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THE ORIGIN AND POSSIBLE SIGNIFICANCE OF SUBSTANCE P IMMUNOREACTIVE NETWORKS IN THE PREVERTEBRAL GANGLIA AND RELATED STRUCTURES IN THE GUINEA-PIG

BY MARGARET R. MATTHEWS[1] AND A. C. CUELLO[1,2]

[1]*Departments of Human Anatomy and* [2]*Pharmacology, University of Oxford, South Parks Road, Oxford OX1 3QX, U.K.*

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The distribution and origin of substance P immunoreactive nerve elements have been studied in the guinea-pig prevertebral ganglia by the indirect immunohistochemical technique, using a monoclonal antibody to substance P. Non-varicose substance P immunoreactive nerve fibres enter or leave the ganglia in all nerves associated with them, traversing the ganglia in larger or smaller bundles. Networks, mainly single-stranded, of varicose substance P immunoreactive nerve fibres also permeate the ganglia, forming a loose meshwork among the neurons. Similar networks are present in the lumbar paravertebral ganglia. In all these ganglia, neuronal somata do not in general show substance P immunoreactivity.

The various nerves connected with the inferior mesenteric ganglion have been cut, in single categories and in various combinations, and the ganglion examined, after intervals of up to six days. Cutting the colonic or hypogastric nerves, which connect the ganglion with the hindgut and pelvic organs, leads to accumulation of substance P immunoreactive material in their ganglionic stumps, extending retrogradely to intraganglionic non-varicose fibres traceable through into the intermesenteric and lumbar splanchnic nerves. There is some local depletion of intraganglionic varicose networks. Cutting the intermesenteric nerve, which connects the coeliac–superior mesenteric ganglion complex with the ganglion, leads to accumulation of substance P immunoreactive material in its cranial stump and depletion of its distal stump; a minimal depletion is detectable in the inferior mesenteric ganglion itself. Cutting the lumbar splanchnic nerves, which connect the ganglion with the upper lumbar spinal cord and dorsal root ganglia, leads to accumulation of substance P immunoreactive material in their proximal stumps and total depletion of their distal, ganglionic stumps; in the ganglion there is subtotal loss of non-varicose substance P immunoreactive fibres and of varicose nerve networks, and the few surviving non-varicose fibres are traceable across the ganglion from the intermesenteric nerve to the colonic and hypogastric nerves. Cutting the intermesenteric and lumbar splanchnic nerves virtually abolishes substance P immunoreactive elements from the ganglion within three days postoperatively. It is concluded that these arise centrally to the ganglion.

Capsaicin treatment of guinea-pigs, which depletes substance P immunoreactivity of sensory neurons, was found to leave no more than minute occasional traces of substance P immunoreactivity in the prevertebral ganglia and in dorsal root ganglion cells and spinal laminae I and II; in the ileum, substance P immunoreactivity was abolished from the paravascular nerves and perivascular nerve networks, and from large nerve varicosities in the submucous plexus. The substance P immunoreactivity of the myenteric and submucous plexuses and of the nerve networks in the muscle and mucosal layers was however otherwise unaffected. Removal of the spinal cord caudally to the seventh thoracic segment without injury to the dorsal root ganglia is without detectable effect either on the substance P immunoreactive elements of the inferior mesenteric ganglion and associated nerves, or on the peri- and paravascular nerves of the hindgut mesenteric vessels. It is concluded that the intraganglionic substance P immunoreactive elements of the prevertebral ganglia are attributable to sensory neurons of the dorsal root ganglia, and that intraspinal and enteric neurons do not contribute significantly to them. It is further postulated with the support of indirect evidence that the intraganglionic networks of varicose substance P immunoreactive fibres, which have been shown to form synapses upon the postganglionic neurons, arise as collateral branches from the substance P immunoreactive sensory fibres which traverse the ganglia, and that these can subserve a short-loop reflex control over the excitability of the ganglionic neurons in advance of, and, or, in support of, or even independently of, the recruitment of central nervous circuits.

Introduction

The prevertebral ganglia are important as reflex coordinating centres placed in the sympathetic pathways between the central nervous system and the alimentary tract and pelvic organs (Crowcroft *et al.* 1971; Kuntz & Saccomanno 1944; Szurszewski & Weems 1976; Gabella 1976; Kreulen & Szurszewski 1979). In recent years a number of neuroactive peptides, including substance P, have been localized in these ganglia and in the enteric nerve plexuses in the alimentary tract (Pearse & Polak 1975; Hökfelt *et al.* 1977; Schultzberg *et al.* 1979, 1980; Furness & Costa 1980; Jessen *et al.* 1980). It is therefore becoming important, to understand further the physiology of the system, to elucidate the parts that may be played by the various peptides in its control. A particular peptide, luteinizing hormone releasing hormone, has been shown to have a transmitter function in a sympathetic ganglion of the bullfrog (Jan *et al.* 1979). It has also been demonstrated that substance P is able to elicit a slow excitatory postsynaptic potential in the sympathetic neurons of the guinea-pig inferior mesenteric ganglion (Dun & Karczmar 1979; Konishi *et al.* 1979; Krier & Szurszewski 1979; Konishi *et al.* 1980), mimicking the function of an endogenous transmitter (Neild 1978); and Konishi *et al.* (1980) have shown that potassium depolarization induces a calcium-dependent release of substance P from the inferior mesenteric ganglion, and have produced evidence to indicate that much of the intraganglionic substance P may originate centrally to the ganglion.

There are three possible sources of substance P immunoreactive networks in the prevertebral ganglia.

(i) Substance P is associated with primary sensory neurons (Lembeck & Zetler 1962) and has been demonstrated immunohistochemically in neurons of the cranio-spinal sensory ganglia (Hökfelt *et al.* 1975; Del Fiacco & Cuello 1980) and also in peripheral branches of these neurons (Cuello *et al.* 1978); and sensory nerve fibres from the alimentary tract are known to traverse the prevertebral ganglia on their way to related levels of the spinal cord (Gabella 1976).

(ii) On the other hand, substance P immunoreactive neurons and networks are profusely present in the enteric nerve plexuses, where substance P can produce similar slow depolarizations (Katayama & North 1978; Katayama *et al.* 1979). These could also contribute to intraganglionic substance P immunoreactive nerve networks in the prevertebral ganglia, which receive inputs from these plexuses (Kuntz 1938, 1940; Kuntz & Saccomanno 1944; Ungváry & Léranth 1970).

(iii) A third possible source of such networks is the intermediate zone of the spinal cord, from which the preganglionic nerve fibres originate and in which a few substance P-containing neurons have been demonstrated following colchicine treatment (Ljungdahl *et al.* 1978).

