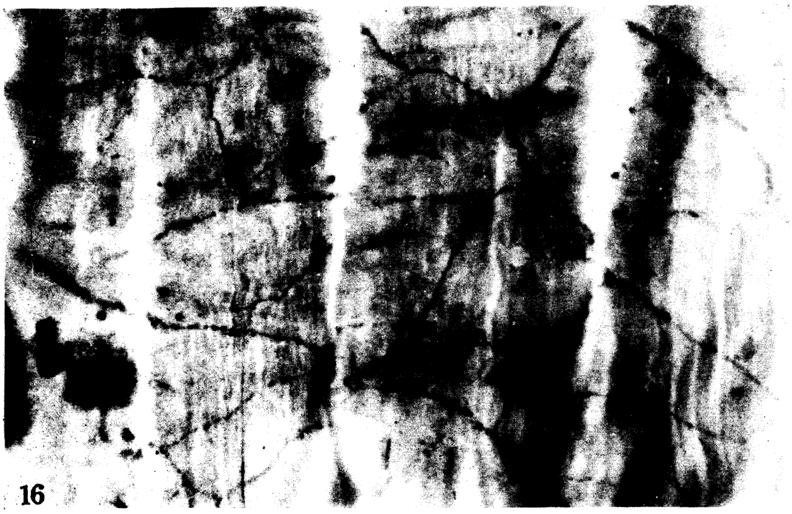
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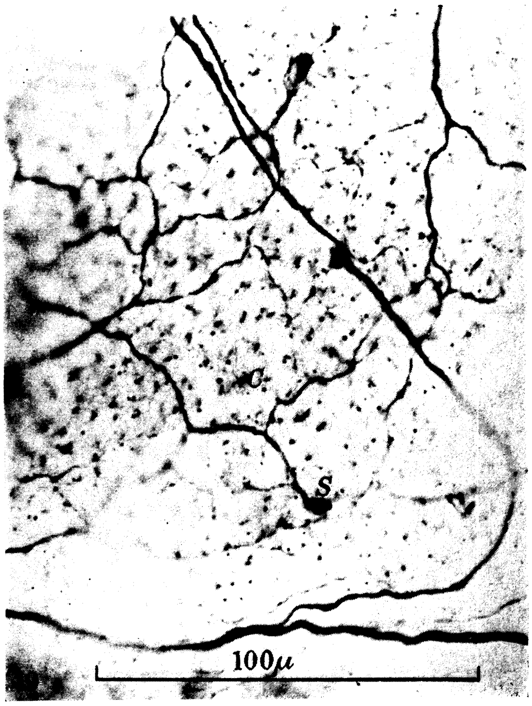

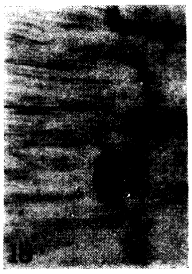

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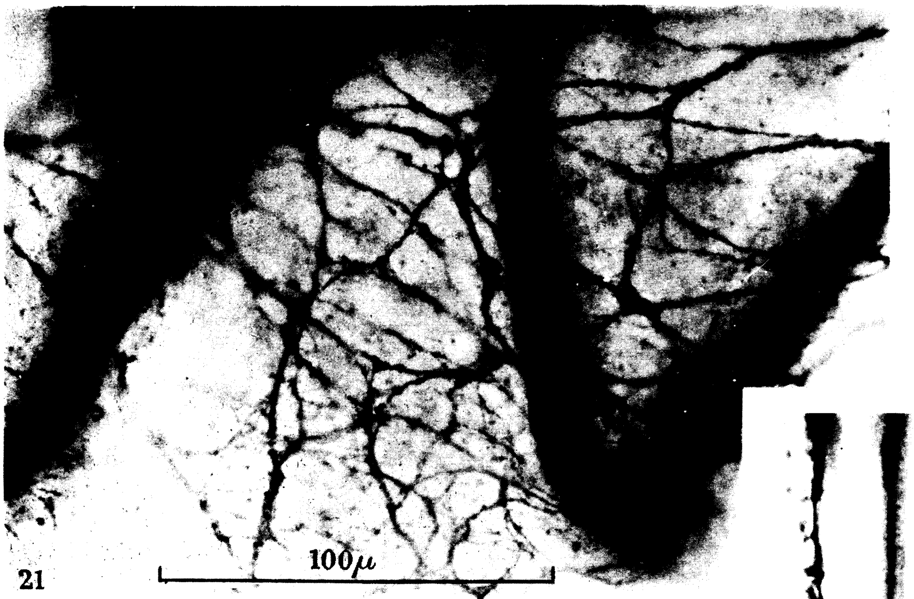

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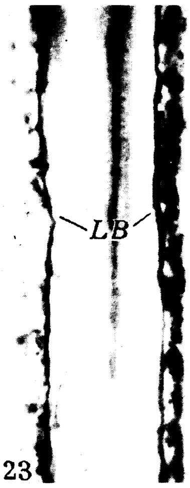

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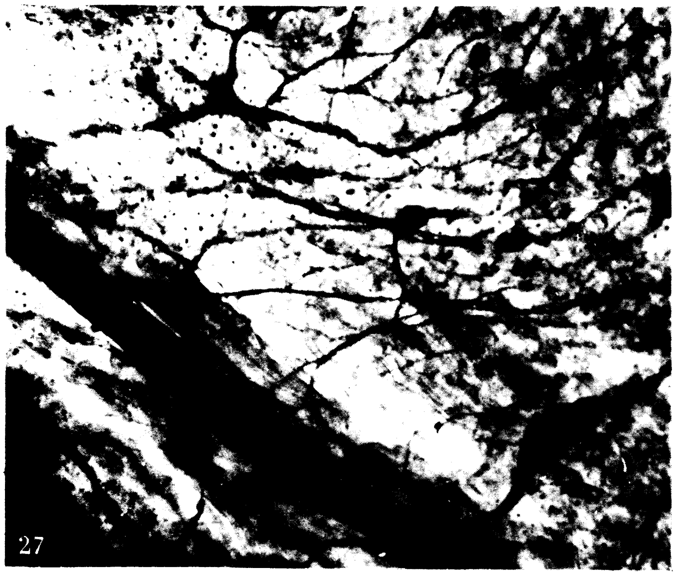

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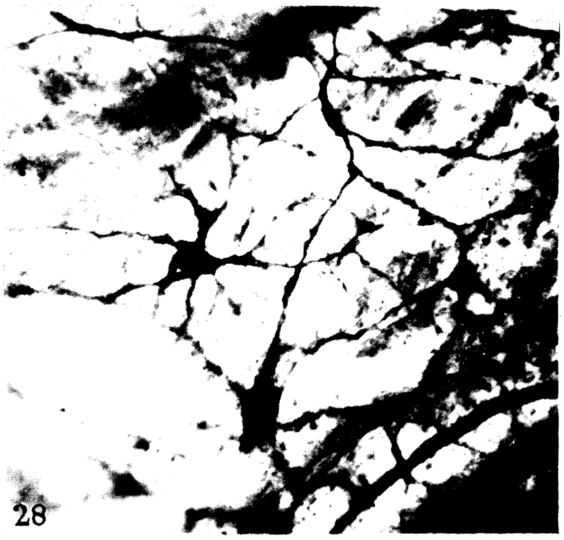

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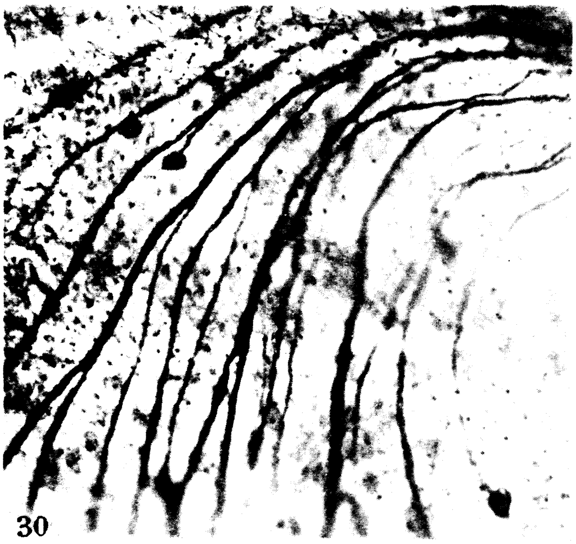

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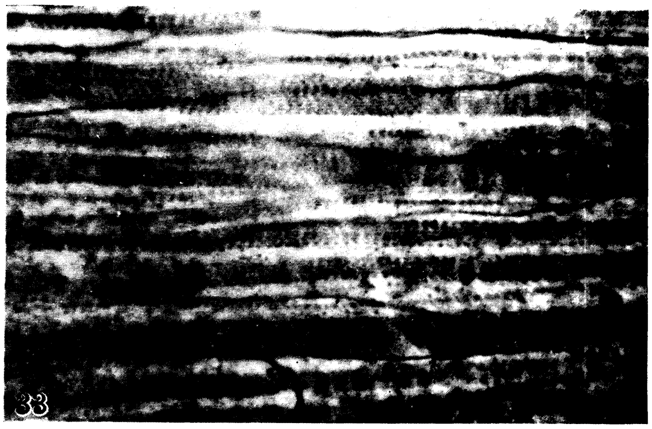

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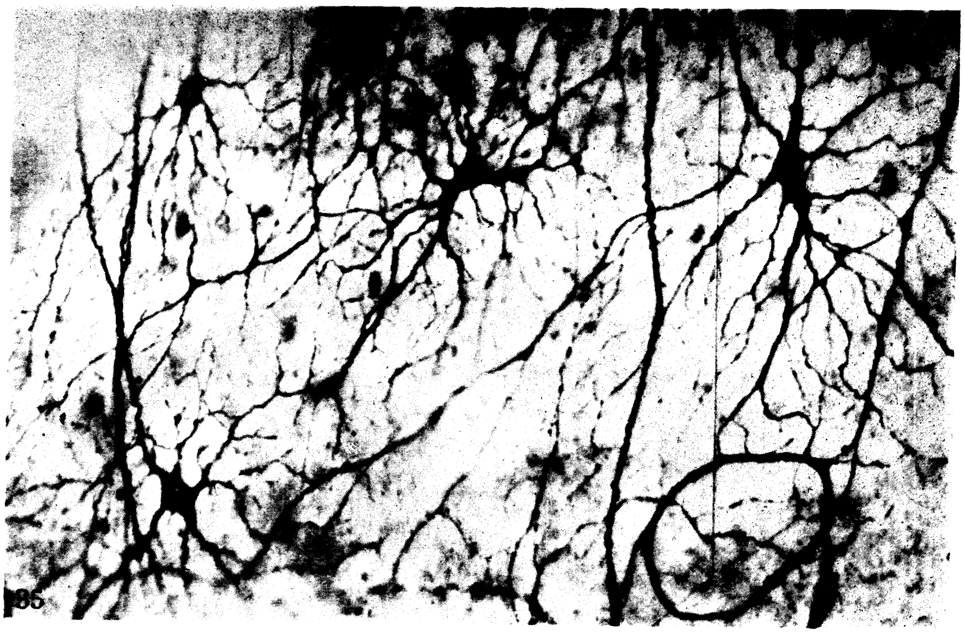
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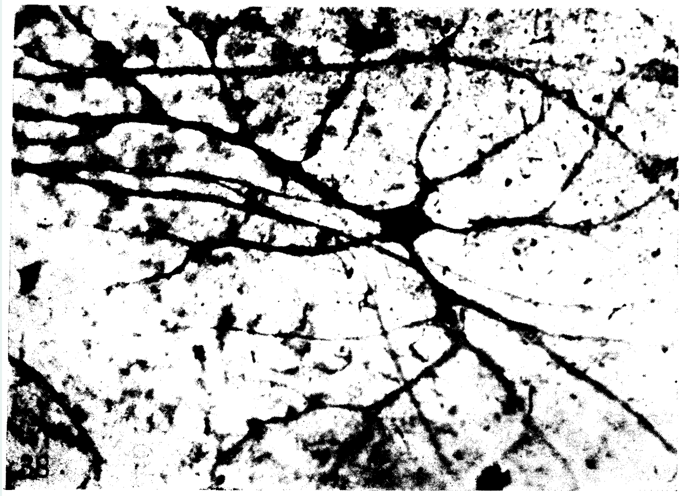
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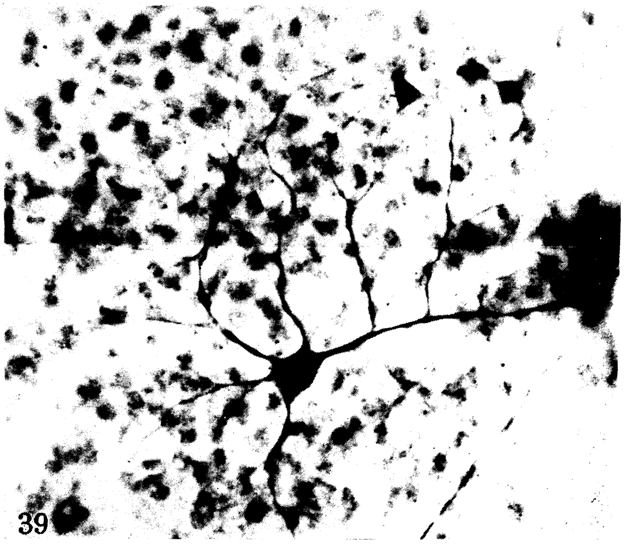
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As these rami pass up the parietal walls, they give off many branches (figure 4) which form a plexus on the upper part of the walls. From this plexus, fibres pass across to the viscera. Amongst the meshes of this plexus, and also lower down on the parietal walls, there are small unipolar sensory cells, much resembling those of the pterygial system. Sometimes their axons pass directly into ascending rami, at other times they pursue rather tortuous courses on the atrial walls, and the cell body itself may even lie so far down the parietal wall, as to be adjacent to the entry of the descending rami. Like the cells of the pterygial plexus, these sensory elements stain but rarely with silver. I have not observed them either on the walls of the posterior extension of the atrium, or upon the inner walls of the somite muscles in that region.

The similarity of these sensory cells to those of the pterygial plexus may argue similarity of function, but at present, it is perhaps best to refrain from attempting to interpret their function from their histological appearance. As will become clear in the later sections of this account, we know so little of the physiology and function of any of the various components of the atrial system, that any interpretation of the functions of particular elements from their histological appearance alone, is insecure. There is great need for functional analysis of the whole system.

The gonad sacs have a plexus of fine fibres upon them (figure 19, plate 62) derived from special branches arising from near the point of division of the descending and ascending rami. These fibres are especially numerous in *Asymmetron*, where a large branch supplies each gonad sac. The function of this plexus is not certainly known, but it seems reasonable to suppose that it is motor to the smooth muscle cells of the wall of the sac. It is known (Cerfontaine 1906; Conklin 1932) that amphioxus spawns only at sunset, and that many animals release their sexual products simultaneously. Spawning involves rupture of the gonad sacs, and release of their contents via the atriopore; thus it seems that some

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Description of plate 63

The innervation of the gill apparatus and the atrio-coelomic funnels.

Figure 20. General view of sub-endostylar plexus. The endostyle has been stripped from the atrial epithelium and the nerve plexus underlying it.

Figure 21. Enlargement of a part of figure 20, to show density of sub-endostylar plexus. The dark bars are the deeply stained bases of the skeletal rods of the primary gill bars (cf. figure 20).

Figure 22. The plexus upon the atrial face of the primary and secondary gill bars, showing the crossing of nerve fibres upon the synapticulae (X). Note thick bundles on the primary bar (PR) and the open plexus of the secondary bar (S).

Figure 23. Similar view to figure 22, but at deeper focus, to show lateral bundles of nerve fibres (LB).

Figure 24. Plexus of fibres around head of gill bar.

Figure 25. Multipolar cell from sub-endostylar plexus.

Figure 26. General view of entry of 28th ascending visceral ramus into the atrio-coelomic funnel of an adolescent specimen. 7 nerve cell bodies (N) are visible, as are some of their processes. Trapezius muscle fibres in focus at T. The rounded dots over most of the area of the field are the nuclei of the atrial epithelial cells composing the funnel.

All from Holmes's silver whole mounts. 500 μ scale refers to 20.

mechanism must exist for rupture of the sacs and extrusion of their contents when the animals receive the (unknown) stimulus for spawning. It may be suggested that the smooth muscle cells of the sac play some role in the release of the sexual products. I have not been able to observe any terminations upon the smooth muscle fibres of the sac wall, but the plexus is unlikely to be sensory, as peripheral cell bodies are rare on the gonad sacs.

FIGURE 4. An ascending visceral ramus on the parietal wall of the atrium. The plexus at the top of the figure is beside the denticulate ligament, and fibres from it cross to the gill apparatus. Sensory cells are more abundant than illustrated.

(iii) *The branchial and sub-endostylar plexuses*

Several of the early workers noted nerve fibres in connexion with the gill apparatus (e.g. Müller 1841; Rohon 1882; Fusari 1889), but it was once again Dogiel (1903) who gave the best description of the system. Kutchin's (1913) later account largely duplicated that of Dogiel.

Dogiel's work gave a picture of a plexus of fine nerve fibres upon each gill bar, connected to the ascending visceral rami via a plexus upon the denticulate ligament. Amongst the meshes of this plexus, he observed occasional multipolar cells. Cross-connexions between the bundle of axons of each bar were found at the synapticulae joining the primary and secondary bars. My own observations confirm those of Dogiel, but certain points may be added; in particular, the present account describes the arrangement of the fibres upon both types of gill bar in more detail, and amplifies Dogiel's description of the connexions of the branchial plexus with the ascending visceral rami. Anteriorly, the first few gill bars receive their innervation from *descending* rami (figure 13), for their heads are attached rather ventro-laterally to folds of atrial epithelium joined to the sheet covering the pterygial muscle. More posteriorly, the nerve fibres pass to the primary gill bars from the plexus on the parietal wall via the denticulate ligament (figure 5). The secondary bars receive their innervation indirectly, no nerve fibres passing directly from the parietal plexus to them; the plexus on the secondary bars is derived from fibres crossing from the primary bars across the synapticulae joining the two types of bar. There is some asymmetry in the fibre connexions of the hinder third of the gill apparatus, for the right-side gill bars (to which is attached the asymmetrical foregut diverticulum) carry in addition to the fibres destined for the branchial plexus, the axons of the sensory cells of the diverticular system. These different types of fibre cannot be distinguished histologically.

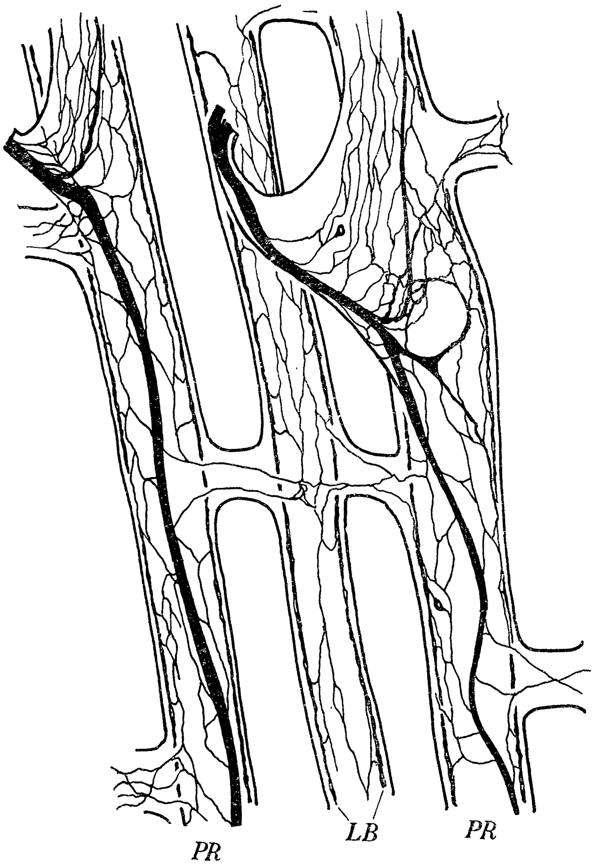

FIGURE 5. The passage of axon bundles on to the upper part of the primary gill bars (*PR*) from the denticulate ligament plexus. The heads of the bars are just above the figure. The smaller lateral bundles (*LB*) of nerve fibres on both primary and secondary bars are viewed by transparency.