It is therefore essential to establish the origin of the intraganglionic substance P immunoreactive networks in the prevertebral ganglia, to arrive at an understanding of the role that may be played by this peptide in the sympathetically mediated regulation of gastrointestinal function. The following experiments were therefore undertaken with these considerations in mind. Preliminary reports have already been presented (Baker *et. al.* 1980; Matthews & Cuello 1982). The findings there described indicate that the substance P immunoreactive networks are almost totally of sensory origin. The present account confirms and substantiates these observations and presents further evidence on the pattern, nature and distribution of substance P immunoreactive nerve elements in the prevertebral ganglia and in the following associated structures: thoracic and lumbar splanchnic nerves, intermesenteric nerve, colonic nerves, hypogastric nerves,

superior and inferior mesenteric arteries, lumbar sympathetic ganglia, dorsal root ganglia, spinal cord; and the nerve plexuses, serosa and associated vessels of the alimentary tract.

Materials and methods

Experimental procedures

Young albino guinea-pigs were used in all experiments.

Animals were anaesthetized by intraperitoneal injection of chloral hydrate (35 g l^{-1} in distilled water) and were perfused through the heart at room temperature (20–22 °C) with 40 g l^{-1} paraformaldehyde, freshly depolymerized, in 0.1 M sodium phosphate buffer (pH 7.2–7.4), preceded by a brief wash with oxygenated Krebs–Henseleit solution to wash out blood. Tissues to be examined were dissected out and were placed in fresh fixative for a period of 2.5–3 h; according to experimental protocol, these included: coeliac–superior mesenteric ganglion complex and inferior mesenteric ganglion, with associated nerves and vessels in each case, lumbar sympathetic ganglia, dorsal root ganglia, spinal cord, mesenteries, samples of terminal ileum.

The fixed tissues were then washed with three changes of 0.1 M phosphate buffer containing 50 g l^{-1} sucrose at 4 °C, for a minimum of 3 h. Cryostat (Dittes, Heidelberg) sections from ganglia (including attached vessels and nerves) and the spinal cord were cut at 8 μm and were collected on gelatinized glass slides. Pieces of ileum were dissected into their component layers and immunostained according to the method described by Costa & Furness (1982). Mesenteries were similarly immunostained and mounted without further dissection.

For all tissues localization of specific substance P-like immunofluorescence was performed by the indirect technique, using a monoclonal anti-substance P antibody (NCl/34-HL) (Cuello *et al.* 1979). Dopamine β-hydroxylase (DβH)-like immunofluorescence was similarly localized, using a well characterized antibody (Rush 1982). All antibodies were diluted in phosphate-buffered saline, pH 7.2 (p.b.s.) containing 2 g l^{-1} Triton-X-100. Preincubation, intervening and last washes were performed with the same solution.

Dilutions were, for the monoclonal anti-substance P antibody, 1 in 200; for the anti-DβH antibody, 1 in 100 and for the fluorescein isothiocyanate conjugated anti-rat immunoglobulin G and anti-rabbit immunoglobulin G (Miles), 1 in 10. Tissues were exposed to the first antibody overnight at 4 °C and to the second antibody either for 1 h at 37 °C or for 2 h at room temperature, and were mounted in a 3:1 glycerol-p.b.s. mixture. Control preparations were run in the absence of primary antibody or by preabsorbing it with 200 ng of substance P per ml. The preparations were viewed in a Leitz epifluorescence microscope and photographed on Kodak Tri-X film. A Leitz Variomat system was used for the photomicrography, and the same exposure intervals were employed throughout, that is for both experimental and control material.

Nerve lesions

In male guinea-pigs of masses 250–700 g the lumbar splanchnic, intermesenteric, colonic or hypogastric nerves (figure 1) were exposed at laparotomy and were cut at a distance of a few millimetres, not less than 4–5 mm, from the inferior mesenteric ganglion, alone or in various combinations, with aseptic precautions under anaesthesia induced by intraperitoneal injection of chloral hydrate. Care was taken not to injure the blood vessels of the region. Four or six days later the animals were re-anaesthetized and perfused as described above. Coeliac–superior

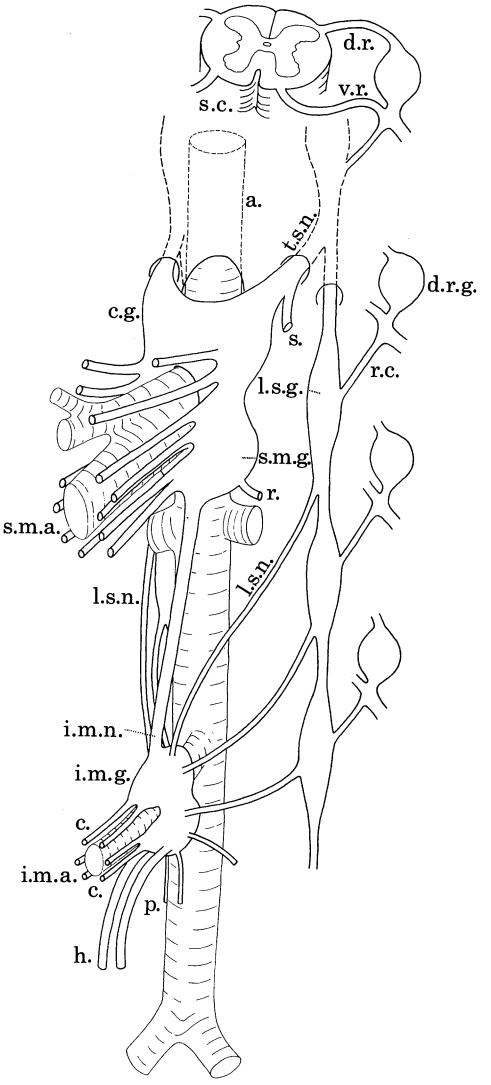

FIGURE 1. Schematic diagram to show the general arrangement of the prevertebral ganglia and principal associated nerves, their connections with the sympathetic chain, spinal cord and ganglia and their relation with the major arteries. (Not to scale.) c.g., Coeliac ganglion; s.m.g., superior mesenteric ganglion (note incomplete separation); i.m.g., inferior mesenteric ganglion; l.s.g., lumbar sympathetic ganglion; d.r.g., dorsal root ganglion; s.c., spinal cord; d.r., dorsal root; v.r., ventral root; r.c., ramus communicans (only one shown at each level), t.s.n., thoracic splanchnic nerve; s., suprarenal branch; l.s.n., lumbar splanchnic nerve; r., aortico-renal nerve; i.m.n., intermesenteric nerve; c., colonic nerves; h., hypogastric nerves; p., caudal (pelvic) branches; a., aorta; s.m.a., superior mesenteric artery; i.m.a., inferior mesenteric artery.

mesenteric and inferior mesenteric ganglia were dissected out for examination. In three preliminary experiments all nerves associated with the inferior mesenteric ganglion were cut except the lumbar splanchnic or colonic nerves. In the main series of experiments, groups of five guinea-pigs were used: one animal served as unoperated control, and in each of the remaining two pairs a different set of nerves was cut; four experiments of each kind were performed. In a further series of three animals the lumbar splanchnic and intermesenteric nerves, with or without the hypogastric nerves, were cut and the animals were allowed to survive from 46 h to 3.8 d; these animals served also for comparison with the results of spinal cord lesions, as described below.

Capsaicin injections

Guinea-pigs weighing 340–400 g were injected subcutaneously with capsaicin under cover of theophylline injections and isoprenaline aerosol, according to the dosage and schedule described by Gamse *et al.* (1981). Control animals were injected with theophylline and with the vehicle used for the capsaicin injections, on the same schedule. Six days after the last injection the animals (three in each category) were anaesthetized and were fixed by perfusion as described above. The inferior mesenteric and coeliac–superior mesenteric ganglia, spinal ganglia, the spinal cord and pieces of ileum and the mesentery were taken for determination of specific substance P immunofluorescence.

Spinal cord lesions

Spinal cord lesions were performed according to the general procedure described by Zelená & Soukup (1974). Guinea-pigs of masses 75–250 g, of ages ranging from a few days to a few weeks postnatally, were used. Anaesthesia was induced by intraperitoneal chloral hydrate and continued with ether. Laminectomy was performed from the mid-thoracic level to the sacrum, the spinal cord was divided between ligatures at the level of the T7 segment, or between T7 and T8, and the caudal section of the spinal cord was excised. Care was taken not to injure the spinal ganglia. After careful haemostasis the erector spinae muscles were approximated over the vertebral canal by a continuous suture, and the skin wound was closed. Postoperatively the animals were kept in a warm environment in cotton wool, with hay, food pellets and water containing 5 g l^{-1} ascorbic acid readily accessible. Urine was gently expressed from the bladder at regular intervals.

In four animals, survivals of 24 h, 2.5 d, 4 d and 5.5 d were obtained, at the end of which tissues were fixed for immunofluorescence either by *in vivo* perfusion under anaesthesia or by immersion fixation shortly after death. Normal control animals were perfused at the same time. To control for the rate of disappearance of substance P immunoreactivity after nerve lesions, in two guinea-pigs the lumbar splanchnic and intermesenteric nerves were cut and the animals were fixed after approximately 2.5 and 3.5 d respectively; a third animal was perfused 46 h after the intermesenteric, lumbar splanchnic and hypogastric nerves had been cut. In these three experiments the lumbar splanchnic nerves were divided as close to the vertebral column as possible. The prevertebral ganglia, spinal ganglia and lumbar paravertebral ganglia were processed for specific substance P immunofluorescence as described above, together with spread preparations of mesentery, omentum and mesocolon.

Reconstruction from tracings

In some of the nerve lesion experiments, tracings were made on acetate film from photographic montages of the inferior mesenteric ganglion sections to show either the non-varicose substance P immunoreactive (s.P i.) fibres of the largest calibre, in the case of hypogastric nerve lesions, or all surviving intraganglionic s.P i. elements, in the case of the lumbar nerve lesions. These were then superimposed by a best fit method to reconstruct the distribution of the s.P i. elements studied.

Results

Normal prevertebral ganglia

The general arrangement and nerve connections of these ganglia are reproduced diagrammatically in figure 1. Non-varicose, axon-like s.P i. nerve fibres and varicose s.P i. nerve networks were found in all the prevertebral ganglia, as earlier illustrated by Hökfelt *et al.* (1977), but there were certain differences between them. Substance P immunoreactive neurons were not seen in these ganglia, with the exception of one cell found in one animal in the superior mesenteric ganglion (figure 3*f*).

Coeliac–superior mesenteric ganglion

The thoracic splanchnic nerves entering the ganglion complex contain many non-varicose s.P i. nerve fibres of axon-like character (figure 2*a*, plate 1). Within the coeliac half of the ganglion these split up into a number of compact brightly immunofluorescent fascicles which run in relatively straight but divergent courses throughout the ganglion complex (figure 2*c*, *f*). Both halves of the ganglion are also permeated by networks of varicose, strongly s.P i. fibres, branching among the neurons and surrounding them in a loose-meshed reticulum (figure 2 and figure 3, plate 2). In some places there are indications that these arise from the bundles of non-varicose fibres (figures 2 and 3). Non-varicose s.P i. fibres radiate away in the branches leaving the caudodistal margin of each subdivision of the ganglion, and form also a substantial contingent of the fibres in the intermesenteric nerve which connects the ganglia with the inferior mesenteric ganglion. In the superior mesenteric half of the ganglion the bundles of non-varicose s.P i. fibres which traverse the ganglion tend to be smaller than in the coeliac ganglion (figure 2) and the fibres may be themselves of smaller calibre and, or, less intensely immunofluorescent.

Inferior mesenteric ganglion

In the inferior mesenteric ganglion s.P i. non-varicose nerve fibres are seen in all four sets of nerves entering and leaving the ganglion (figure 4, plate 3), that is, in the lumbar splanchnic, intermesenteric, colonic and hypogastric nerves. The lumbar splanchnic nerves usually seem to have the most intensely immunofluorescent and largest diameter fibres; they also have the highest density of s.P i. nerve fibres. The other nerves have s.P i. fibres of apparently slightly lesser calibre and a less intense, sometimes grainy immunofluorescence, with those of the colonic nerves being possibly of least calibre, and those of the intermesenteric nerve being at lowest density. In all the nerves close to the ganglion the s.P i. nerve fibres are diffusely dispersed, rather than locally grouped or clustered (figure 4). Both the hypogastric nerves and the intermesenteric nerves on occasion seem to contain a few varicose or beaded nerve fibres also, though this appearance is difficult to differentiate from grainy immunofluorescence.

From the entry of the lumbar splanchnic nerves, intensely s.P i. nerve fibres spread distalward for a short distance along the border of the inferior mesenteric ganglion and penetrate it in larger or smaller bundles. Other large coherent bundles run right across the ganglion, particularly between the lumbar splanchnic and hypogastric nerves (figure 5, plate 4) but also toward the colonic nerves (figure 4*a*). Groups of varicose branching nerve fibres emerge at right angles from these bundles and enter the intraganglionic networks (for example, figure 8*a*, *b*). S.P i. nerve fibres of intermediate calibre also spread out from or converge upon the bases of the hypogastric nerves, (i) turning toward the entry zone of the lower lumbar splanchnic nerves;

(ii) streaming round the arterial margin of the inferior mesenteric ganglion toward the intermesenteric nerve and upper lumbar splanchnic nerves (or vice versa) in the flattened interganglionic bands which connect the two ganglionic lobes round the inferior mesenteric artery (figure 1). Non-varicose nerve fibres run in small bundles throughout the ganglion, principally in an oblique dorsoventral, craniocaudal direction, that is, from lumbar splanchnic nerves and intermesenteric nerve to colonic and hypogastric poles. Finer varicose or beaded s.P i. nerve fibres composed of fine intervaricose segments and brilliantly fluorescent varicosities form perineuronal networks throughout the inferior mesenteric ganglion except within areas of the 'small intensely fluorescent cells' of catecholamine fluorescence microscopy (s.i.f. cells) which usually form one or several compact peripherally situated masses, often near to one or other pole of one or both of the ganglion subdivisions, and sometimes in the base of the intermesenteric nerve. In s.i.f. cell groups no more than an occasional solitary fibre or minute fascicle of s.P i. fibres is seen, penetrating part way in from the margin of the group toward its centre or traversing it in company with a blood vessel (figure 12).