The plexus of fine fibres observed by Dogiel is found on both primary and secondary gill bars, in addition, the primary bars carry a single large bundle of fibres (which subdivides towards the bottom of the bar). This large bundle pursues a characteristic sinuous course along the primary bar (figure 22, plate 63) for it curves round the expanded bases of the synapticulae linking the primary to the secondary bars. In cross-sections of the bars (figure 6), it is evident that the fine plexus and the thick bundles lie just underneath the atrial epithelium covering the atrial faces of the bars. Linked with this plexus, are two lateral longitudinal fibre bundles (figure 23, plate 63) which lie just at the junction of the atrial epithelial cells and the ciliary cells of the lateral ciliary tracts. The lateral bundles are formed by fibres which pass from the fine plexus around the coelomic space (on the primary bars), or half-around the skeletal rod (on the secondary bars). In the majority of my preparations, there are no other nerve fibres visible on the gill bars. That is, there are none under the ciliary tracts, nor upon the pharyngeal face of the bars. It is known, however, that the lateral tracts of cilia (though not the pharyngeal) are under nervous control (Bone 1958*a*) and it seems a reasonable assumption that many if not most of the fibres upon the gill bars are ciliary-motor in function, and that their connexions with the lateral ciliary tracts have not been impregnated. In occasional favourable preparations, fine branches can be observed passing from the lateral bundles towards the ciliary cells of the lateral ciliary tracts. In some instances, appearances such as that shown in figure 7 are observed. Rows of wedge-shaped cells lie along the gill bars, with their basal apices in connexion with fine fibres joining the lateral bundles, their expanded heads (sometimes of rather irregular shape) lying against the beginning of the lateral ciliary tract. It is difficult to reconcile the appearance of these cells in whole mounts, with any of the types of cell observable in sections, but it seems that they lie at the beginning of the lateral ciliary tract in the position shown on figure 6.

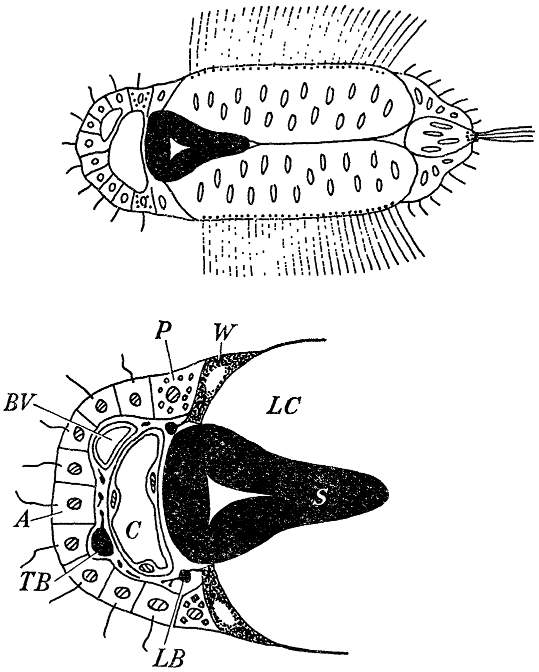

FIGURE 6. Diagrammatic cross-section of primary gill bar to show relation of nerve fibres to other structures of bar. The lower portion of the figure is an enlargement of the atrial face of the bar, seen on the left of the whole bar at top. The arrangement of the secondary bars is similar, except that they do not possess a septal coelomic space, nor thick bundles of nerve fibres. *A*, atrial epithelium; *BV*, blood vessel; *C*, septal coelomic space; *LB*, lateral bundles of nerve fibres; *LC*, lateral ciliary tract; *P*, pigmented cells; *S*, skeletal rod of bar; *TB*, thick bundle of nerve fibres; *W*, wedge-shaped cells.

These cells are too numerous and too regularly arranged to be interpreted as sensory cells (which they resemble); in my opinion, they are probably to be interpreted as some sort of ciliary-motor element. Since there seem to be no nerve fibres on the gill bars except upon their atrial faces, it is natural to imagine that the nervous impulse for ciliary regulation arrives at the atrial end of the band of ciliary cells and is propagated across the band through connexions between the ciliary cells themselves. The connexion of the wedge-shaped cells with the lateral fibre bundle, and their position at the beginning of the ciliary tract may imply that they play some role in this process. I do not believe that observations with the light microscope will assist greatly in solving such problems, but it is clear that an investigation with the electron microscope would yield results of much interest upon the question of the mechanism of nervous regulation of ciliary cells.

Occasional nerve cells are found at intervals throughout the branchial plexus, as Dogiel remarked; they vary in form but probably belong to two types. The first, multipolar or occasionally bipolar, was described by Dogiel. In my preparations, a second type of cell, unipolar, is more common. These are usually found at the top of the gill bars, near the nephridia, and at their bases, adjacent to the sub-endostylar plexus. Cells of the first type are similar to those found below the endostyle, and upon the gut; those of the second type resemble some of the cells of the atrio-coelomic plexuses. Both types of cell are relatively rare in my silver preparations (one may be visible upon every tenth bar examined), but they are probably actually much more abundant, as both Dogiel and Kutchin figured them; again, the silver method is inappropriate for the demonstration of these sensory elements. Nevertheless, it is probable that most of the axons of the branchial plexus belong to ciliary-motor neurons whose cell bodies lie within the central nervous system, and only a small proportion to peripheral sensory cells.

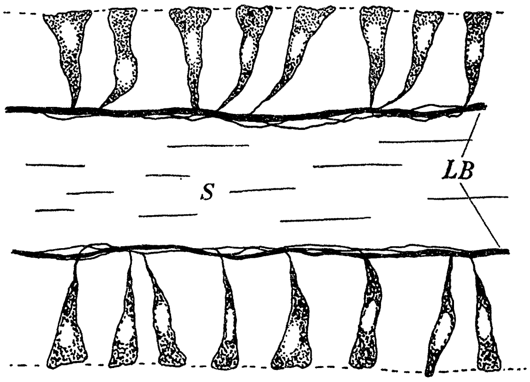

FIGURE 7. Wedge-shaped cells upon a primary gill bar, viewed from atrial surface, showing their connexions with the lateral bundles of nerve fibres (*LB*). Drawn from a whole mount in which the lateral ciliary tracts were unstained. *S*, skeletal rod of bar.

The primary gill bars are attached to the denticulate ligament (and receive their innervation) some way below the heads of the bars. A proportion of the fibres entering the gill apparatus therefore pass *upward* after entering the branchial plexus, and innervate the upper parts of the gill bars (figure 5). A dense meshwork of nerve fibres (figure 24, plate 63) surrounds the hemisphere formed by the top of the bar. Some of these fibres probably innervate the smooth muscle fibres which join adjacent bars and are no doubt partially responsible for the recovery of shape of the gill apparatus after its deformation by contraction of the pterygial muscle. Others are ciliary-motor to the lateral ciliary tract, which is continuous from primary to secondary bar around the heads of the bars. It is possible that other fibres may supply the nephridia, which also lie in this region, but although fibres pass close to the nephridia, it is not certain that these organs are actually receiving nerve fibres from the branchial plexus. Occasional sensory cells are found in the region, their function is not known.

At their bases, the gill bars are attached to the endostyle, and the branchial plexus is in connexion with the sub-endostylar plexus lying just under the layer of atrial epithelium which covers the endostyle ventrally. Something of the richness of the sub-endostylar plexus is seen in figures 20 and 21, plate 63. It contains occasional multipolar nerve cells (figure 25, plate 63), which resemble those of the foregut, and thus, although connected with the branchial plexus, may be functionally distinct from it. The branchial plexus seems to be predominantly ciliary-motor, whilst the cilia of the endostyle do not appear to be under nervous control. Perhaps the sub-endostylar plexus is chiefly concerned with the regulation of mucus secretion by the endostyle, but no connexions have been observed between the plexus and the gland cells of the endostyle. Indeed, if the atrial epithelium is stripped off the endostyle (as in figures 20 and 21, plate 63) the sub-endostylar plexus in its entirety comes off with it; if there are connexions beween the plexus and the endostyle, they are below the limit of the light microscope, or do not stain. I am unable to say whether the cell bodies in the sub-endostylar plexus belong to motor or sensory neurons. They resemble those of the gut plexus which are assumed to be sensory.

To the posterior gill bars of the right side is attached the diverticulum of the foregut. The diverticulum joins with each primary bar by a thin tube of epithelium (formed as the diverticulum pouches forward in development); across these tubes pass the axons derived from the cells of the diverticular plexus. Axons therefore pass into the branchial plexus from the diverticular plexus in a regular way, but since axons course up and down the diverticulum between the attachments to the bars, they pass in to the branchial plexus both segmentally and non-segmentally. Anteriorly, the atrial epithelial sheath that covers the diverticulum runs forward past its tip, as a narrowing tube, and finally terminates a few bars farther ahead. In this anterior end of the atrial sheath there are about 20 axons. Occasional nerve cell bodies are seen upon the tubes of epithelium joining the diverticulum to the gill bars, but they are rare, possess no dendrites, and are most probably to be interpreted as cells about to enter the diverticular plexus, rather than as cells belonging to the branchial plexus.

Arrangements within the diverticular plexus will be described in a subsequent section; this brief account of the connexion between the diverticular and branchial plexuses has been given at this point in order to explain the asymmetry of axon numbers upon the more posterior gill bars.

(iv) *The innervation of the atrio-coelomic funnels*

These enigmatic organs were first discovered and described by Lankester (1875, 1889). They consist of small pouches of atrial epithelium projecting into the dorsal coelom at the level of the hinder end of the gill apparatus. Each funnel is overlain by a thin sheet of striated muscle fibres, inserting at one end into the connective tissue sheath of the notochord, at the other, into the midgut wall. This muscle was discovered by Schneider (1879) and named the trapezius muscle by Legros (1902). Large branches of the ascending visceral rami of the 28th spinal nerves pass to these organs, and were observed by Legros and by Franz (1927). The arrangement of the organs, and their relations with other organs in the atrium is seen in figure 8. An essentially similar arrangement is found in *Asymmetron*.

Lankester thought that at their anterior ends, the funnels were open to the dorsal coelom, and so suggested that they might function to equalize pressures between this cavity, and that of the atrium. This suggestion did not meet with general acceptance, and it now seems most probable that the anterior ends of the funnels are in fact blind (Holmes 1953). Zarnik (1904) and Franz concluded that the trapezius muscle functioned to alter the shape of the funnels (Zarnik stated that he observed these changes in shape in the living animal), but what these changes in shape might mean, and what the function of the organs might be, remained obscure.

Boeke, in his description of the 'autonomic' of amphioxus, made the interesting observation that there were many multipolar nerve cells associated with the trapezius muscle. These nerve cells he supposed to be motor to the muscle, stating 'the processes of these ganglion cells were in connexion with the muscle fibres, either by means of small end-rings, or by very delicate reticula and end-nets, which seemed to pass onto the substance of the cross-striated muscle fibres, but then became invisible and could not be followed any further'.

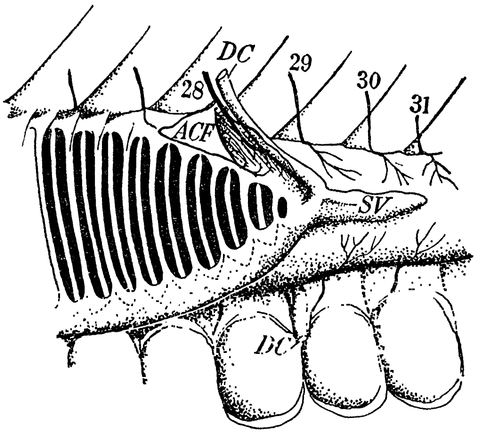

FIGURE 8. An atrio-coelomic funnel seen from below after removal of pterygial muscle. Anterior to left. *ACF*, atrio-coelomic funnel; *DC*, ductus Cuvieri; *SV*, sinus venosus; 28 to 31: ascending visceral rami sending branches to funnel and to foregut.

Boeke also found similar multipolar nerve cells in a dense plexus on the gut, and concluded that both types of cell were motor, the one to striated, the other to unstriated muscle fibres. When Holmes reopened the question by suggesting that the nerve cells of the gut plexus were sensory cells, it was natural for him to re-examine the neurons of the atrio-coelomic funnels. Observing a number of possible sensory terminations in the atrial epithelium of the funnel, he concluded that these organs were probably sensory structures, perhaps sampling the water within the atrium. Holmes emphasized the richness of the innervation of the funnels, and the numbers of nerve cells within the atrio-coelomic plexus, and stated '...the cell bodies of the neurones lie in, or slightly below, the atrial epithelium (*of the funnel*) the axons lie between it and the fibres of the trapezius muscle, and very likely some innervate the muscle, though I have not seen any special end-plates'. Holmes's view was that some at least of the vast numbers of neurons associated with the funnels were sensory elements, whilst others were possibly motor to the fibres of the trapezius muscle.

In sections of the region (which both Boeke and Holmes used) it is not possible to make out much of the connexions or relations of the atrio-coelomic plexus. The present account is based chiefly upon silver-impregnated whole mounts of the funnels dissected out after impregnation of the whole of the portion of the animal containing them.

A general view of such a preparation is seen in figure 9. There are great numbers of nerve cells associated with the funnels and trapezius muscles. Their number is so great that at first it appeared almost incredible that they could be nerve elements at all, and

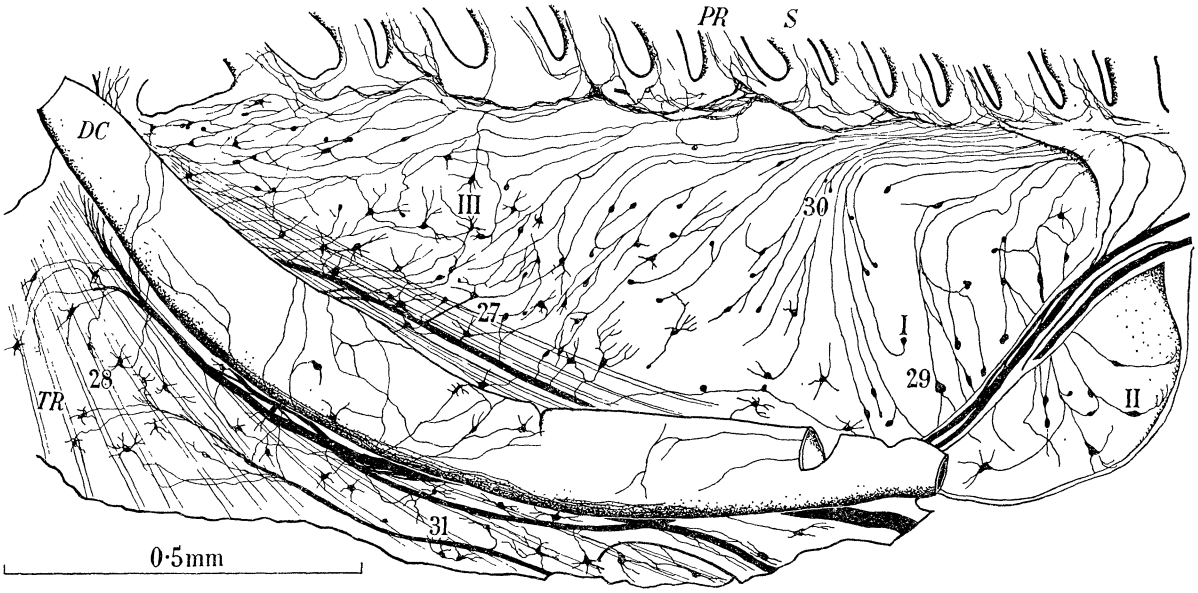

Figure 9. Left atrio-coelomic funnel (enlargement of same funnel as shown in figure 8). The number and position of the neurons in the plexus are shown, but their finer processes have not been drawn. *DC*, ductus Cuvieri; *PR* and *S*, primary and secondary gill bars; *TR*, trapezius muscle fibres; I, II, and III, types of nerve cell within plexus. The figures 27 to 31 indicate the sites in the funnel whence figures 27 to 31, plate 64, were taken (not all from this dissection).

Description of plate 64

The innervation of the atrio-coelomic funnels. The sites in the funnel whence these details were taken are shown in figure 9, above.

Figure 27. Cells of types I and III showing dense network of processes.

Figure 28. Multipolar type III cells upon trapezius fibres.

Figure 29. Type II cells, and groups of cells with little cytoplasm at the anterior end of the funnels. These may perhaps be interpreted as developing cells.

Figure 30. Type I cells and the axon entry at the anterior end of the funnel beside the gill bar bases (above top of figure).

Figure 31. Type III cells with many processes forming a dense network.

Figures 32 and 33. Nerve plexus upon trapezius fibres. Nuclei visible in 32 belong to atrial epithelial cells.

All from Holmes's silver whole mounts.

not connective tissue cells, but comparison with the undoubted nerve cells of the gut, and recognition of their connexion with the nerve trunks, makes it certain that they are actually nerve cells. Some of these cells are seen in figures 27 to 31, plate 64.

It is not easy to be certain upon the point, but it seems that these neurons may be divided into three morphological types. At the anterior blind ends of the funnels, and also less frequently elsewhere in the plexus, there are small unipolar nerve cells (8 to 10 μ across the cell body). The axons of these type I cells can sometimes be followed to the collecting rami of the branches of the ascending visceral rami associated with the plexus, and thus they seem to be sensory cells sending their axons towards the central nervous system. A second type of cell, type II, is also found at the anterior ends of the funnels. These cells are much larger (25 μ along the length of the cell body), and are essentially bipolar elements. Their peripheral processes branch at some distance from the cell body into a fan of dendrites, which end apparently freely amongst the atrial epithelial cells forming the funnel. The type II cells that lie near the anterior blind ends of the funnels are arranged so that their peripheral processes curve around the end of the funnel, whilst their axons pass posteriorly and dorsally to join the longitudinal collecting rami in which they are lost.

In some respects similar to these type II cells, are other cells found chiefly at the open posterior ends of the funnels, lying over the trapezius muscle. These type III cells bear many dendrites (usually 5 to 7), which radiate from the cell body in a more or less symmetrical manner, and connect with the processes of neighbouring cells. Very few of these dendrites end freely, and even where it seems that they do, this probably indicates that impregnation has been incomplete; it is probable that the dendrites of the type III cells always end in relation to the processes of other cells.

These cells are very similar to those found in the gut plexus (as Boeke recognized), not only in form, but also, for example, in the occasional presence of cells with 2 nuclei in

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Description of plate 65

The gut plexus.

Figure 34. Multipolar neuron from top of diverticulum, its processes directed towards the side of the diverticulum.

Figure 35. Several multipolar neurons from plexus upon mid-gut. Note axon loop, and interconnexion of processes. Apparent free endings actually join with other processes but are out of the focal plane.

Figure 36. Multipolar neuron from top of diverticulum as it joins the gill bars (to right).

Figure 37. Neuron from mid-gut region. Note great extent of the dendritic processes, many of their interconnexions are out of the focal plane. The axon of the cell runs out of the figure to the left.

Figure 38. Neuron from diverticulum, axon to left. Few other neurons in this area, probably resulting in the expansion of the cell body.

Figure 39. Neurons from diverticulum. A large neuron (its axon passing to the right) is in connexion with smaller neurons at upper right.

Figures 36 and 39 from 35μ sections impregnated by Palmgren's silver method, remainder from Holmes's silver whole mounts.

either plexus. In the gut plexus, where cell bodies are not so abundant as they are in the atrio-coelomic plexus, it is often possible to identify the axons of the neurons, and to follow them towards the ascending visceral rami. In the atrio-coelomic plexus, cell bodies lie so close together (chiefly those of types II and III), and there are so many processes in a small area, that only rarely can the axons of the neurons be identified. However, the general impression gained is that as in the gut plexus, the neurons of the atrio-coelomic plexus send their axons to the central nervous system.

Where cells of types I and II are found amongst the more numerous type III cells, it is clear that they are morphologically different from one another. This is not to say that these differences must reflect differences of function between the three types of cell (although this is perhaps probable), since it is just possible that cells of types I and II may represent stages in the development of the type III cells. The observations refer only to adult animals, but with our present knowledge of the elements of the atrial nervous system, we do not know whether it is reasonable to assume that it may alter during the adult life of the animal by the migration and alteration of neurons. Figure 26, plate 63, shows the entry of the 28th ascending visceral ramus onto the atrio-coelomic funnel of an adolescent specimen, probably under two years old. Seven neurons only are visible, and these lie over most of the area of the funnel, which is smaller than in an adult specimen. Evidently, between this stage, and that of the larger fully adult animal, the funnels increase in size, and the atrio-coelomic plexus increases in numbers of neurons. It is noteworthy that in the young animal all the neurons seem to be of type III.

The muscle fibres of the trapezius (figures 32 and 33, plate 64) are long and thin, coarsely cross-striated, and bear large oval nuclei laterally. They resemble the fibres of the anal sphincter, or those fibres of the pterygial muscle found at the extreme anterior end of the atrium behind the velum, but they are quite unlike the fibres of the somite muscles. Neither Holmes nor Boeke were able to satisfy themselves definitely as to the innervation of these trapezius muscle fibres. Unfortunately, I am unable to dispel this uncertainty.

The difficulty is that the nerve fibres and cells of the atrio-coelomic plexus lie between the funnel and trapezius muscle, and it is not at all easy to see with which layer the cell processes relate. Furthermore, as there are no special end-formations upon the trapezius fibres, it is not certain *which* of the nerve fibres running on the muscle do in fact innervate them. I have never been able to trace the processes of any of the three types of neuron actually onto a muscle fibre of the trapezius. There are two possibilities, for each of which there is some evidence. First, all the neurons of the plexus may be sensory, in which case the trapezius muscle fibres must be innervated by axons passing into the plexus from central cells. Secondly, some of the cells of the plexus may be motor to the muscle.

On the whole, I have come to favour the first alternative. If any of the types of neuron are motor, then all that can be said is that they are indistinguishable from the few of each type whose axons are traceable as they pass toward the central nervous system.

The trapezius muscle has much the same structure as the pterygial muscle, presumably being derived in ontogeny from the common source of the lateral plate mesoderm; hence it seems reasonable to suppose that it is innervated in a similar manner to the pterygial muscle, that is, by the axons of central cells. At no other site within the atrium are there nerve cells whose motor nature can be ascertained. I conclude therefore, that all the neurons within the atrio-coelomic plexus are sensory cells of various types, and that the trapezius muscle fibres are innervated by the axons of central cells.

Holmes's suggestion, that the atrio-coelomic funnels are water-sampling organs, collecting information about the water within the atrium (e.g. chemical composition, particle content etc.), is a reasonable one, but it is difficult to understand why the animal should require a relatively complex organ at this position within the atrium to obtain such information. There are many other sensory systems apparently concerned with conditions in the water stream flowing through the atrium. Indeed, this is one of the puzzling aspects of the atrial nervous system as a whole, there is an *embarras de richesse* of sensory elements making it hard to disentangle what the function of each may be.

These sensory systems contain very many peripheral nerve cells, and (as Alexandrowicz (1960) remarked of cephalopods) 'there appears to be everywhere...a profusion of nerve elements, one might say, an uneconomical use of nervous substance...'.

Associated with the atrio-coelomic funnels, and conveniently considered as a part of the atrio-coelomic system, are the large vessels of the ductus Cuvieri and heart. The ductus Cuvieri pass inwards from their origin from the lateral vein at the level of the gonads, and join to form a 'sinus venosus' in the midline just behind the origin of the gill bars. Nerve bundles follow the ductus Cuvieri to pass onto the gut (they are mainly composed of ascending axons derived from the cells of the gut plexus), and the large 28th ascending visceral ramus supplying the atrio-coelomic funnels also passes along on or near the ductus Cuvieri. Small bundles are given off from it to the funnels as well as the larger recurrent rami and the large bundles to the posterior open ends of the funnels. Figure 9 illustrates the course of these rami and the position of the ductus Cuvieri. The walls of these vessels are thus covered with nerve fibres, amongst which there are frequently scattered cells of type III. It seems unlikely that these cells have any specific function related to their position upon the vessels. Perhaps there may be vaso-motor fibres upon the larger vessels, but I have never observed endings related to their walls. Ayers (1921) stated that vaso-motor fibres derived from a special ramus of the ventral root nerves pass to the larger vessels. This statement has been accepted, and is to be found in the literature in such discussions as that of Goodrich (1930); Nicol (1952); and Johnels (1956). The fibres which Ayers observed to pass out of the ventral roots are very probably somatic-sensory proprioceptive fibres, passing to the somite muscles (Bone 1960) and it is most unlikely that the ventral root nerves contribute anything to the atrial nervous system.

(v) *The innervation of the foregut and diverticulum*

Although the nervous tissue upon the diverticulum and upon the portion of the gut within the atrium is the best-known part of the whole atrial system, thanks to the accounts of Boeke and Holmes, there is still much that is perplexing about this system of neurons, and it must be admitted that the conclusions that have emerged from the work of these authors are not quite so clearly defined as they might be.

The interconnexion of these neurons, for example, is still puzzling, and nothing is known of their function. In the present account, since I have already discussed the results of Boeke and Holmes in some detail (Bone 1958*b*), I shall confine myself to a brief description so as to permit comparisons between this and other parts of the atrial system, and to reporting new observations which have been made since the publication of the earlier account.

The entire diverticulum and 'atrial' portion of the gut are covered with an extremely dense meshwork of nerve fibres amongst which lie many nerve cell bodies (figure 10). All workers on this subject have been impressed by the abundance of these neurons. Van Wijhe (1913) described the system as containing 'an enormously large number of splendid multipolar cells'; Boeke (1935*a*) spoke of the 'quite astonishing abundance of stellate multipolar nerve cells'. That these impressions were not exaggerated can be seen from the excellent figure of Holmes (his figure 1) and from the figures of the present account. A typical concentration of cell bodies is 150/mm^2, a density greater than in any part of the atrial system except the atrio-coelomic plexus. The cells vary in size, from small cells 8 to 10 μ across the width of the cell body, to cells as large as 50 μ across the cell body; they also vary a good deal in form. However, they all appear to belong to the same type. Almost all are multipolar, with 5 to 7 branching dendrites spreading out from the cell body (figures 34 to 39, plate 65), the dendritic processes cover a large area, they may spread up to 200 μ from the cell body itself. The axons of the cells pass away from the cell body (this will be discussed below), to the collecting rami of the appropriate ascending visceral ramus, and

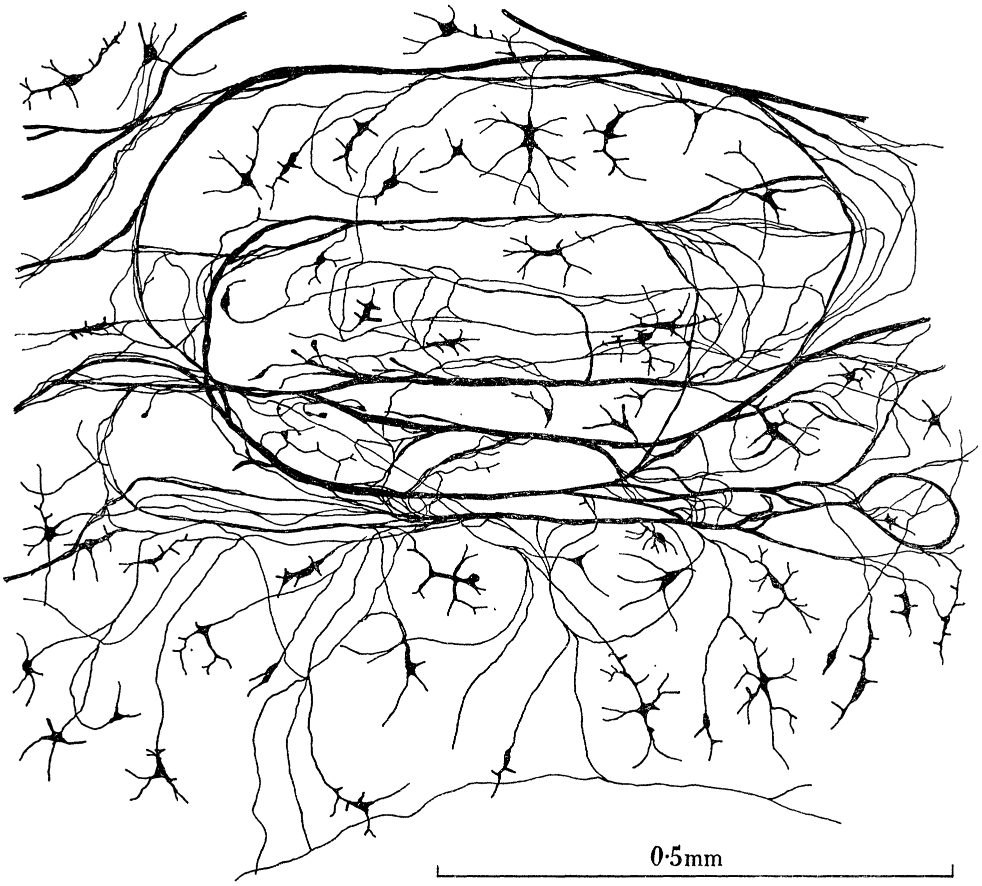

FIGURE 10. The course of axon bundles and the positions of cell bodies upon the roof of the mid gut. An ascending ramus passes from the gut just above the figure. The number and size of the nerve cells are shown, their finer processes have not been drawn.

run towards the central nervous system. Processes similar to the dendrites are given off along the course of the axon, even at a considerable distance from the cell body. Both 'axonic' and dendritic processes connect with the processes of neighbouring cells. This connexion is of a remarkable kind, for as will be seen, it seems that the processes of neighbouring neurons actually fuse together asynaptically. For this reason, I have avoided terming the system a *plexus*; yet it is not correct to describe it as a nerve net in the strict sense of the *Nervennetz* of earlier workers (see Beccari (1943)), for axons leave the system to pass toward the central nervous system. The gut neurons will therefore be described under the non-committal title of the 'gut system'.

In well-impregnated preparations, it is difficult to find freely ending dendritic processes. Where they do appear to end freely, it is likely that impregnation has been incomplete; only very occasionally do dendritic processes terminate in *dendritlamellae* in the manner Boeke illustrated. The appearance of the junctions between neighbouring neurons is such that it is easy to see why Boeke supposed that there was actual fusion between the processes of neighbouring neurons; he supposed, that is, that all the neurons of the gut system were so intimately connected that they were in actual cytoplasmic continuity, anastomosed together. The gut system of amphioxus, in fact, looks remarkably like the classical pictures of nerve-nets (if the axons are not considered) where neurons were supposed to be fused together in a continuous network.

Despite the fact that every example of a nerve-net hitherto suggested has not retained this status upon further investigation, it has seemed to me worth emphasizing that upon *histological* grounds, it is not possible to exclude Boeke's view of cytoplasmic continuity between the neurons of the gut system. The closest examination of the most successfully impregnated preparations has failed to convince me that the junctions between neighbouring cells are synaptic rather than asynaptic; on the whole, I believe (with some reluctance) that the neurons of the gut system are in cytoplasmic continuity with each other.

It is unreasonable, however, to support such a view upon histological grounds alone, and further investigation, both physiological, and with the electron microscope, will be required to determine the true nature of the interconnexion of the neurons of the system. The assumption that they may be in cytoplasmic continuity does not raise any functional difficulties, for we know nothing of the function of the gut system, save that it is probably sensory. The evidence for this conclusion will be discussed below.

Although Boeke supposed that, one with another, the neurons of the gut system were fused together asynaptically, he imagined that synaptic contacts were made upon them by the fibres of central cells, passing into the atrial nervous system from the central nervous system. He regarded the neurons of the gut system as motor, innervating smooth muscle cells of the gut wall. Holmes questioned this interpretation, and showed that the axons of the gut neurons themselves probably passed into the central nervous system, hence, that the gut neurons were sensory elements. My own whole mounts confirm this conclusion, but the degeneration experiments reported in the previous account (which it was supposed gave decisive proof of this conclusion), I have now to admit did not actually do so. At the time when they were carried out, it was supposed that the only peripheral neurons in the region of the atrium served by the interrupted dorsal root nerves, were those within the gut system. The discovery of the pterygial sensory cells (§ i), whose axons run in the same nerves, means that some, at least, of the centrally degenerated fibres must belong to the latter cells.

Definite proof that the gut system neurons send their axons into the dorsal roots can therefore only be obtained by interrupting their axons before they join with those of the pterygial sensory cells, i.e. before the junction of ascending and descending rami. Unfortunately, this junction lies within the atrium itself, and it would be extremely difficult to perform such experiments. Nevertheless, the evidence from whole mounts, where axons can in many instances be traced from the cell body for long distances towards the ascending rami, and the complete absence of degenerated fibres within the gut system after the degeneration experiments, make it almost certain that the neurons of the gut system send their axons to the central nervous system, and are sensory elements.

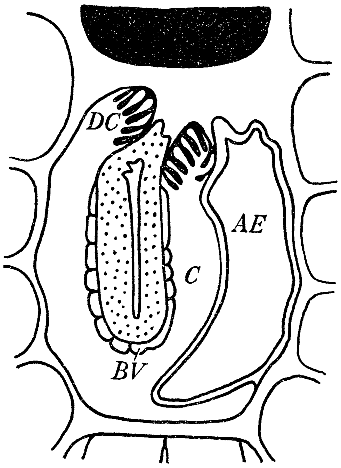

Figure 11. Cross-section of hindgut region (simplified after Franz 1926). Gut dotted. *AE*, atrial extension; *C*, coelom; *BV*, blood vessels; *DC*, dorsal cells.

The function of this rich sensory system is obscure. It is conceivable that it may play some part in the regulation of the cyclical activities of the gut in feeding, possibly giving information as to the amount of food collected in the gut. There does not appear to be any regional differentiation of the gut system, which might afford a clue as to its function, except that the post-atrial hindgut is much more sparsely innervated (§ vi), and this region is concerned with absorption rather than digestion (Barrington 1937).

(vi) *The innervation of the hindgut*

The hindgut lies outside the atrium, beside the tube of atrial epithelium which forms the asymmetrical post-atrioporal atrial extension. A good account of the anatomy of the region has been given by Franz (1926); figure 11 (simplified from one of his figures) will recall the topography. The hindgut itself depends from the roof of the coelomic space which encloses it, it is not covered with atrial epithelium. Along its ventral and lateral walls there is a plexus of blood vessels, which are probably concerned with the transfer of the food substances which are absorbed in this region. Beside the hindgut, on the right side, lies the post-atrioporal extension of the atrium, which runs back so far as the level of the anus, where it ends blindly. Information about the innervation of this region is scanty. Dogiel and Kutchin described a plexus of fine nerve fibres on the rectum, and around the anus (where the cross-striated muscle fibres of the anal sphincter are innervated by rami from left-side dorsal root nerves, since the anus opens asymmetrically). Heymans & van der Stricht described nerve fibres upon the parietal and visceral walls of the mucosa behind the atriopore, and these fibres Kutchin believed to pass to the hindgut and atrial extension, but neither workers traced these fibres to their terminations. Holmes confined his observations to the foregut region, for he found in his silver preparations that impregnation of nerve elements in the hindgut region was interfered with because the cells of the post-atrioporal extension of the atrium were loaded with pigment which took up silver. This difficulty was also found by the present writer, and in addition, it was found that the curious cells projecting into the coelom around the dorsal part of the hindgut (figure 11, *DC*) take up silver deeply and overlie and obscure views of the nerve fibres upon the gut. Nevertheless, I have obtained good results both with whole mounts, and with thick frozen sections, and am able to add some points to previous accounts.

First, some of the fibres in the ascending visceral rami of the hindgut region, are the axons of multipolar neurons lying on the hindgut. These neurons resemble those of the gut system, but they are much less numerous in my preparations, and since they are widely spaced upon the hindgut, their processes are more symmetrically disposed around the cell body than are those of the neurons within the gut system. The dendrites of the hindgut neurons have not been fully impregnated, and I cannot say whether they normally terminate freely or join with the processes of neighbouring neurons. On the whole, since the ascending rami to the hindgut region are much smaller than those to the foregut, and since (as will be seen below) there appear to be free nerve terminations upon the hindgut, I am inclined to think that the sparsity of cell bodies on the hindgut does not result from incomplete impregnation. It is unsafe to base conclusions as to the absence or rarity of elements upon preparations made with a single silver method, but it does seem probable that the system on the hindgut contains many fewer neurons than that of the foregut.

Secondly, the general appearance of the innervation of the hindgut (figure 12) is rather different to that of the foregut because, in addition to the rarity of cell bodies on the hindgut, the nerve fibres of the ascending visceral rami connected with the hindgut, pass onto it in a different manner to those in connexion with the foregut system.

On the foregut, the axons of the sensory cells of the system fan out from the point of entry of the ascending ramus onto the foregut, running downwards from this point over the foregut. On the hindgut, nerve fibres do not run downwards around the hindgut at each point of entry of an ascending ramus, but instead, they run anteriorly and posteriorly parallel to the axis of the gut. Fibres derived from anterior rami may pass so far along as the anus without terminating or branching. Whilst running along the upper third of the gut in this way, in a roughly parallel loose bundle, fibres may loop and recurve and join with other small bundles, so that dorsally, the system is made up of more or less parallel fibres, interconnected by loops and curving cross-connexions (figure 12). From this loose dorsal plexus, occasional fibres pass downwards to innervate the more ventral portions of the hindgut. Sometimes, these ventrally directed fibres seem to terminate in small irregular loops, at other times, they are seen to be the axons of multipolar neurons.

At the anus, conditions are more complex. Fibres from the left dorsal roots of the region supply a plexus on the walls of the anus, which must be partially, at least, motor to the cross-striated muscle fibres of the anal sphincter. The fibres of this muscle resemble those of the trapezius. Some of the fibres from these 'anal' rami pass a short distance anteriorly along the rectum (figure 12), amongst the fibres derived from ascending rami in connexion with more anterior regions of the hindgut, but they then curve back and rejoin the anal plexus; they take no part in the innervation of the hindgut. I have not observed any special sensory terminations at the anal lips, but this is not to say that they do not exist there; on the whole, it would seem probable that a part of the anal plexus is sensory, perhaps concerned with the activity of the anal sphincter muscle, but this point requires further investigation. The ciliary tracts of the rectum and anus beat continuously, and it is unlikely that the innervation of the hindgut region includes a ciliary-motor component.

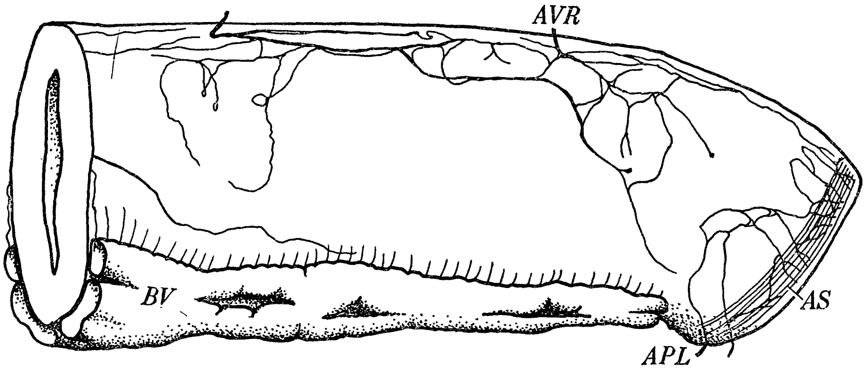

FIGURE 12. The innervation of the rectum. *AVR*, exit of ascending visceral ramus; *APL*, entry of anal plexus nerve fibres; *AS*, anal sphincter muscle; *BV*, blood vessels.