Lumbar paravertebral ganglia

At L3 level brilliantly s.P i. varicose nerve networks are focally concentrated in one or more regions of the ganglion, and scanty networks with smaller or less strongly immunoreactive varicosities are present elsewhere (figure 3*a*). Non-varicose s.P i. nerve fibres traverse the ganglion, mainly in coherent bundles, and become more closely packed in the interganglionic trunk and rami.

In all these ganglia the mesh of the perineuronal networks is relatively open-textured, composed mainly of single strands; and individual varicose nerve fibres are seen to form trails extending through the perisomatic territories of a number of neurons, criss-crossing through several meshes (figure 3). There are seldom appearances suggesting pericellular baskets (as in figure 26*a*, *b*); rather the impression is of a loosely woven network of spreading fibres (figure 3*b–e*). Points of branching are sometimes seen, though the relatively thin cryostat sections (8 μm) do not often reveal them (figures 3, 20*d*, 27). Examples are seen of the possible origin of varicose trails and side branches from non-varicose fibres (figure 3*b*). The network is somewhat elongated along the long axis of the ganglion, its meshes conforming approximately to the dimensions of the neurons, being one or two neurons wide and, or, long, and the fibres running in the interneuronal neuropil, branching occasionally but not profusely. Individual fibres curl round the neurons and conform closely to their contour. The varicosities appear to range in diameter from somewhat less than one half to 1–2 μm and are intensely immunofluorescent. The intervaricose segments of the trails are very slender with a diameter distinctly less than that of the non-varicose nerve fibres, but they tend to fluoresce more brightly so they are clearly resolvable when in critical focus. The fluorescence in the non-varicose nerve fibres has a somewhat granular texture and is generally fainter than in the varicose trails (figure 3). A somewhat beaded or granular fluorescence in a non-varicose fibre might thus, in the absence of more brightly fluorescent varicose fibres for comparison, be mistaken for a truly varicose fibre. Interpretations were made cautiously, with this possibility in mind.

Consequences of nerve lesions

In two preliminary experiments nerves were cut simultaneously in the following combinations: colonic, intermesenteric and hypogastric; lumbar splanchnic, intermesenteric and hypogastric.

These were expected to have the effect of completely disconnecting the inferior mesenteric ganglion from the periphery and from the central nervous system respectively. The first of these combinations of nerve lesions gave rise to accumulations of s.P i. material in the ganglionic stumps of the colonic and hypogastric nerves and in the cranial stump of the intermesenteric nerve, with persistence of s.P i. nerve elements in the inferior mesenteric ganglion, whereas the second combination of lesions led to total depletion of s.P i. from the inferior mesenteric ganglion and from the colonic nerves that had been left intact. There was however in each case a certain amount of tissue reaction which displaced and distorted the anatomical relations; and it was suspected that the vascular supply of the ganglion might have been disturbed, since all of these nerves, and notably the lumbar splanchnic and intermesenteric nerves, regularly contain sizeable blood vessels which might contribute to the blood supply of the ganglion (figures 2, 4, 5*e*). In view of the possibility that the blood supply of the ganglion might be impaired by such extensive lesions it was decided to investigate the various nerves singly, one category at a time.

Intermesenteric nerve lesions

The ganglionic stump of the intermesenteric nerve, when it was located in the cryostat sections, was depleted of s.P i. non-varicose nerve fibres but showed in two examples a few s.P i. varicose trails (figure 5*e*). Its cranial strump showed a massive accumulation of s.P i. material in distended nerve fibres (figure 5*c*), which extended proximally almost to the superior mesenteric ganglion. The networks in the inferior mesenteric ganglion were in all experiments still brightly fluorescent (figure 5*a*, *b*) and did not seem grossly altered or thinned except in the immediate neighbourhood of the entering intermesenteric nerve stump, where there was some evidence of thinning-out of networks. All remaining ganglionic nerves (lumbar splanchnic, hypogastric, colonic) still contained s.P i. non-varicose nerve fibres, and no accumulations of s.P i. material were seen in these nerves or in the inferior mesenteric ganglion. Bundles of brightly fluorescent non-varicose fibres were still seen running through the ganglion and the inter-ganglionic bands from the bases of the hypogastric nerves toward the lumbar splanchnic nerves, or vice versa, (figure 5*b*), and between the lumbar splanchnic nerves and the colonic nerves. No change was seen in the innervation of the inferior mesenteric artery (figure 14*d*).

Hypogastric nerve lesions

These were performed furthest from the ganglion, at a distance of 6–7 mm. In all experiments the ganglionic stumps of the hypogastric nerves showed intensely fluorescent accumulations of s.P i. material in distended nerve fibres (figure 6, plate 5), which in some cases extended with diminishing calibre and intensity back into the inferior mesenteric ganglion and turned toward the lumbar pole of the ganglion and into the lumbar splanchnic nerves, or spread across toward and into the intermesenteric nerve.

The appearance of the varicose s.P i. networks in the ganglion was in some places indistinguishable from the normal, but in other places these networks seemed, regionally, to be less in evidence, especially in the neighbourhood of distended non-varicose s.P i. fibres (figure 6*f*, *g*). Apparent regenerative sprouting of s.P i. nerve fibres was seen from the cut ends of the hypogastric nerves, that is, from their ganglionic stumps (figure 6*a*). The colonic nerves did not show any of these abnormalities, and no distended s.P i. nerve fibres were associated with them.

In one experiment successive montages of the inferior mesenteric ganglion were traced to show the distended or accentuated non-varicose s.P i. fibres, as described in the experimental procedures. Comparison and superimposition of the tracings enabled the enlarged fibres to be followed back across the ganglion toward the entry points of the lumbar nerves and of the intermesenteric nerve.

Colonic nerve lesions

These were followed immediately by vigorous activity of the hindgut leading to complete and rapid emptying of faecal pellets from the distal colon, that is, there was evidence of removal of a tonic inhibitory influence, mediated via the inferior mesenteric ganglion. As in

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Description of plates 1–4

Figure 2. S.P immunofluorescence in the splanchnic nerves and prevertebral ganglia of normal guinea-pigs. (*a*) Thoracic splanchnic nerve, just below the diaphragm, oblique section; (*b*) lumbar splanchnic nerve fascicles, transverse and oblique sections: note central blood vessels, seen as filling defects. (*c*), (*f*) Coeliac ganglion ((*f*) is an enlargement partly overlapping with (*c*)); (*d*), (*g*) superior mesenteric ganglion; (*e*), (*h*) inferior mesenteric ganglion. In the coeliac ganglion, and from place to place in the inferior mesenteric ganglion, the s.P i. non-varicose nerve bundles are particularly large, coarse and strongly immunofluorescent. In the superior mesenteric ganglion the fibres of the bundles are of finer calibre or less strongly immunofluorescent. Single non-varicose fibres are seen to enter or leave the bundles from place to place. Varicose nerve networks are also present, permeating each ganglion, and in places the fibres forming these networks are seen to branch. Scales, 100 μm (*a*), (*c*)–(*e*); 50 μm (*b*), (*f*)–(*h*).