The tube which forms the posterior extension of the atrium is composed of thin hexagonal atrial epithelial cells. These are ciliated, and in life, a current of water passes into the tube (Pusey 1958). Bundles of nerve fibres from the ascending rami run over the walls of this tube, but so far as I am able to observe, they do not terminate there, but run further and enter the hindgut system. It seems, in fact, as though the atrial extension is not innervated, the nerve fibres upon it are merely crossing it on their way to the hindgut system. No clues can be gained as to the function of the atrial extension from its lack of innervation, except that, although a current of water is carried into it through the action of the cilia of its walls, the absence of sensory terminations seems to preclude the suggestion that it can be a sampling organ of any sort.

(vii) *The innervation of the buccal and velar regions*

The stream of water that enters the pharynx passes first through the coarse sieve of the buccal cirrhi, then through the circular aperture formed by the velar sphincter muscle (onto which are attached the velar tentacles), then flows through the gill apparatus to reach the atrium itself. On both cirrhi and tentacles there are numerous sensory cells (Dogiel 1903; Franz 1923), as there are in the epithelium of the oral hood; the responses of these cells to both mechanical and chemical stimulation directly affects the atrial nervous system. Thus, stimulation of these cells can elicit contractions of the pterygial musculature, or again, the muscular systems of the buccal and velar regions affect the rate of entry of water into the atrium and hence affect the atrial receptor systems. It is therefore reasonable to treat briefly of the buccal and velar regions in the consideration of the atrial nervous system as a whole.

Sensory cells are grouped in the small lateral projections of the buccal cirrhi, and along the edges of the velar tentacles, it has been suggested that the former are tactile receptors and the latter chemoreceptors (Franz 1923). Between the bases of the velar tentacles there are regularly arranged papillae on the surface of the sphincter muscle, which rather resemble vertebrate taste buds in appearance. Figure 13 shows these arrangements. Nerve cells were figured both by Fusari and by Kutchin amongst the bundles of motor fibres to the muscles of the buccal cirrhi and velum, but I have never seen nerve cells at these sites, and in my opinion, they misinterpreted the nuclei of connective tissue cells as those of neurons.

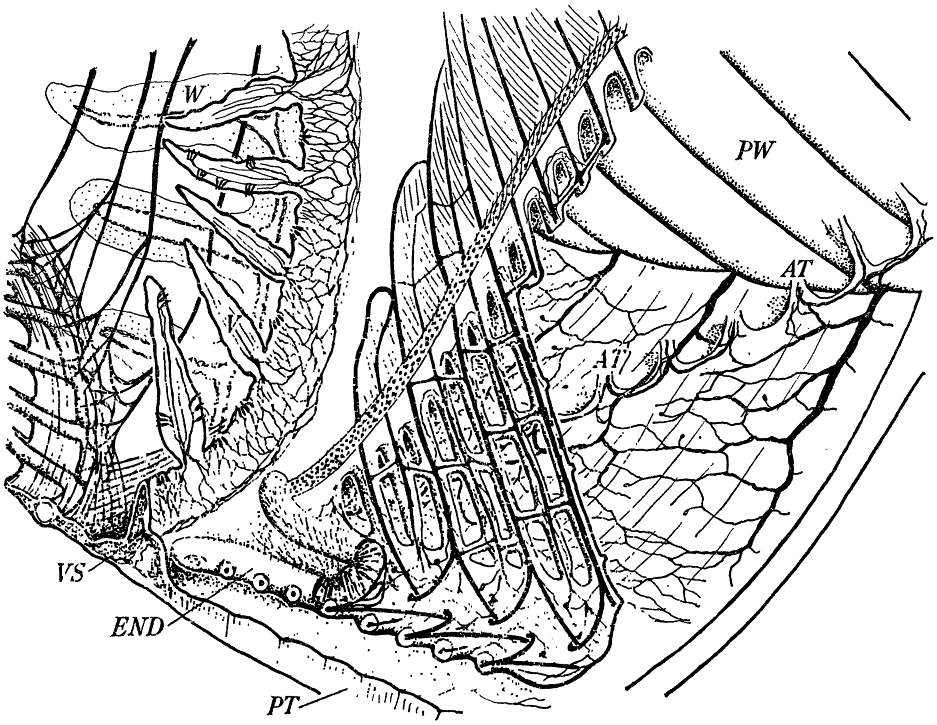

FIGURE 13. The innervation of the anterior end of the pharynx, as if from inside the gill apparatus, looking out of the velar ring. Only a few of the sensory cells of the velum, buccal cirrhi, pterygial and branchial plexuses are shown. Note innervation of anterior gill bars from descending rami (*AT*). Ascending rami are absent until the gill attachments reach the parietal walls of the atrium more posteriorly (to right of figure). The motor fibres to the velar sphincter muscle lie on its anterior face, those illustrated are sensory. *END*, endostyle; *V*, velar tentacle; *VS*, velar sphincter muscle; *PT*, pterygial muscle; *PW*, parietal wall of atrium; *W*, wheel organ of oral hood.

The velar tentacles hang across the water stream entering the pharynx and passing to the atrium, the 'taste buds' lie at the edge of this water stream. It appears that both types of sensory cells receive stimuli when water is entering to flow to the atrium. If the velar tentacles are touched with a glass hair, contraction of the velar muscles flicks them rapidly forward, and a contraction of the pterygial muscle is usually evoked. Very light touch results in small movements of the velar tentacles, and does not evoke a 'cough' reaction of the pterygial muscle. The velar tentacles are also responsive to vibration (such as tapping on the bench, or walking past the container in which the animals are lying); this response is a good sign that the animal is in a healthy condition.

It seems therefore, that in addition to (or possibly instead of) the chemoreceptive function suggested for velar sensory cells by Franz, the velar receptors are mechanoreceptors, sensitive to light touch. In life, these receptors presumably respond when they are struck by a particle in the inhalant stream, and, indeed, it is sometimes possible to observe a large particle touching a velar tentacle as the animal feeds, and this results in the 'cough' reaction.

Discussion

(a) *The function of the atrial nervous system*

Histologically, the atrial nervous system of amphioxus is a complicated system in which there are several specialized categories of components. The arrangements of these components of the system are summarized in figure 2 and in table 1. *Asymmetron* possesses a very similar system (the plexuses upon the diverticulum, the atrio-coelomic funnels, gill bars, and pterygial muscles are identical with those in amphioxus).

We can reasonably infer the functions of some of these components, but of others, we simply do not have any idea what function they may perform. It must be emphasized that only for the motor fibres to the pterygial muscle and for the ciliary-inhibitor fibres upon the gill bars, do we have any clear experimental evidence of the function of any of these components of the atrial nervous system. We may assume that the sub-endostylar plexus (for example) contains secreto-motor fibres, or that the pterygial sensory cells respond to variations in the flow of water through the atrium, but these remain assumptions, unsupported by any experimental evidence.

This uncertainty about the functions of the components of the system handicaps any discussion of the system as a whole, but nevertheless, we can feel fairly safe in supposing that the atrial nervous system is concerned with the regulation of the feeding process, and, as Holmes suggested, with spawning. The motor fibres upon the smooth muscle coat of the gonad sacs presumably assist gamete release, and it is likely that the stimulus for this is received by one of the many kinds of receptor in the atrial system.

The main function of the whole atrial nervous system, however, is almost certainly the regulation of the feeding process. Amphioxus feeds upon particles in the stream of water drawn through the pharynx, which it entraps in the gill apparatus upon mucus produced by the endostyle. This process might appear to be a simple one, but in fact, it is far from simple, for at every stage, a delicate regulatory control is exerted by the atrial nervous system, and this results in a most efficient mode of feeding, capable of coping with varying external conditions, and co-ordinating them with the cyclical internal processes of digestion and absorption.

The flow of water into the pharynx, and thence to the atrium, depends both on the rate of beat of the cilia producing the current, and the degree of contraction of inhalant and exhalant apertures. As the stream flows into the pharynx, it is tested by the anterior sensory systems of the velum and buccal region (perhaps also by the pterygial sensory cells); the presence of noxious material causes the animal to expel the water within the atrium by closing the atriopore and contracting the pterygial muscle. The inflow of water is halted by the cessation of ciliary beat, and the continued closure of the atriopore. In dense food suspensions, the coarse filter of the buccal cirrhi is cleansed when necessary by the 'cough' reaction and the rate of flow through the atrium is less than in suspensions poor in food material. My observations suggest that the animal can distinguish between suspensions of food material and of inorganic particles, even dense suspensions of the latter are treated in the same manner as water containing few food particles, and the animal filters at the maximum rate. When the animal has collected sufficient food, the rate of flow through the atrium slows, and the collection of food is abandoned until the food previously collected is digested.

All these reactions observable during the feeding process are evidently under the direct or indirect control of divers of the components of the atrial nervous system. There is, perhaps, nothing surprising in its complexity, when we realise the manifold problems which face the filter-feeding animal in the regulation of its feeding pattern. It would, indeed, be more surprising if amphioxus had not evolved special control mechanisms for regulating the feeding process and increasing its efficiency.

(*b*) *The atrial nervous system and the visceral nervous systems of other groups*

It is natural to attempt to compare the atrial nervous system with systems of visceral regulation found in other groups, but such comparisons are difficult for two reasons. On the one hand, the visceral nervous systems of the two groups which filter-feed in a comparable manner to amphioxus, and which are (however remotely) presumably related to the Acrania, the Tunicata and Hemichordata, are almost unknown. These two groups have as yet proved refractory to the usual neurohistological techniques; as a result, we do not know whether they may possess regulatory mechanisms concerned with their feeding processes comparable to the amphioxus atrial system. On the view of the atrial nervous system here taken, viz., that it has arisen to regulate the filter-feeding of the animal, it would be expected that the Tunicata and Hemichordata should possess similar systems. There are hints in the literature that this hypothesis may prove to be correct, for example, in *Doliolum*, nervous control of the gill bar cilia has been demonstrated, and fibres innervating the endostyle presumably have a secreto-motor function (Fedele 1923). In the sessile tunicates apparent nerve cells are found scattered over the viscera (Bone 1959); and in the Hemichordata, Knight-Jones (1953) has shown that the cilia producing the feeding current are apparently under nervous control. It would be too much to expect to find in these groups exactly the same solutions to the problems posed by the nervous regulation of the feeding process as those which amphioxus has achieved with its atrial nervous system, but it is not unlikely that analogous solutions have been found, and that further research will demonstrate the existence of an 'atrial system' in these two groups.

On the other hand, the most nearly related craniate to the Acrania, the ammocoete larva, does not feed in an entirely comparable manner, and again the condition of its visceral nervous system is rather poorly known. The work of Johnels indicates that the organization of the visceral nervous system is of the craniate type, and has little similarity to the atrial nervous system. With the change over in the Gnathostomes to feeding on large pieces of food, with the development of jaws, and of muscular systems in the gut wall to propel the food through it, and the development of the respiratory function of the gill slits, the craniate has evolved an entirely different regulation of the feeding mechanism, which has culminated in the complex autonomic system found in the higher forms. This autonomic system is mainly *motor* (unlike the atrial system), and the connexions and position of its constituent nerve cells are not equivalent to those found in the atrial system.

There has been much discussion concerning the sensory components of the craniate autonomic system, but it now seems fairly clear that, at least in the enteric plexuses, the sensory neurons are concerned in local reflexes (Bulbring, Lin & Shofield 1958). We may conclude that the atrial nervous system of amphioxus is a specialization peculiar to the Acrania, which has evolved in relation to the method of feeding; it is not evidently homologous with any part of the craniate autonomic system.

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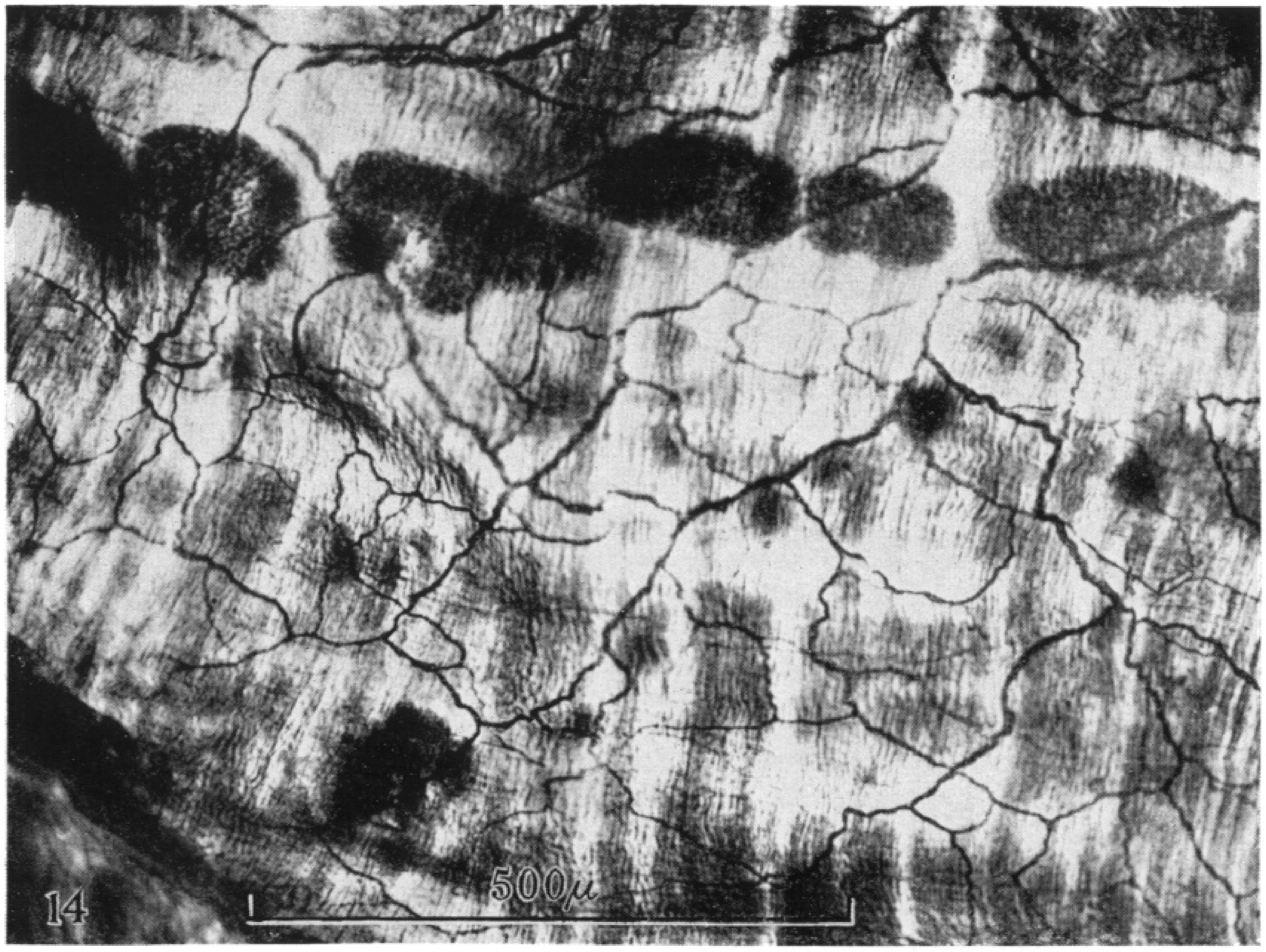

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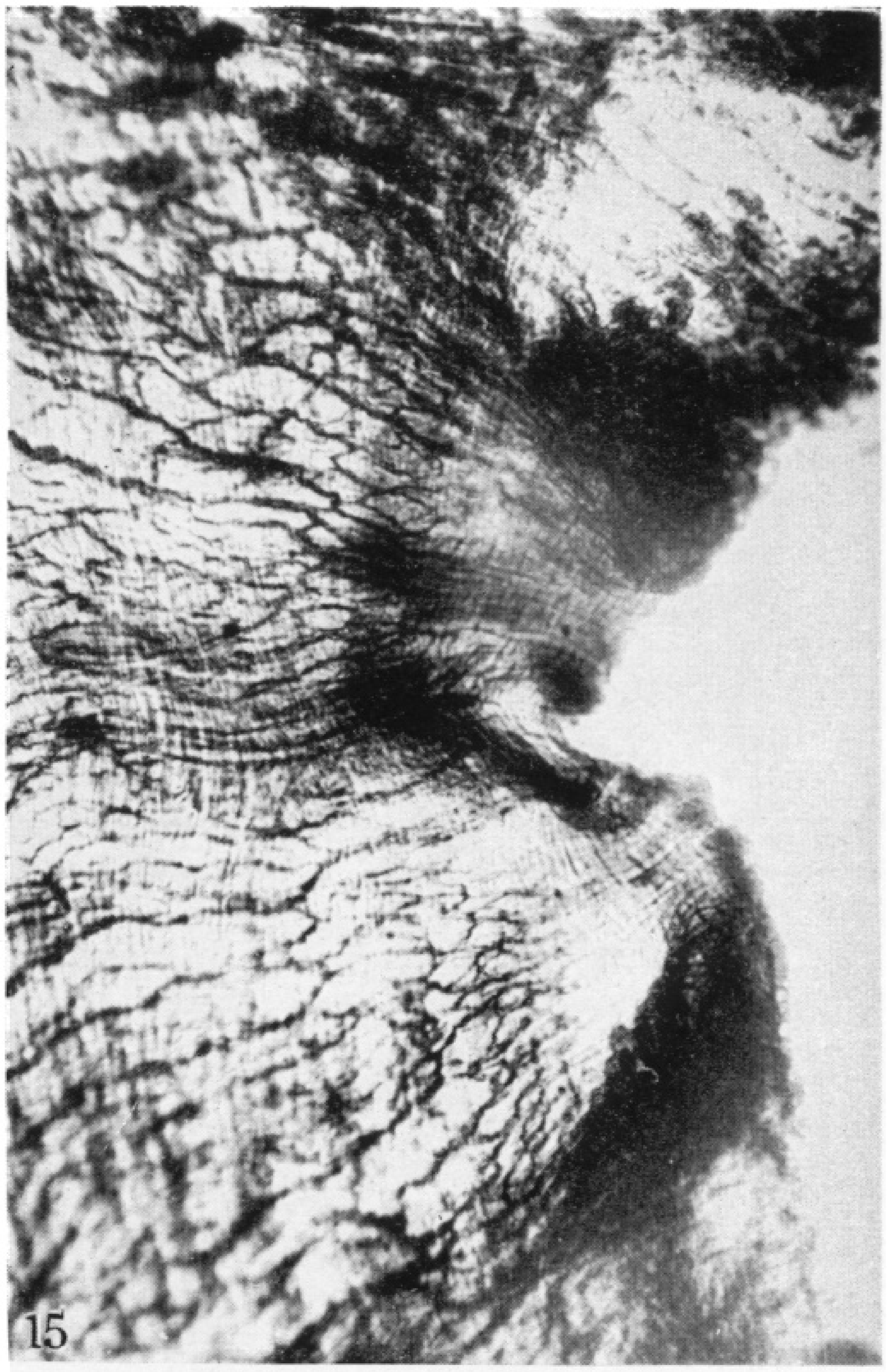

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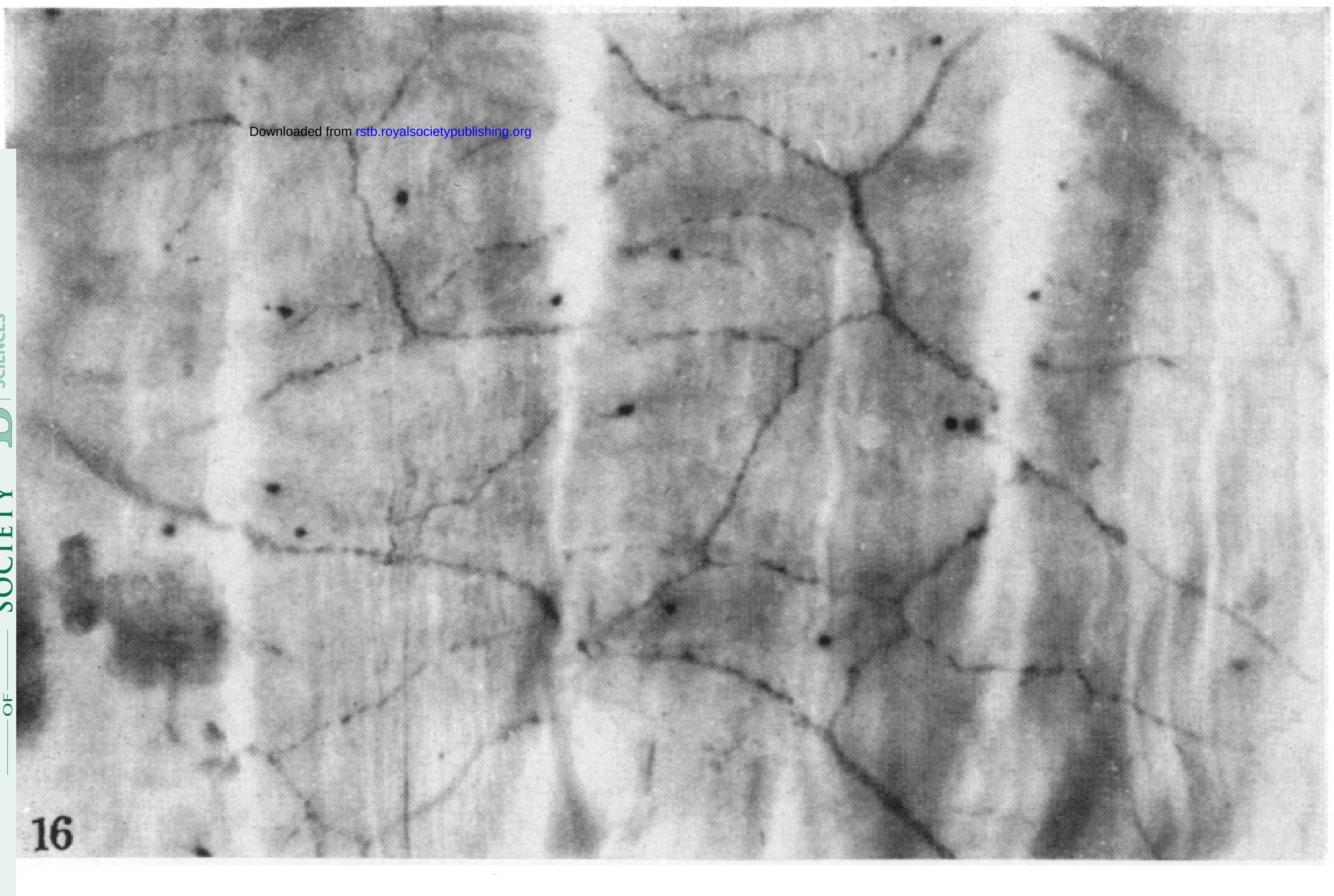

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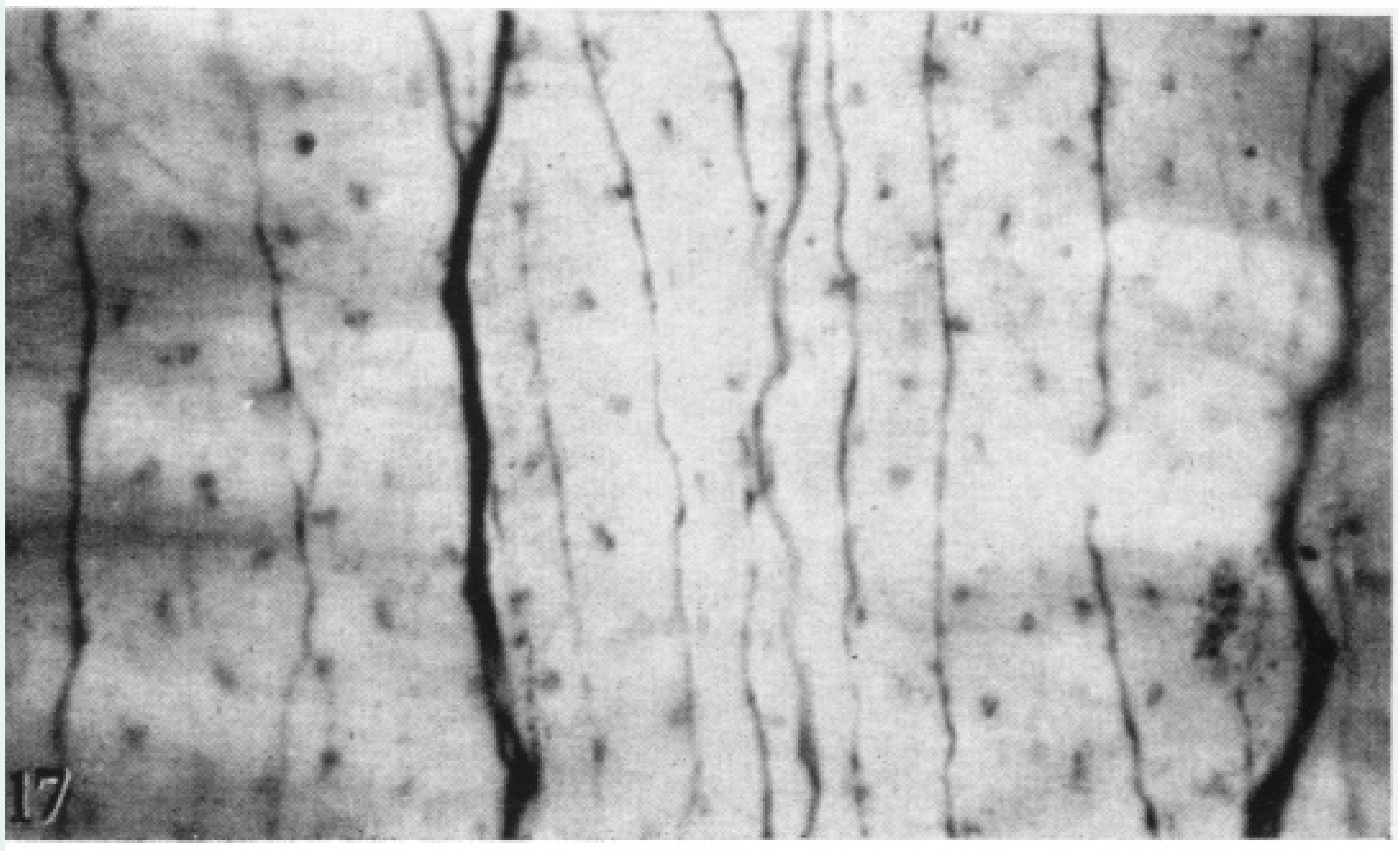

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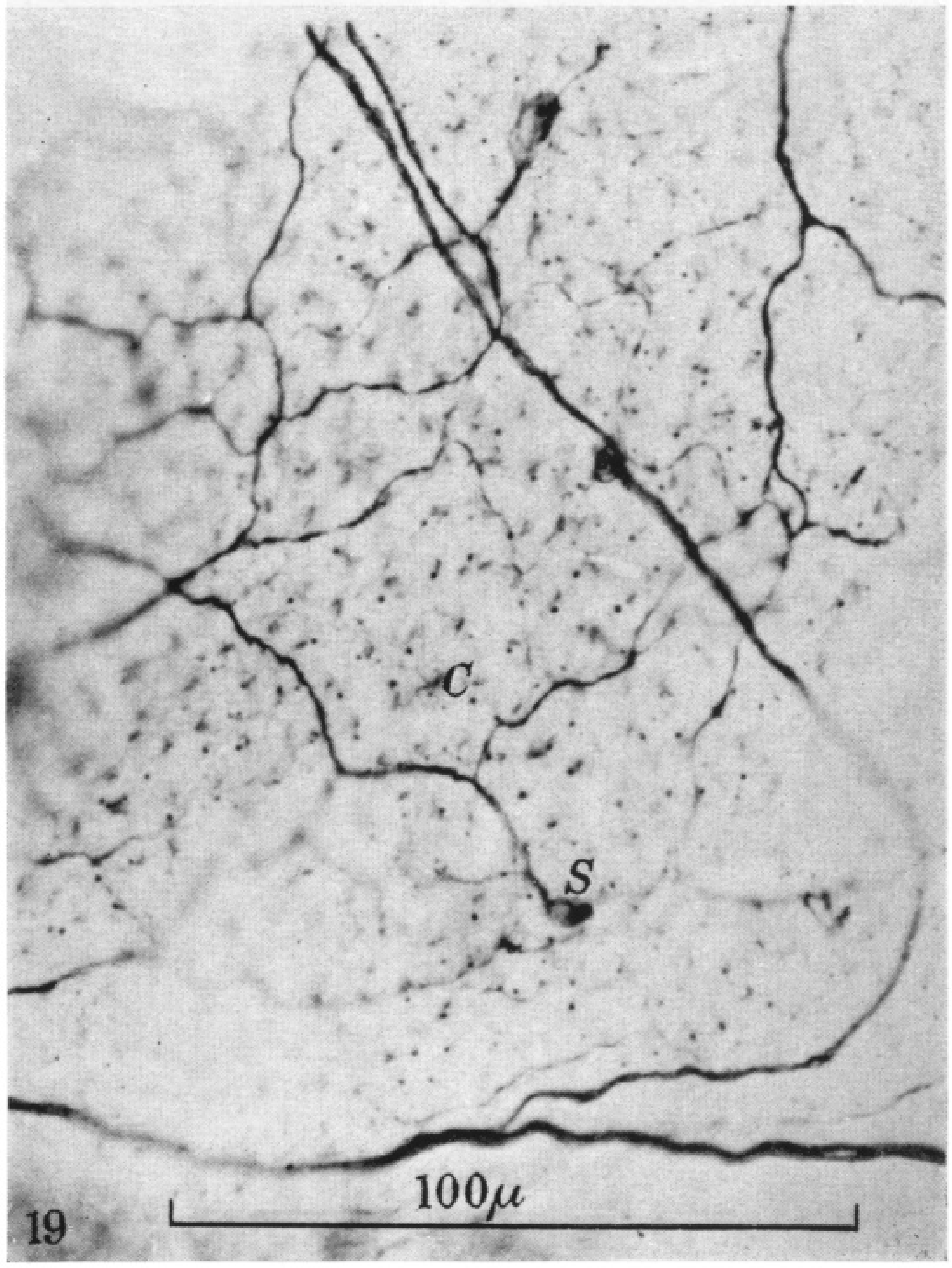

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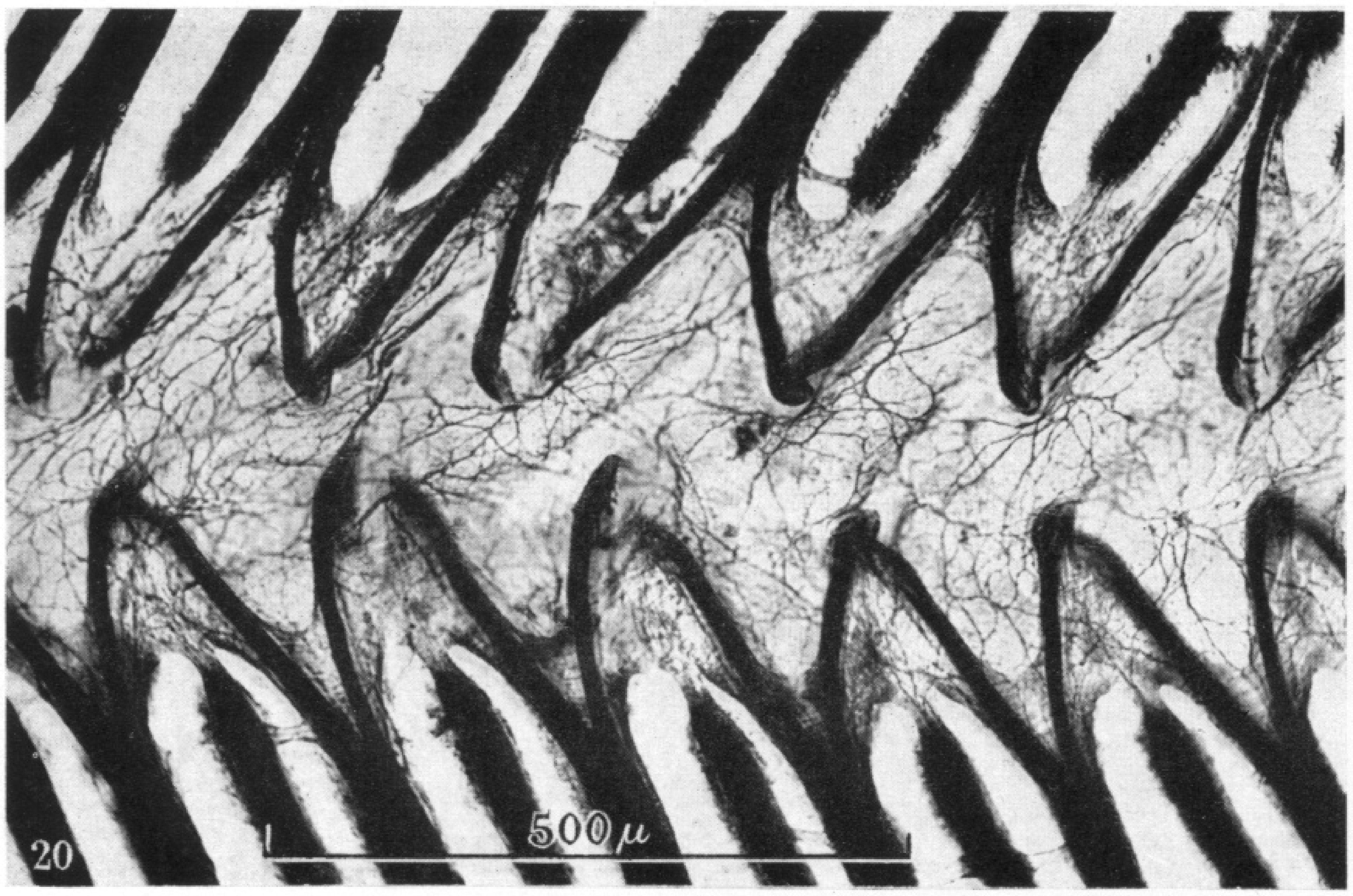

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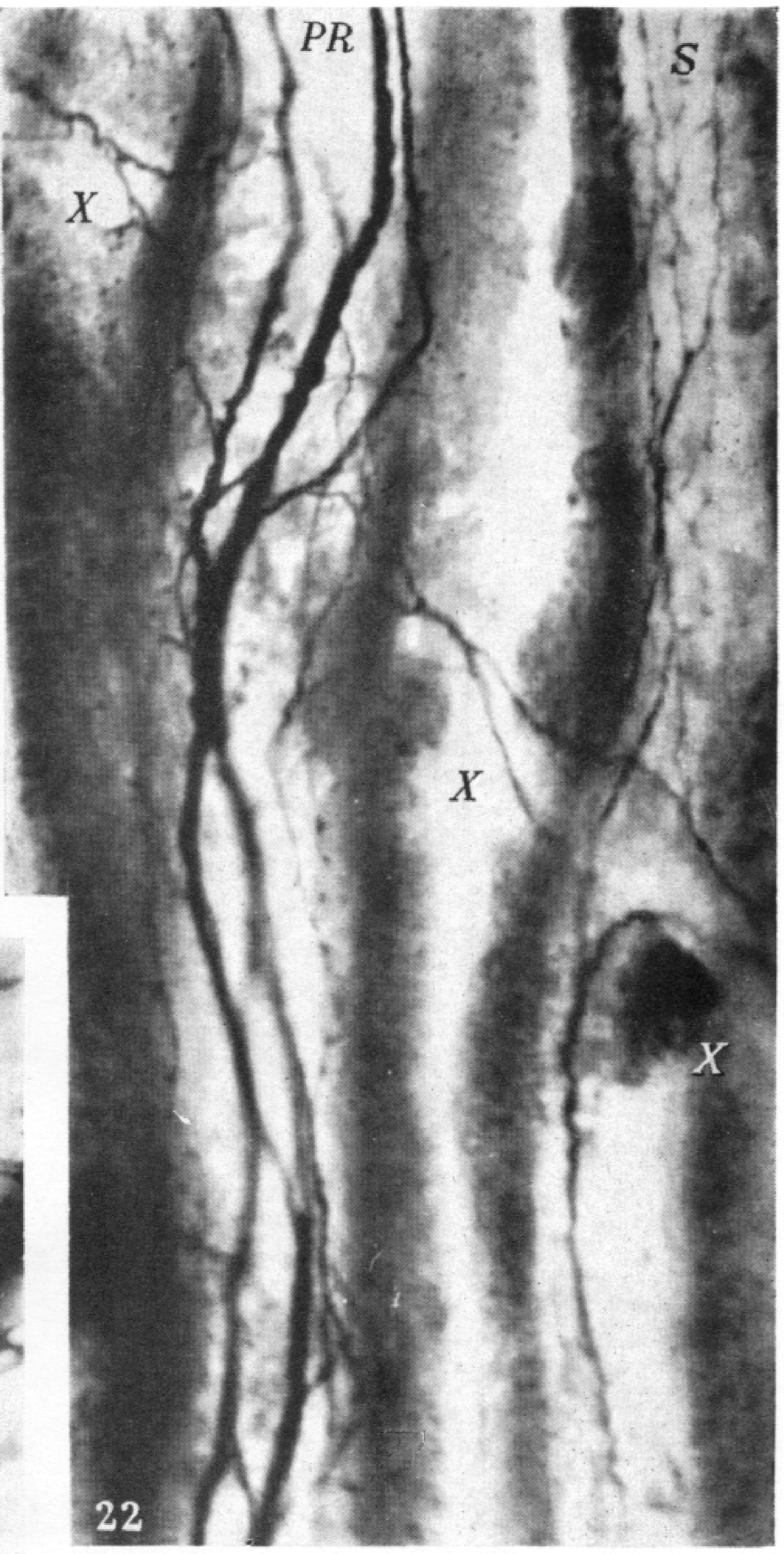

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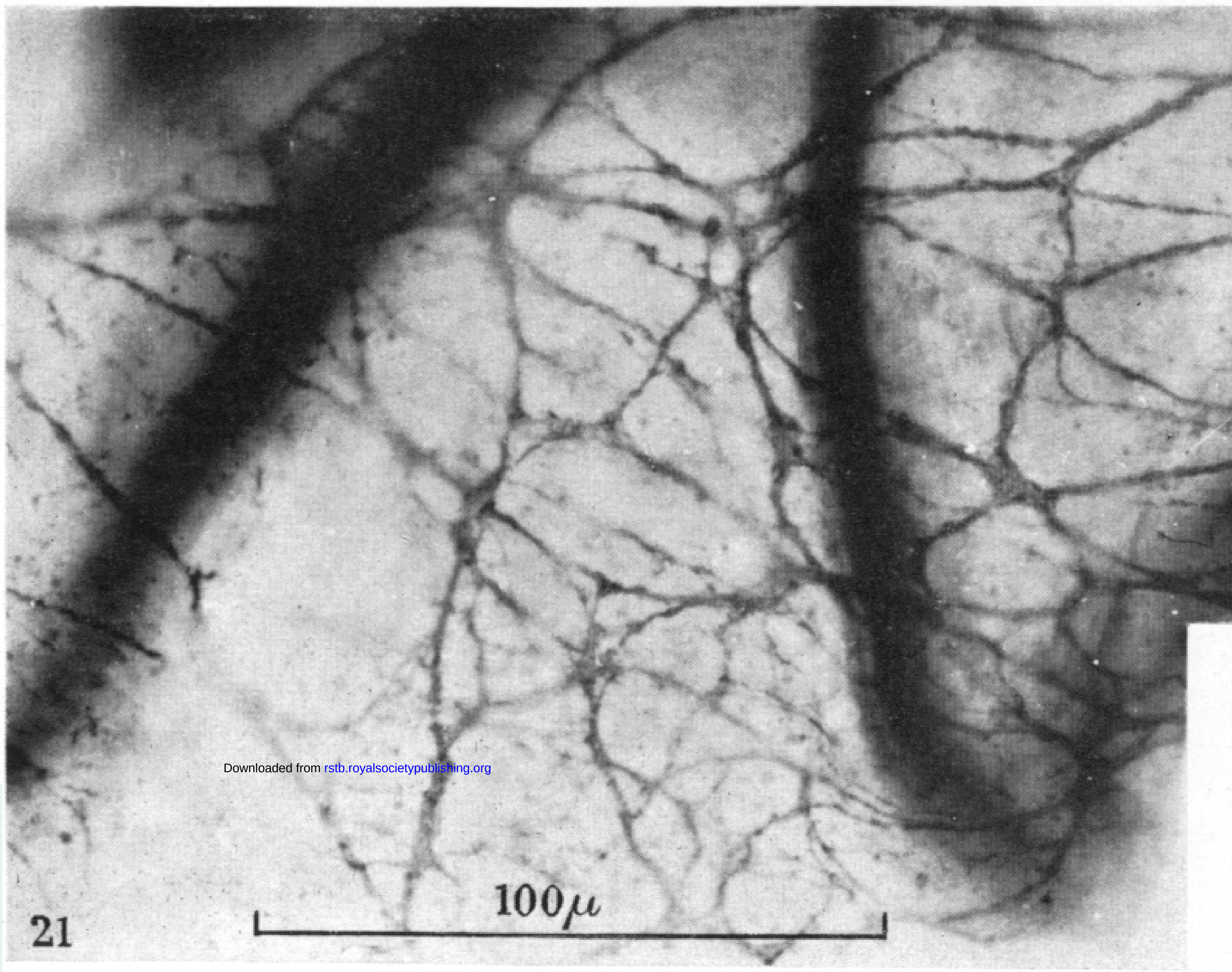