Figure 3. Characteristics of s.P i. nerve networks in normal pre- and paravertebral ganglia; and a single s.P i. neuron. (*a*) Third lumbar paravertebral ganglion; (*b*), coeliac ganglion; (*c*), (*d*), (*f*), superior mesenteric ganglion; (*e*), inferior mesenteric ganglion. Not all these ganglia were processed simultaneously, and this fact may account for some of the relative differences in brilliance of the immunofluorescence, notably between (*a*) and the others; the lumbar paravertebral ganglion in (*a*) appears, however, to have many particularly large s.P i. nerve varicosities. Note (i) the mesh size of the networks ((*b*), (*c*), (*e*)); (ii) the evidence of branching of networks ((*b*) to (*e*)); (iii) the apparent origin of varicose fibres from a non-varicose fibre (arrows in (*b*)). (*f*) A single example of what seems to be an s.P immunoreactive neuron, in the superior mesenteric ganglion. The cell is of small size and gives rise to at least three processes, which run parallel with other s.P i. fibres in this region of the ganglion. Scale, 50 μm.

Figure 4. Nerves associated with a normal inferior mesenteric ganglion and after cutting lumbar splanchnic nerves. (*e*) A montage showing a normal ganglion with bundles of intensely s.P i. non-varicose nerve fibres spreading through from the points of entry of lumbar splanchnic nerves (l.s.) toward and into the colonic nerves (c., and (*b*)) or vice versa. The normal lumbar splanchnic nerves ((*a*), fascicles in longitudinal section, with insets showing transverse sections) contain many intensely s.P i. non-varicose nerve fibres. They are traversed longitudinally by small vessels, enclosed within the fascicles and often centrally placed; the longitudinal and transverse sections show these as filling defects within the nerve bundles. The normal hypogastric nerves (h., and (*d*)) contain many non-varicose s.P i. nerve fibres of a rather lesser calibre or less intense s.P i.; and s.P i. non-varicose fibres with a somewhat grainy fluorescence are seen in the intermesenteric nerve (*g*). At 4 d after cutting the lumbar splanchnic nerves, fewer s.P i. nerve fibres are seen in the colonic nerves ((*c*), (*f*)) and hypogastric nerve ((*h*), (*i*)) but those which remain are of normal calibre. In all figures note 'filling defects' produced by blood vessels within the nerves. S.P i. nerve fibres are seen intimately associated with some of these vessels. Scales, 50 μm (*a*)–(*d*), (*f*)–(*i*); 100 μm (*e*).

Figure 5. (*a*), (*b*) Sections of inferior mesenteric ganglia following section of the intermesenteric nerve 4 d previously (see inset in (*a*)). Non-varicose and varicose s.P i. nerve fibres and networks show little difference from the normal. (*a*) S.P i. nerve fibres still cross the ganglion in the interganglionic bands to enter the hypogastric nerves (h.). (*b*) S.P i. nerve fibres are still seen in the colonic nerves (c.); here, note the brightness of the s.P i. of the lumbar splanchnic nerves (l.s.).

(*c*)–(*e*) Stumps of intermesenteric nerve, 4 d after section of the nerve. (*c*) S.P i. accumulations in grossly distended nerve fibres in proximal stump; (*d*), virtual disappearance of s.P i. nerve fibres from cut end of distal stump; (*e*), level of entry of intermesenteric nerve into inferior mesenteric ganglion, showing a few beaded s.P i. nerve fibres, which might either be pre-existing fibres of distal origin or newly formed sprouts from intra-ganglionic s.P i. nerve fibres. Note, in the lower part of the figure, filling defects associated with blood vessels entering or leaving the ganglion. Scales, 100 μm (*a*)–(*d*); 50 μm (*e*).