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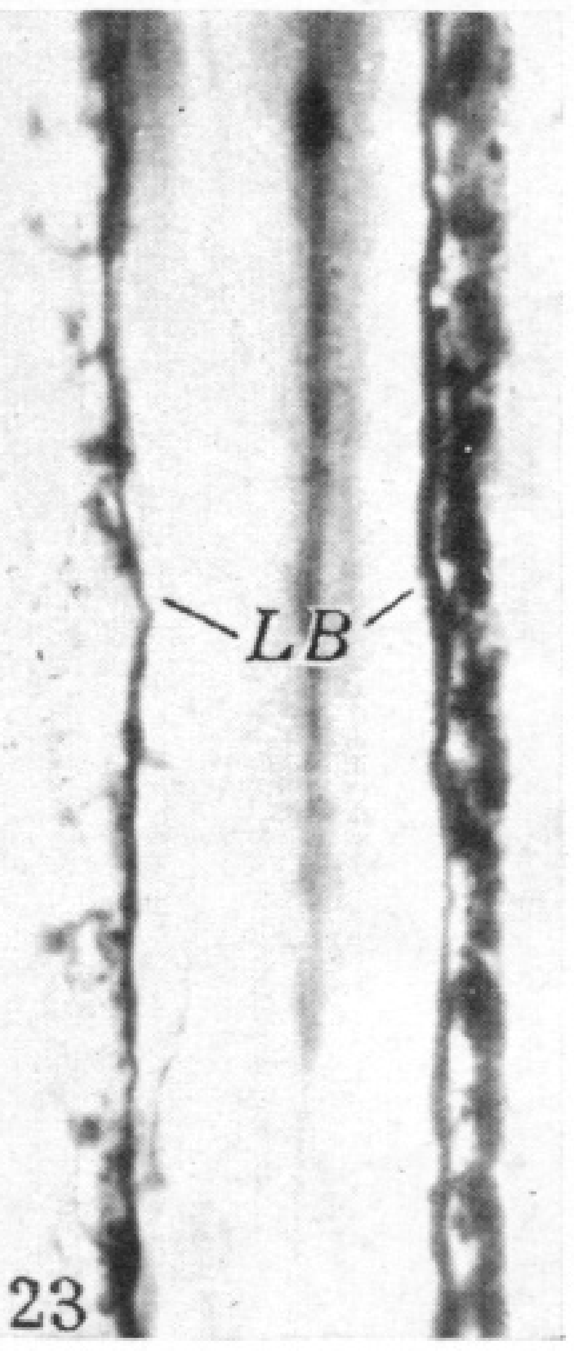

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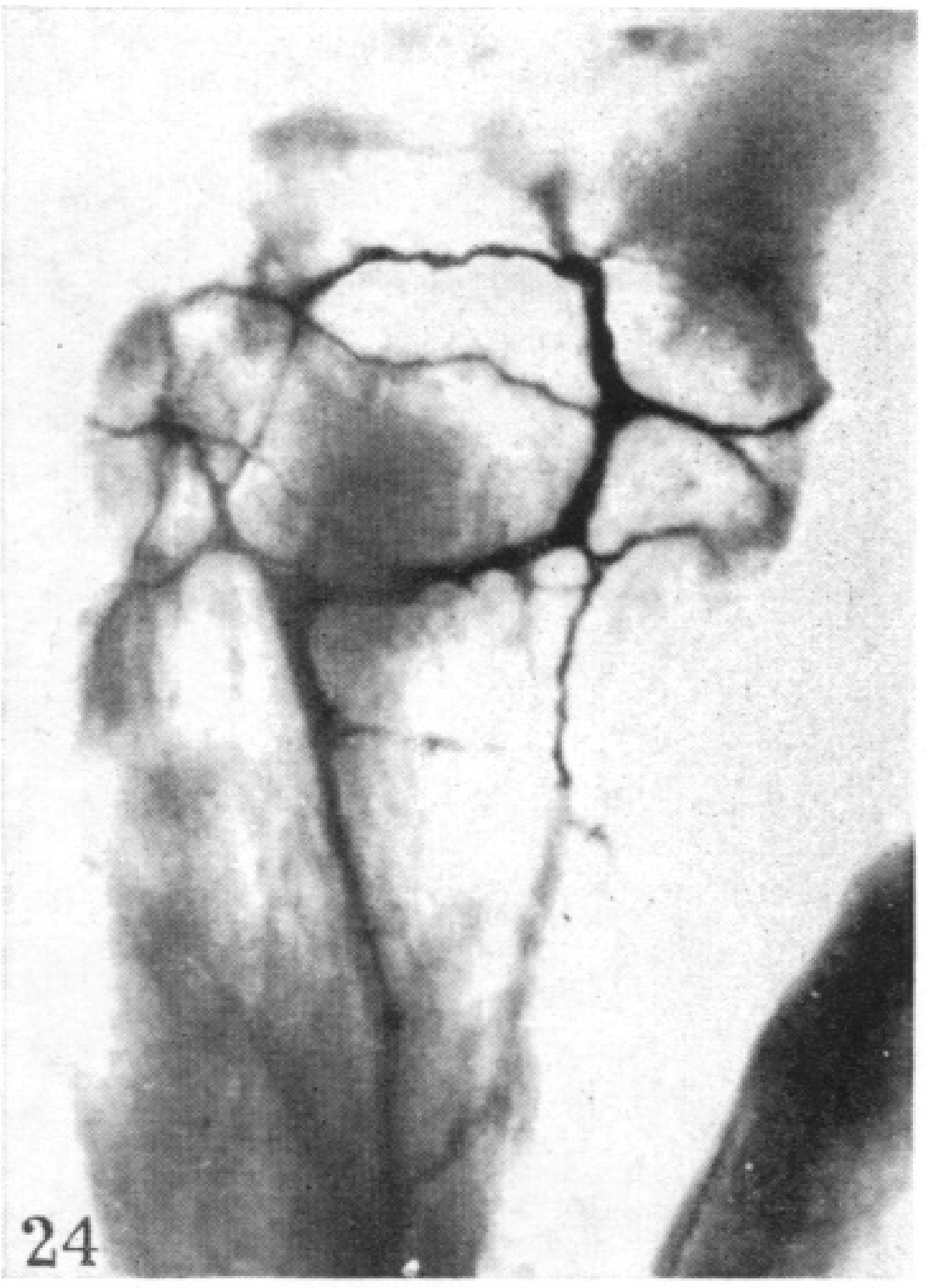

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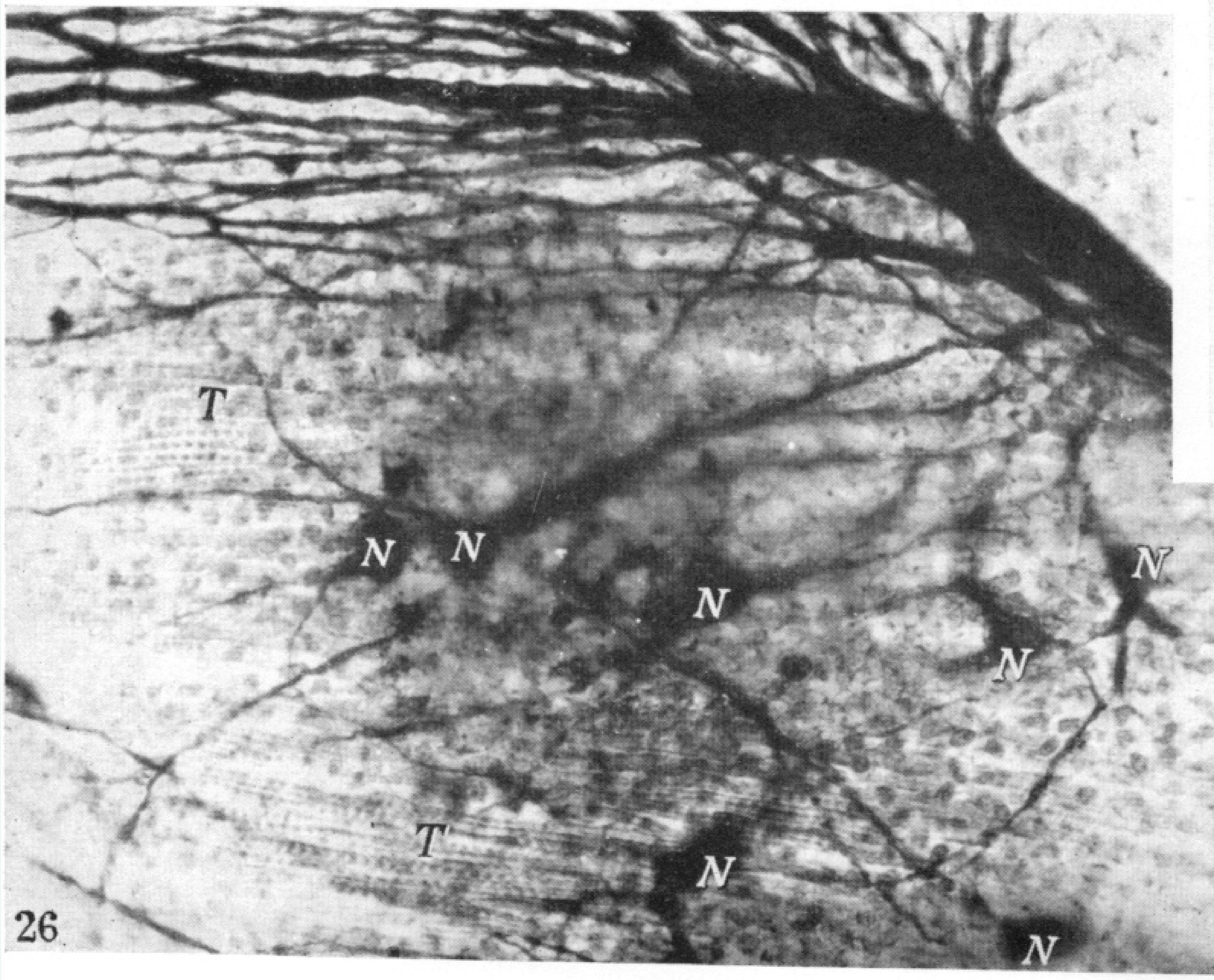

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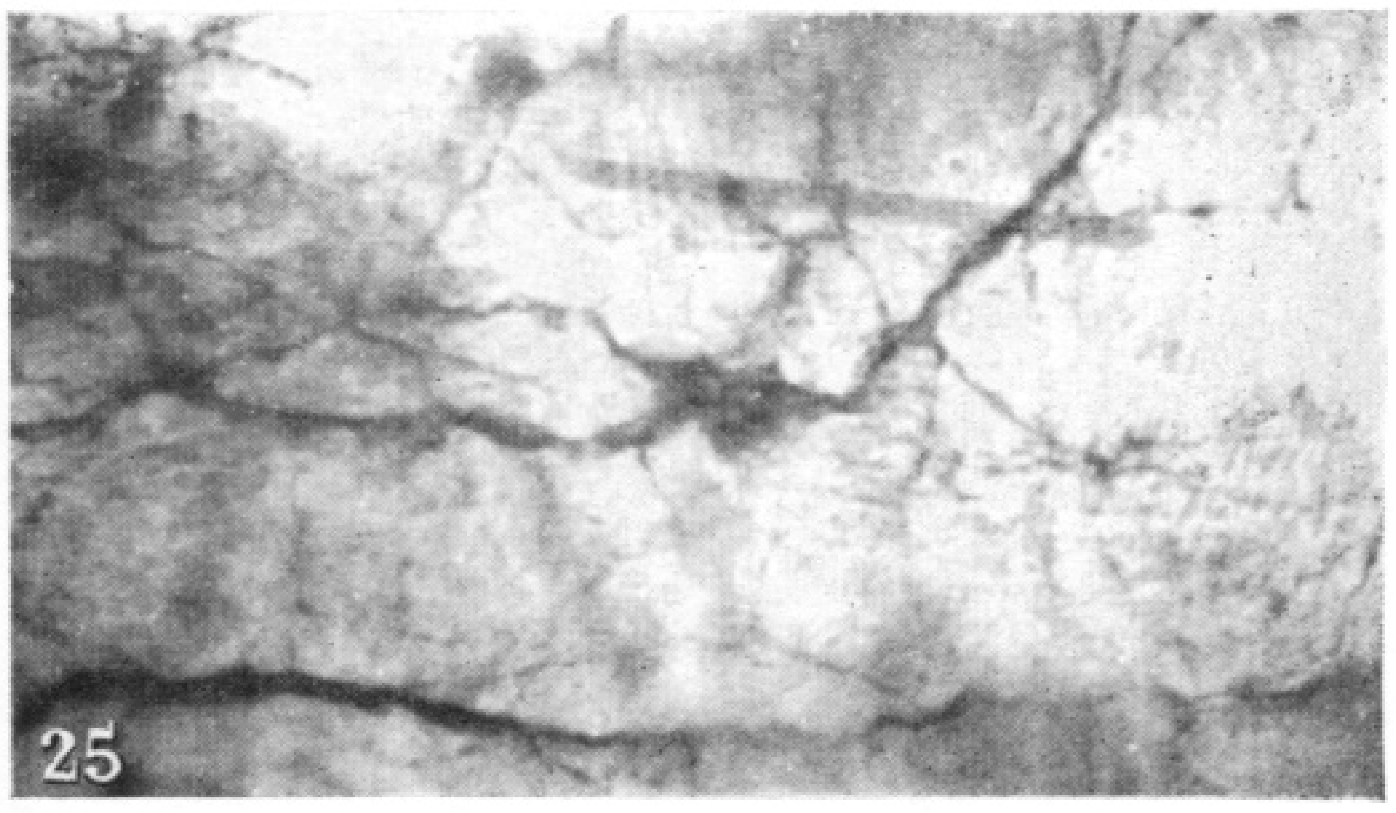

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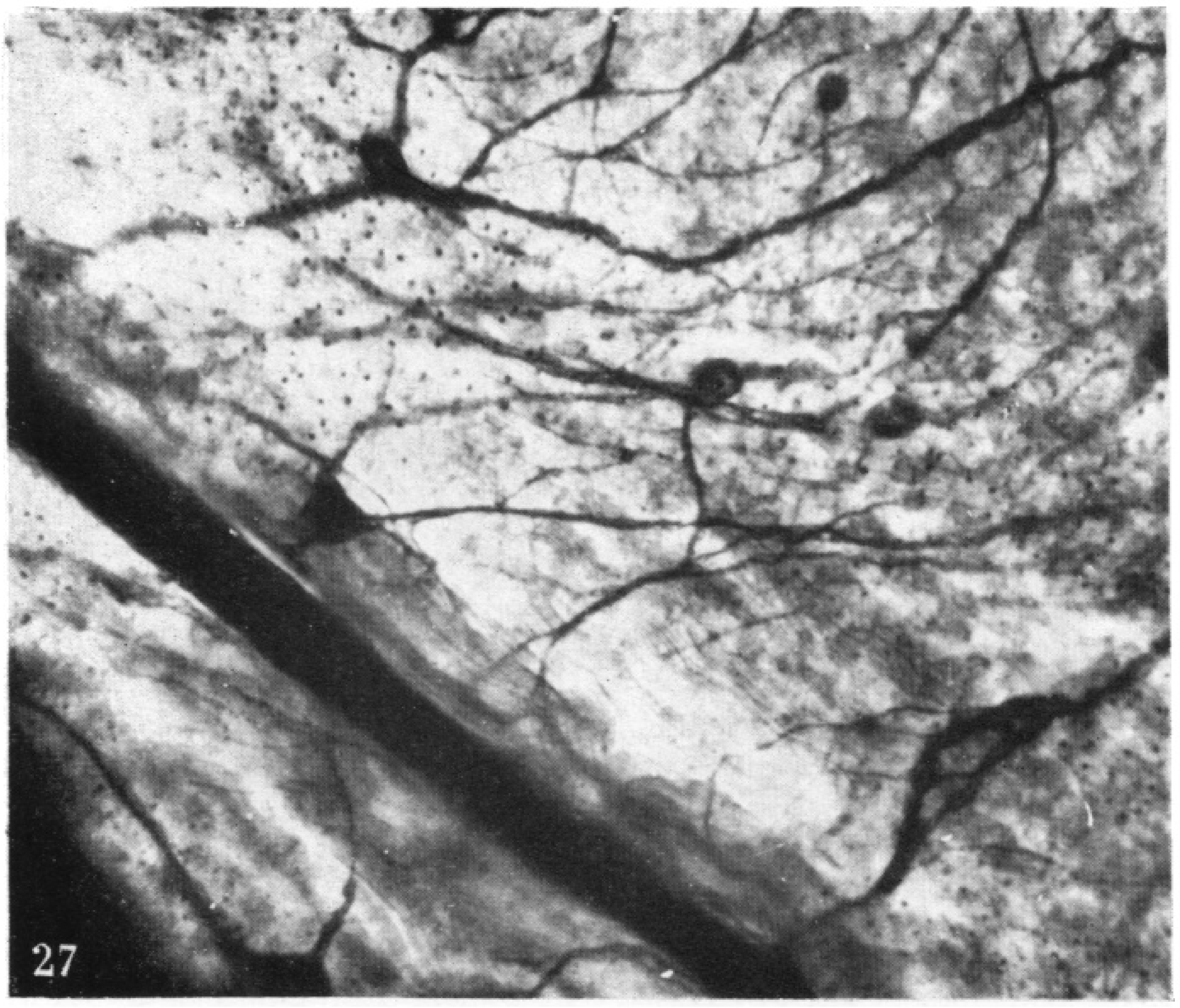

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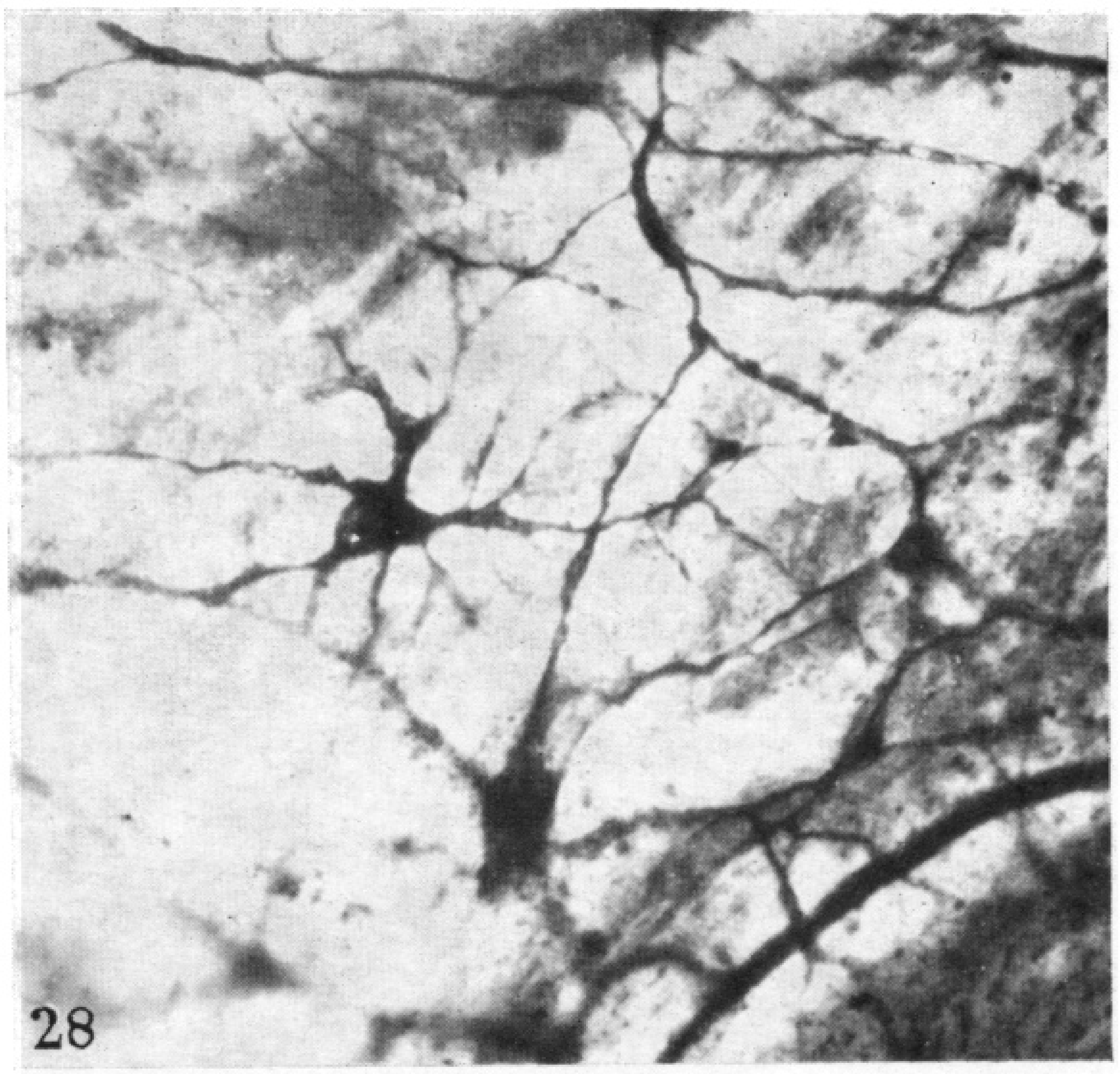

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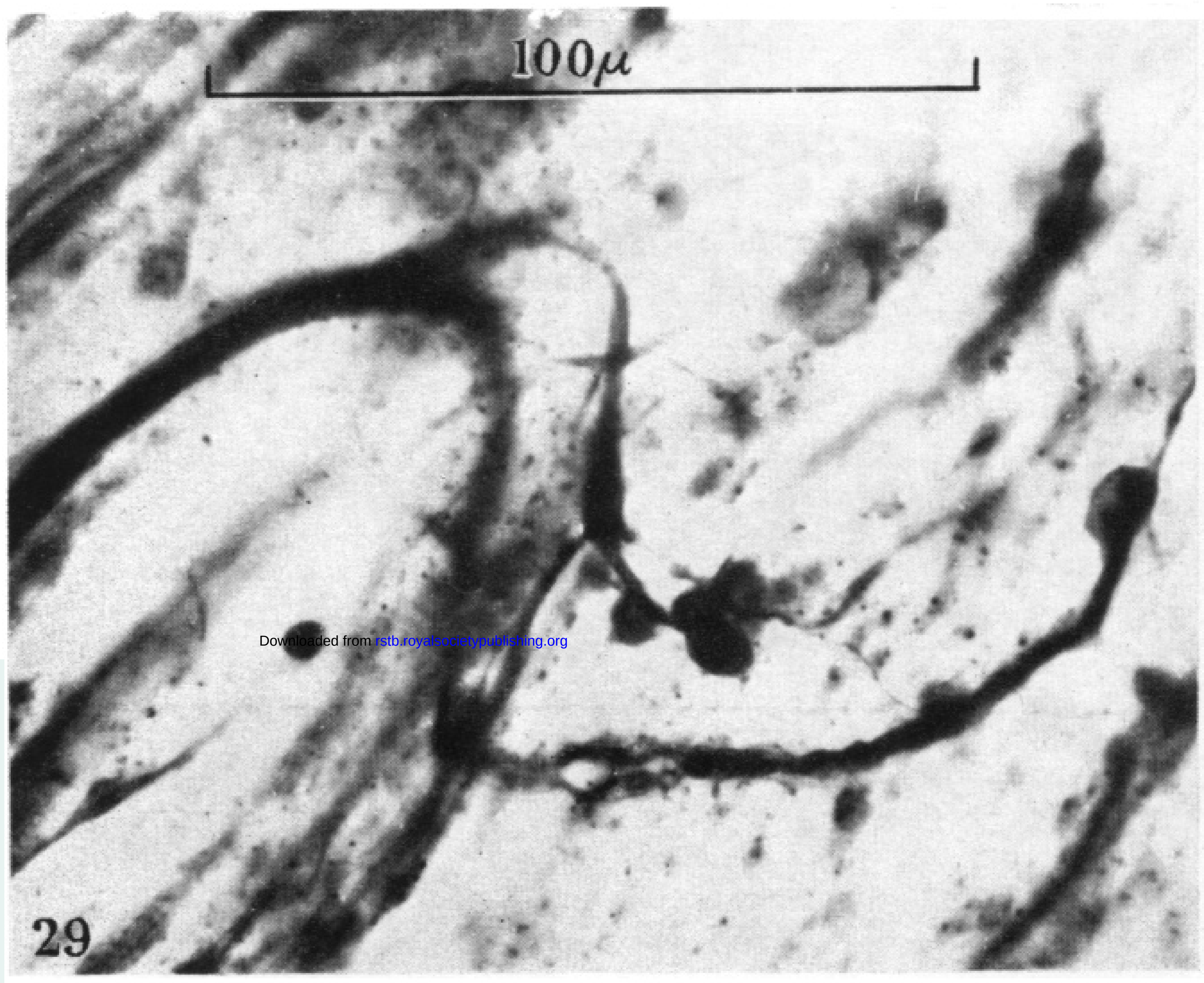

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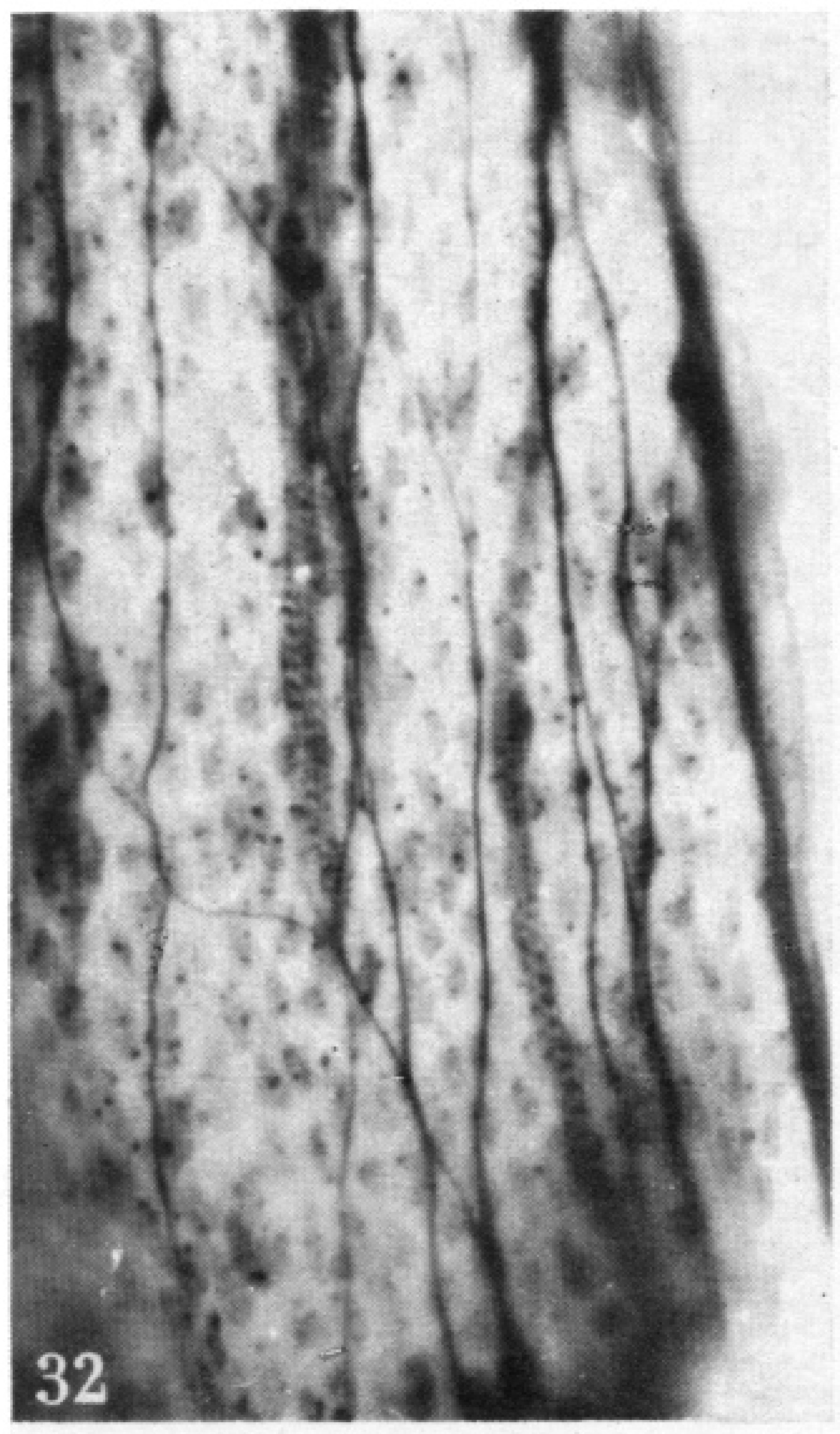

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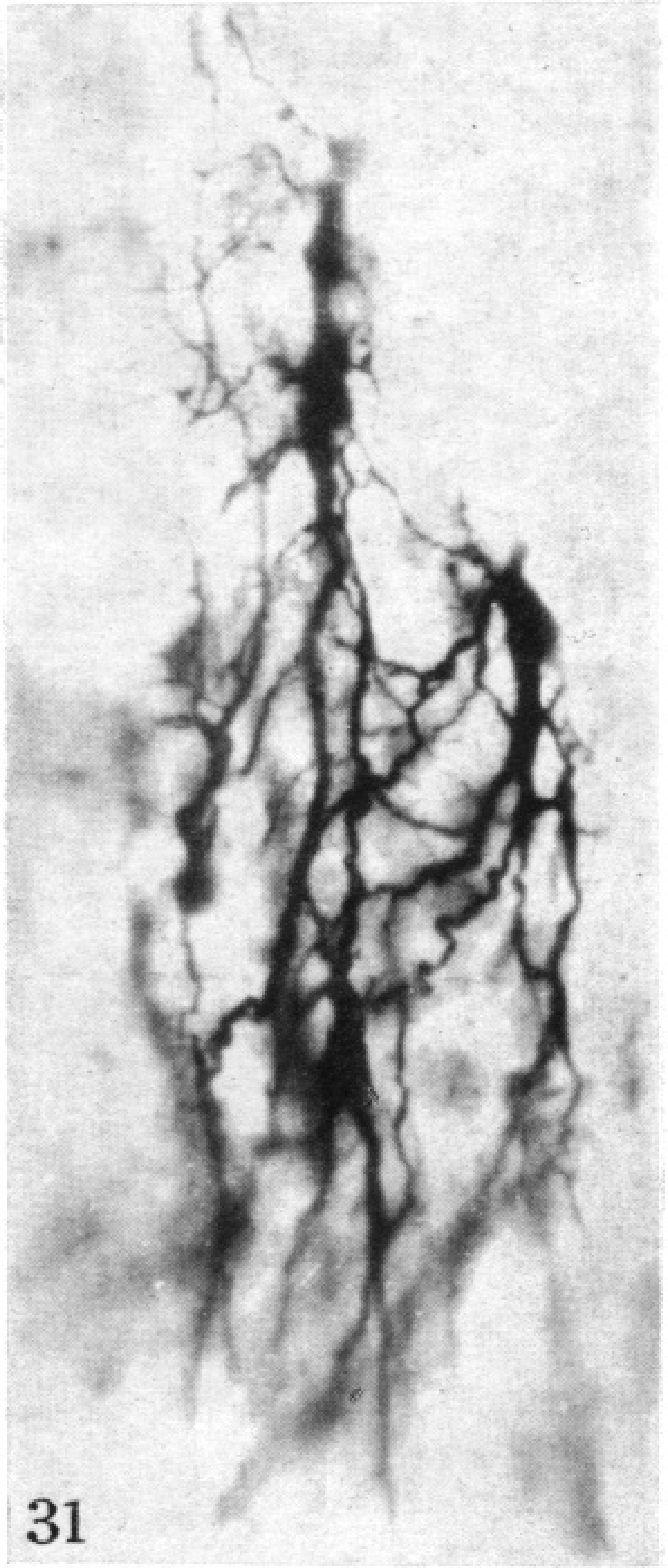

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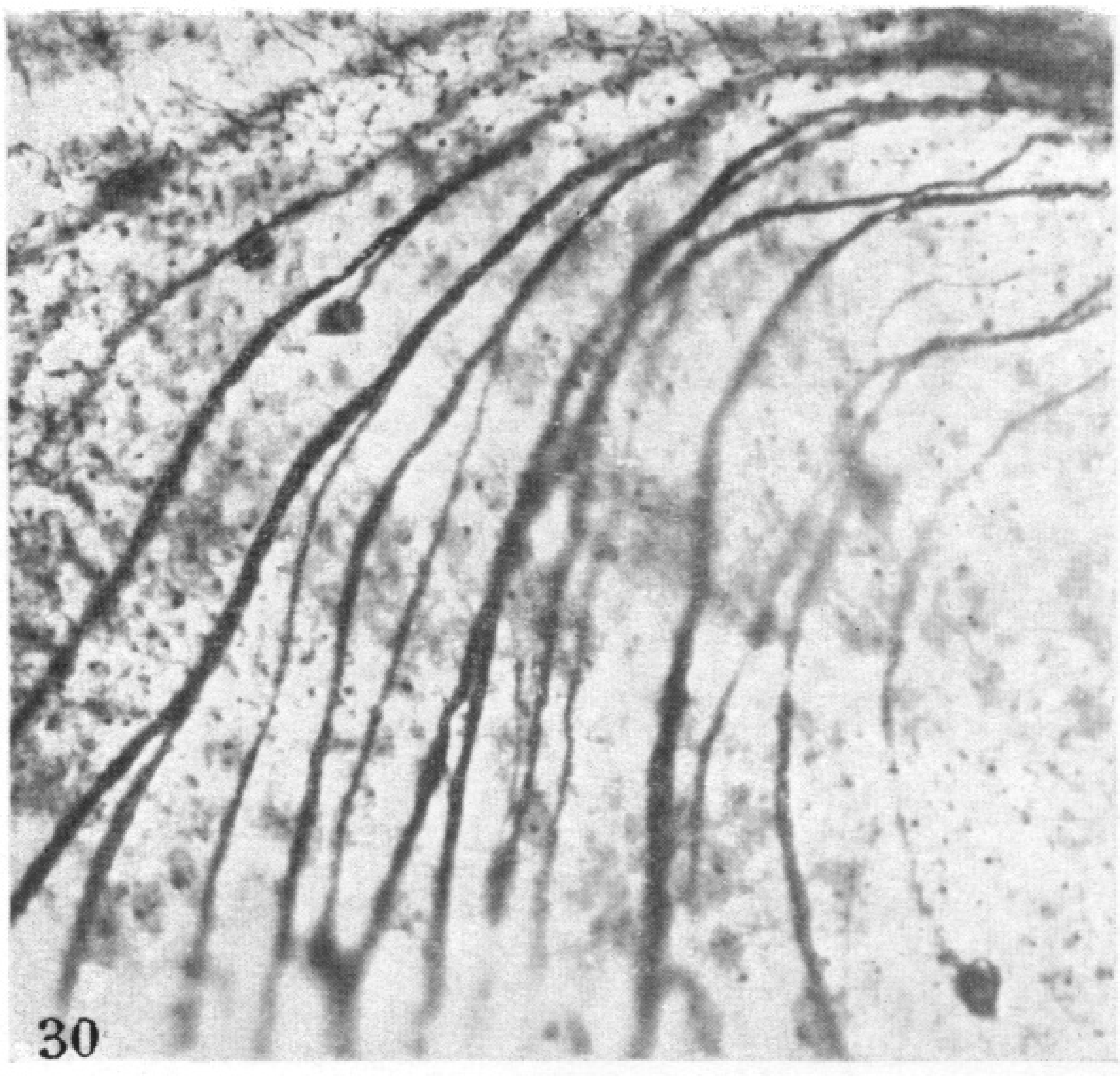

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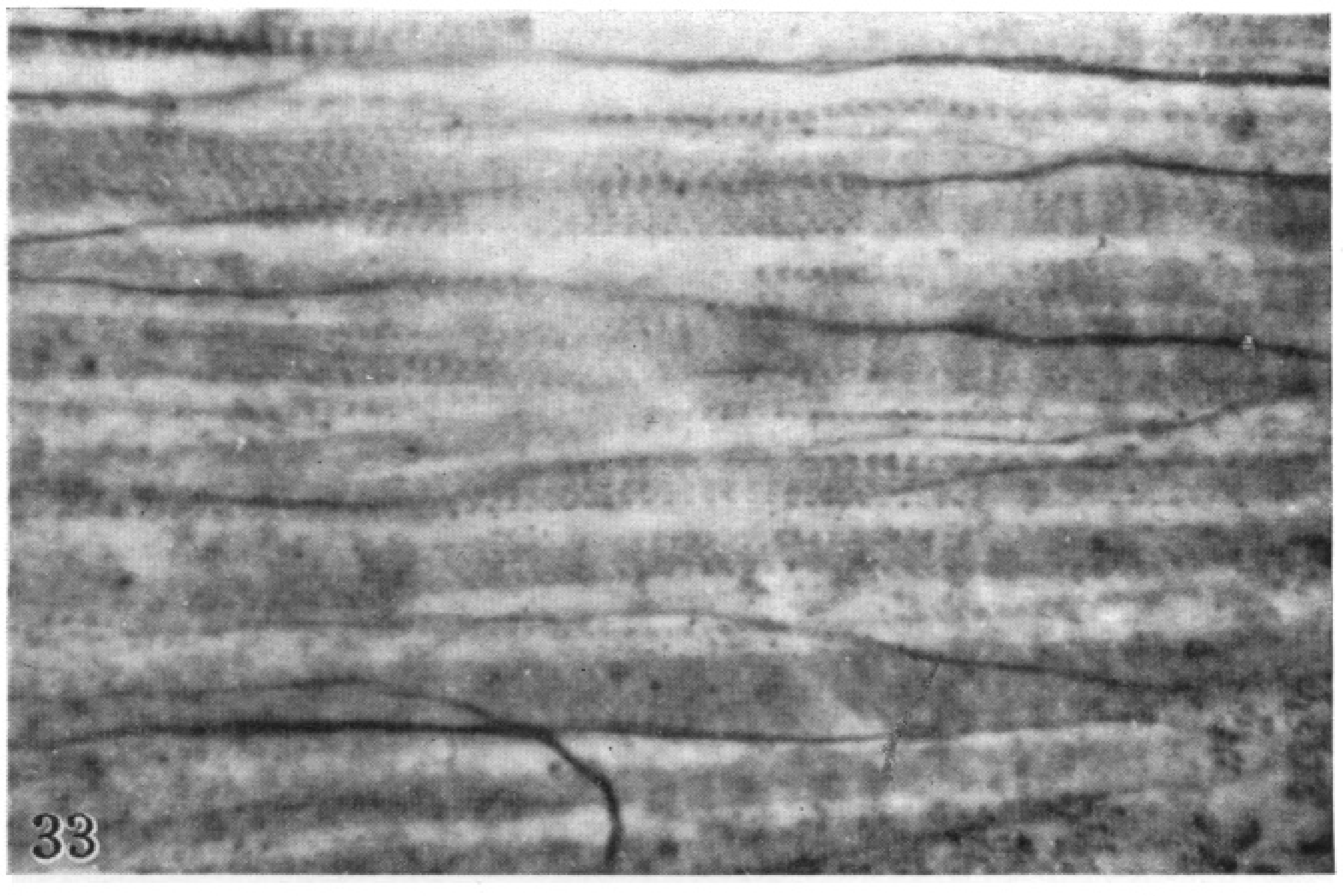