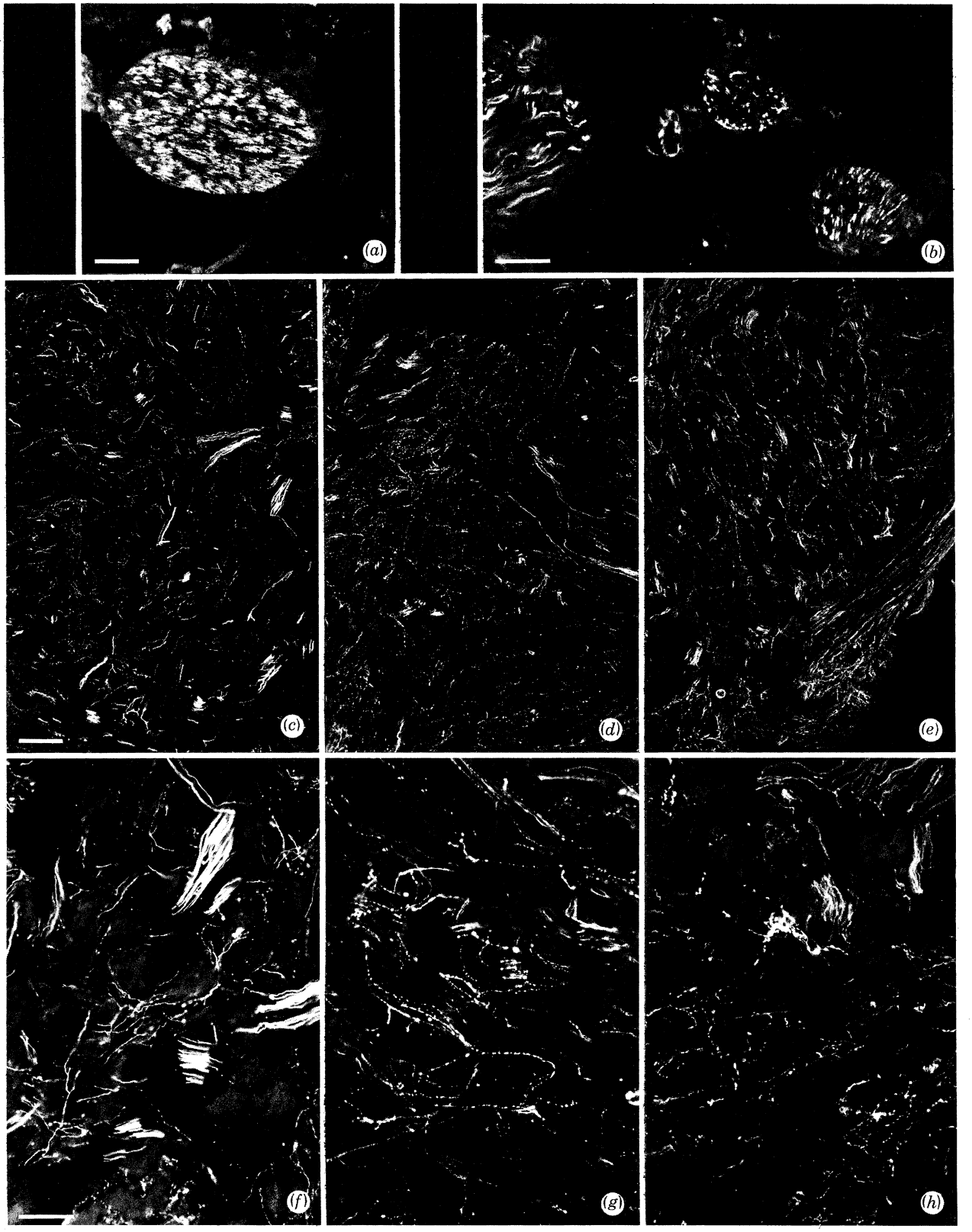


FIGURE 2. For description see opposite.

(*Facing p.* 256)

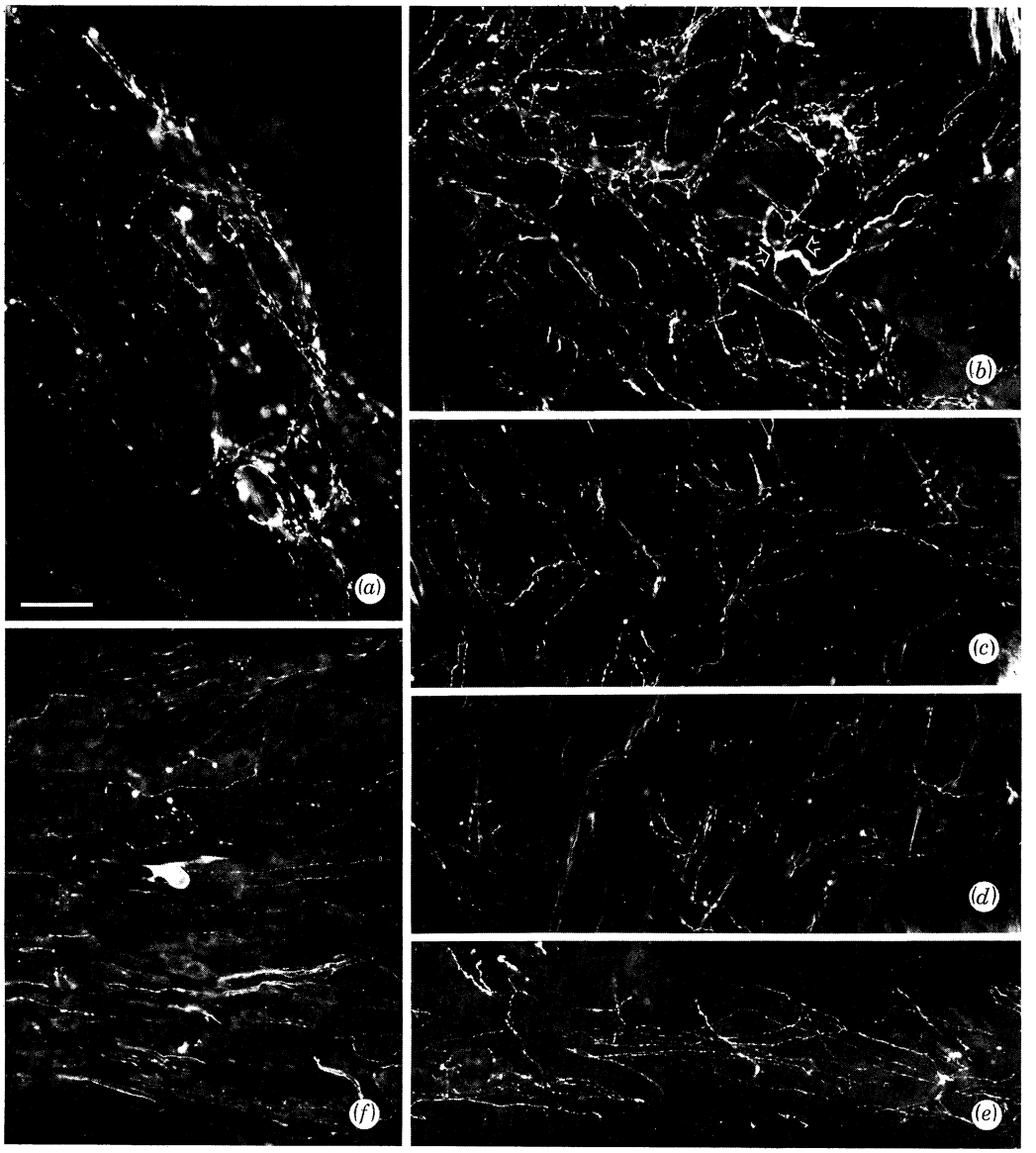
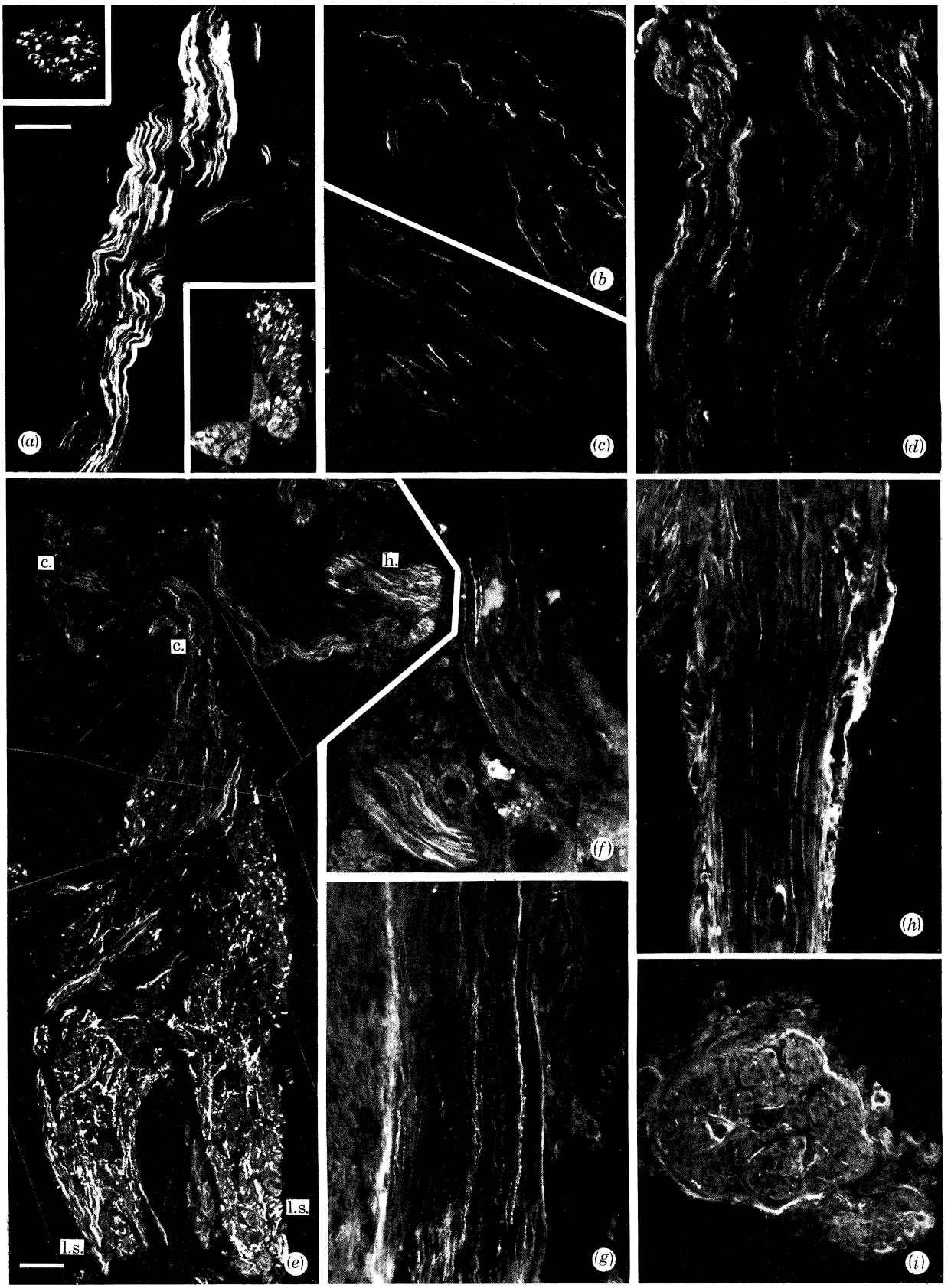