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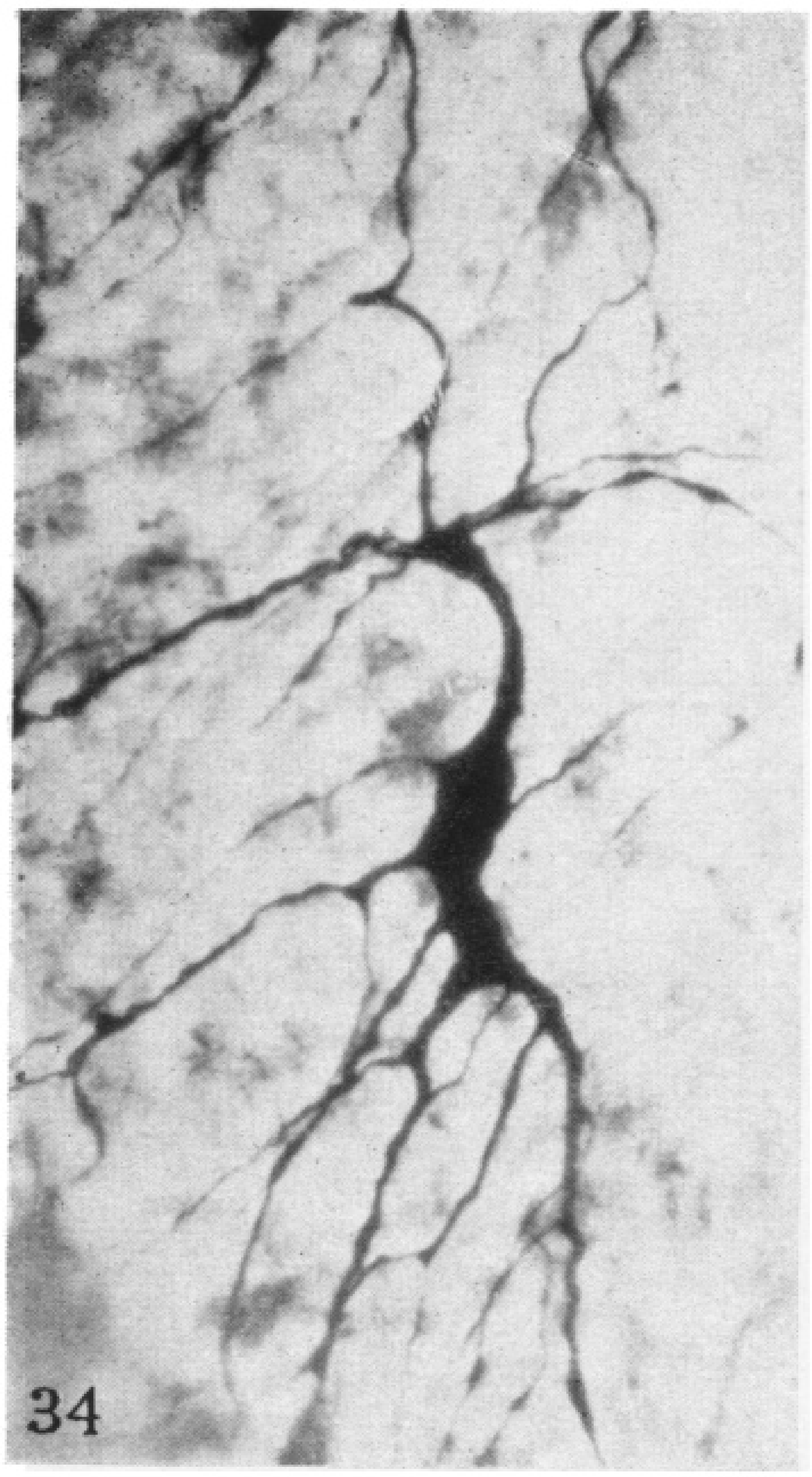
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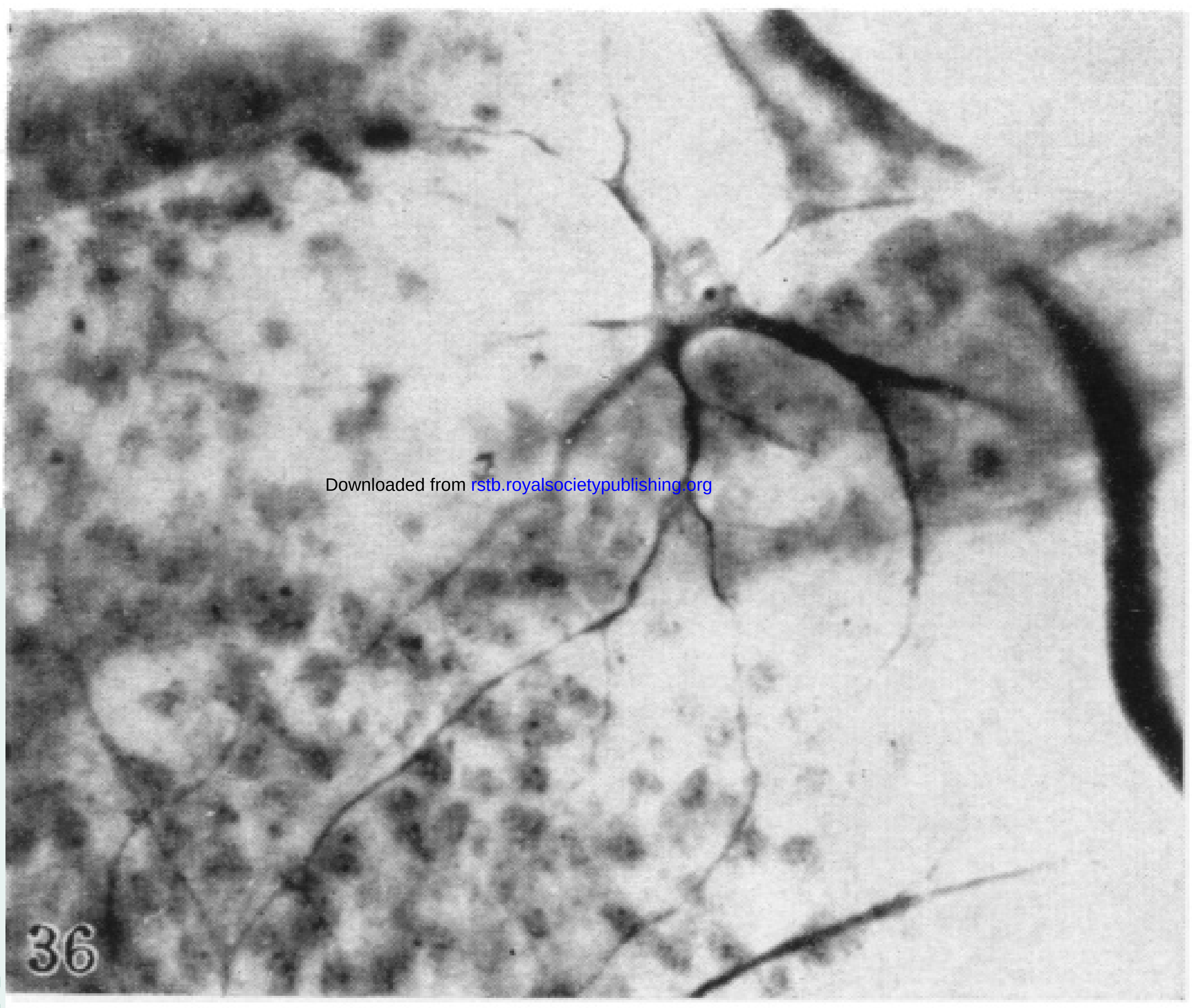
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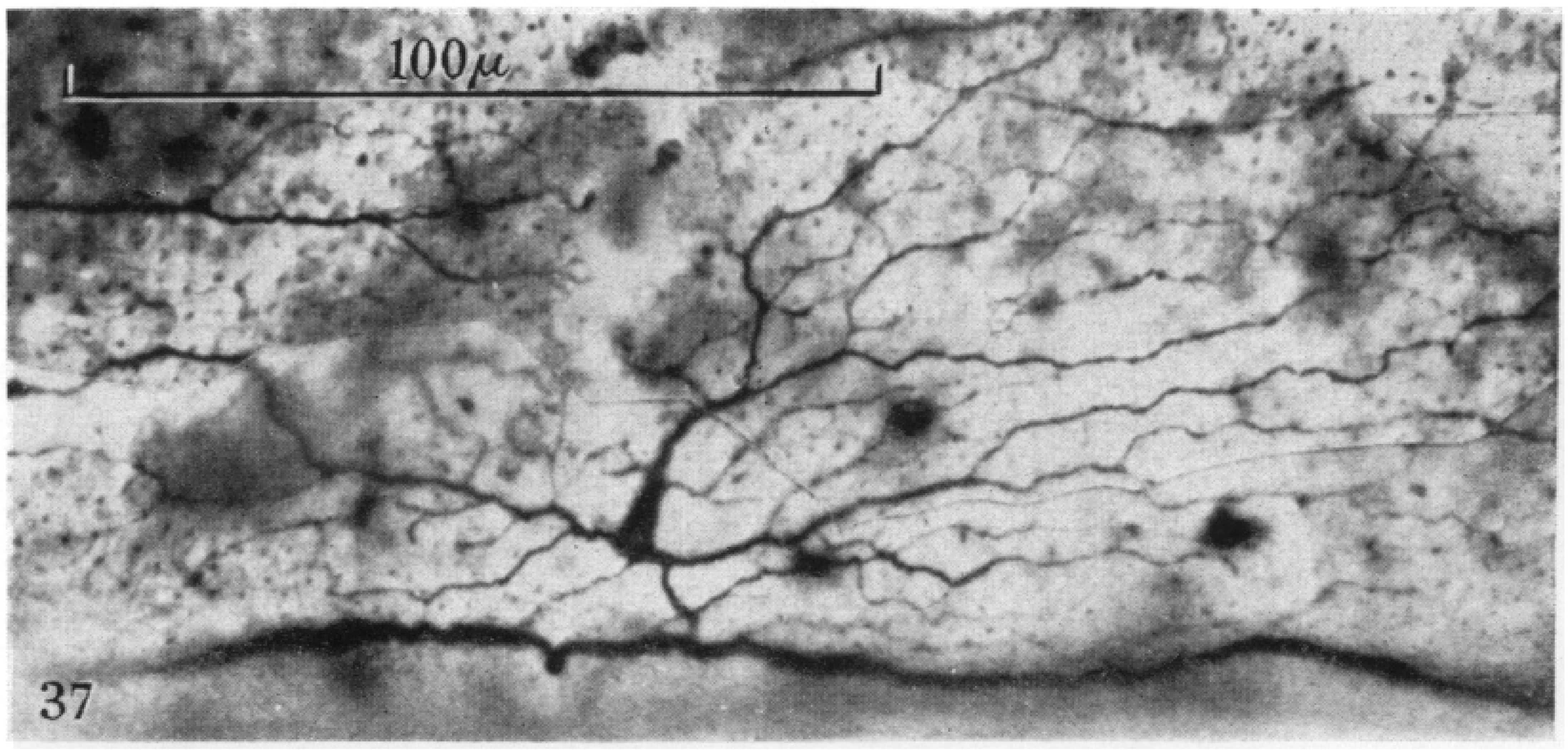

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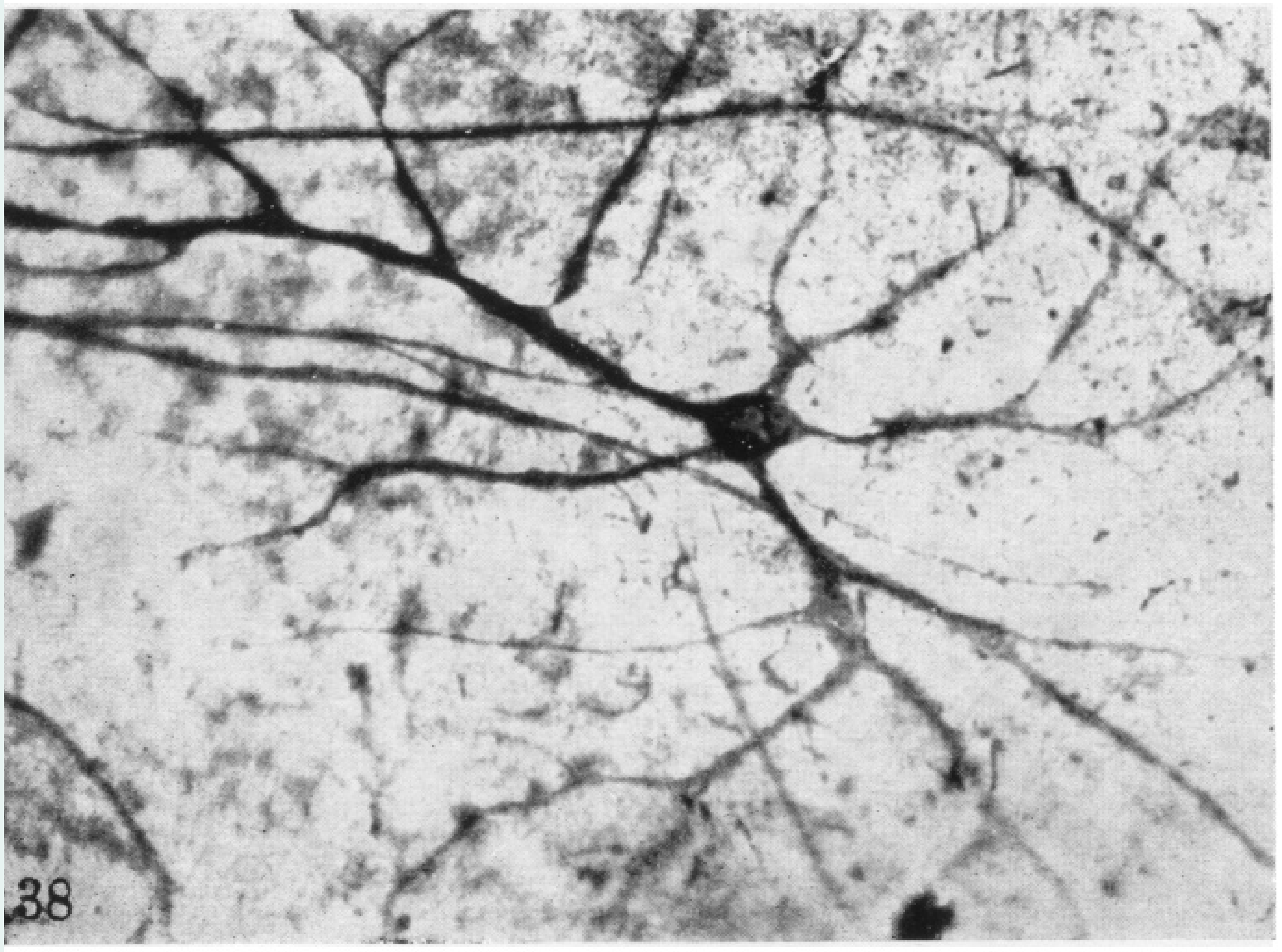
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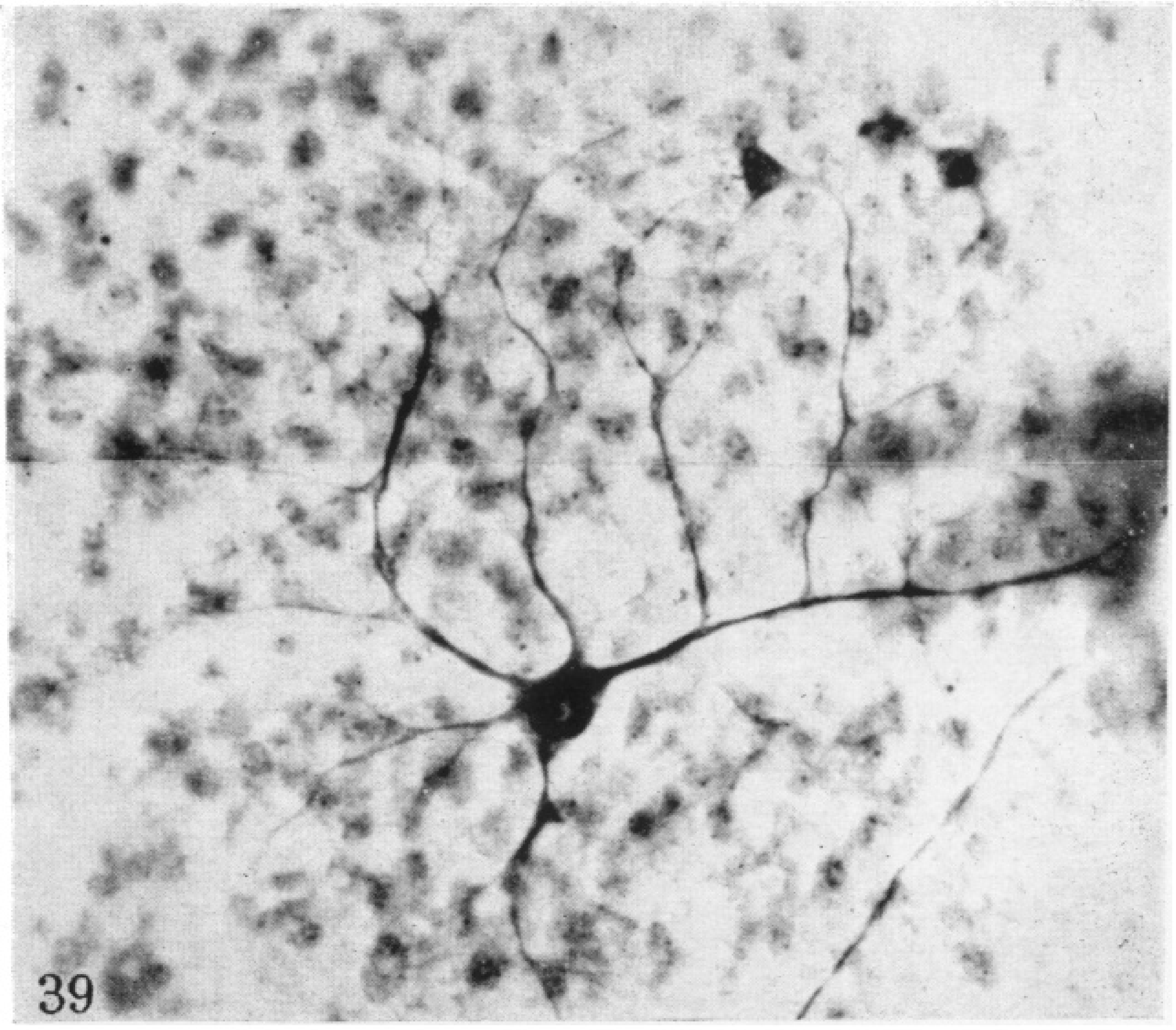
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