FIGURE 3. For description see p. 256.



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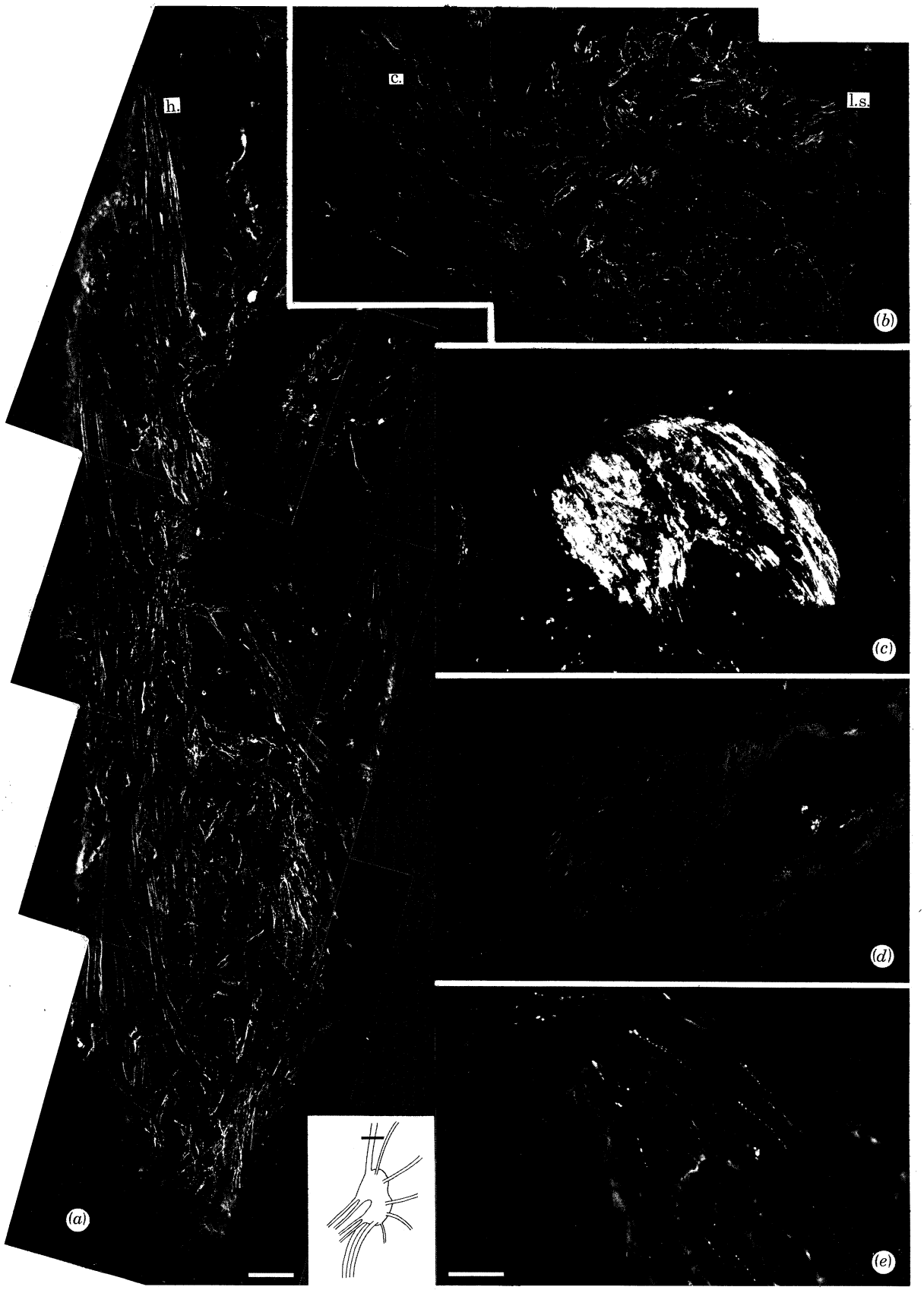

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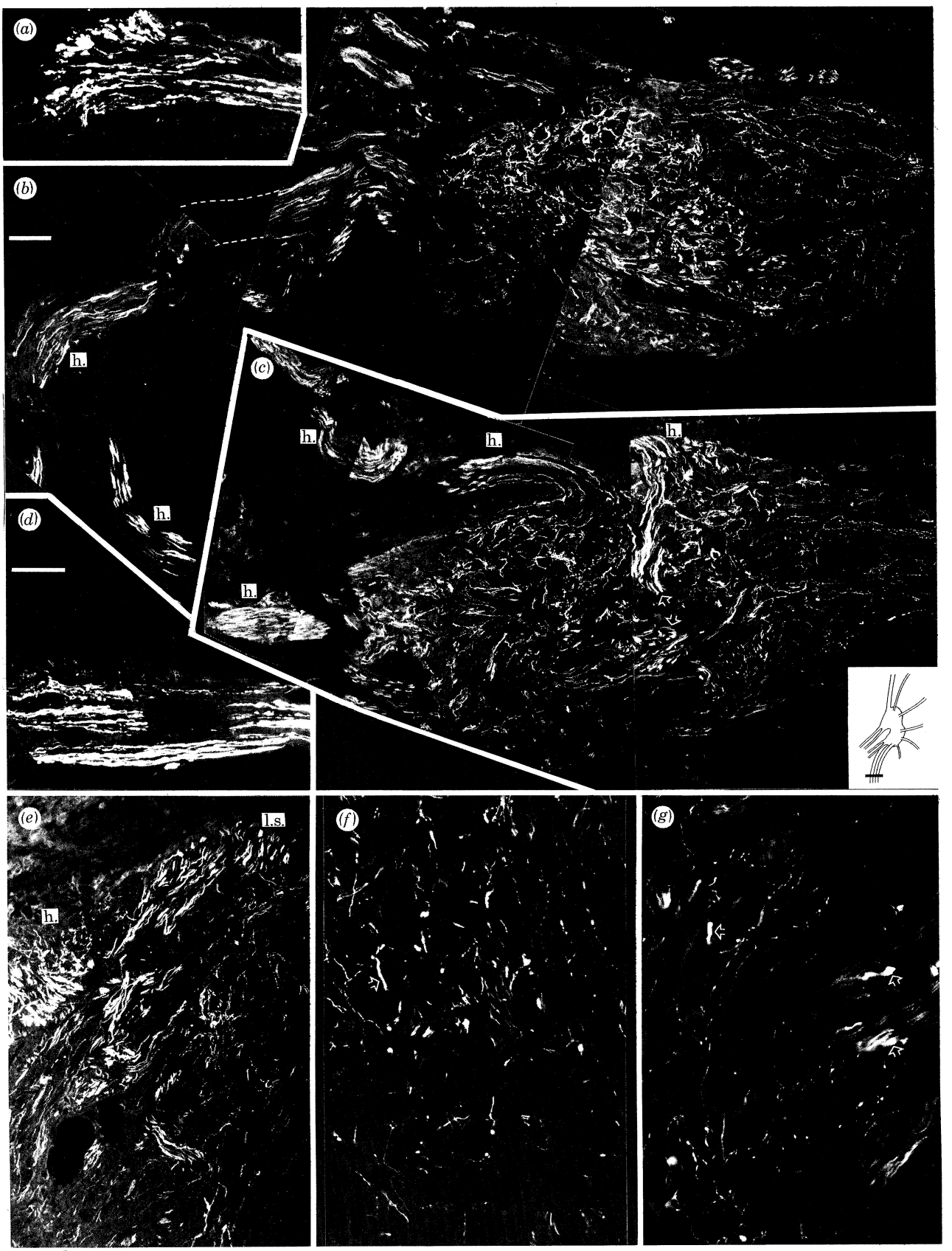

FIGURE 6. For description see p. 257.

FIGURE 7. For description see p. 257.

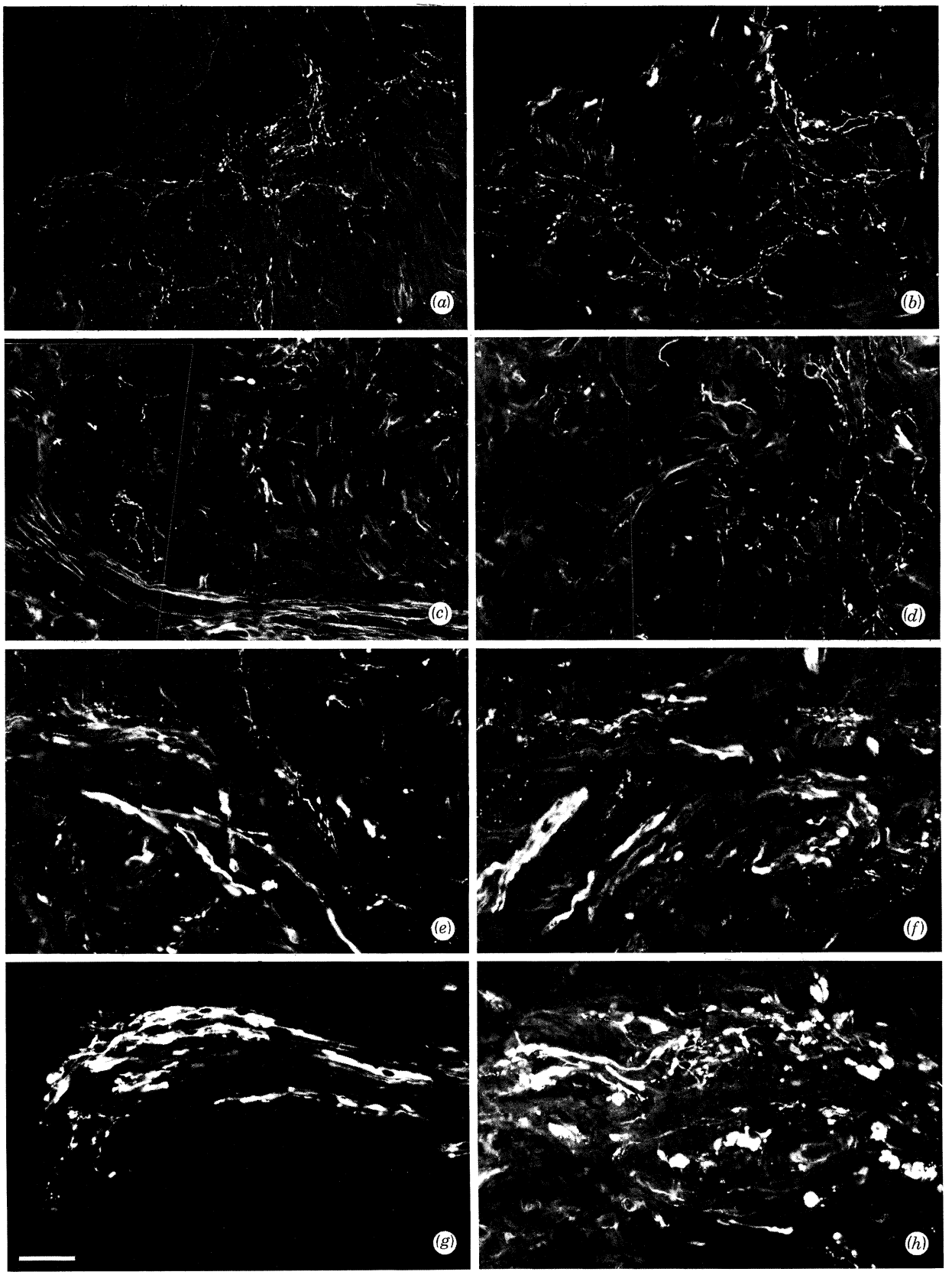

FIGURE 8. For description see p. 257.

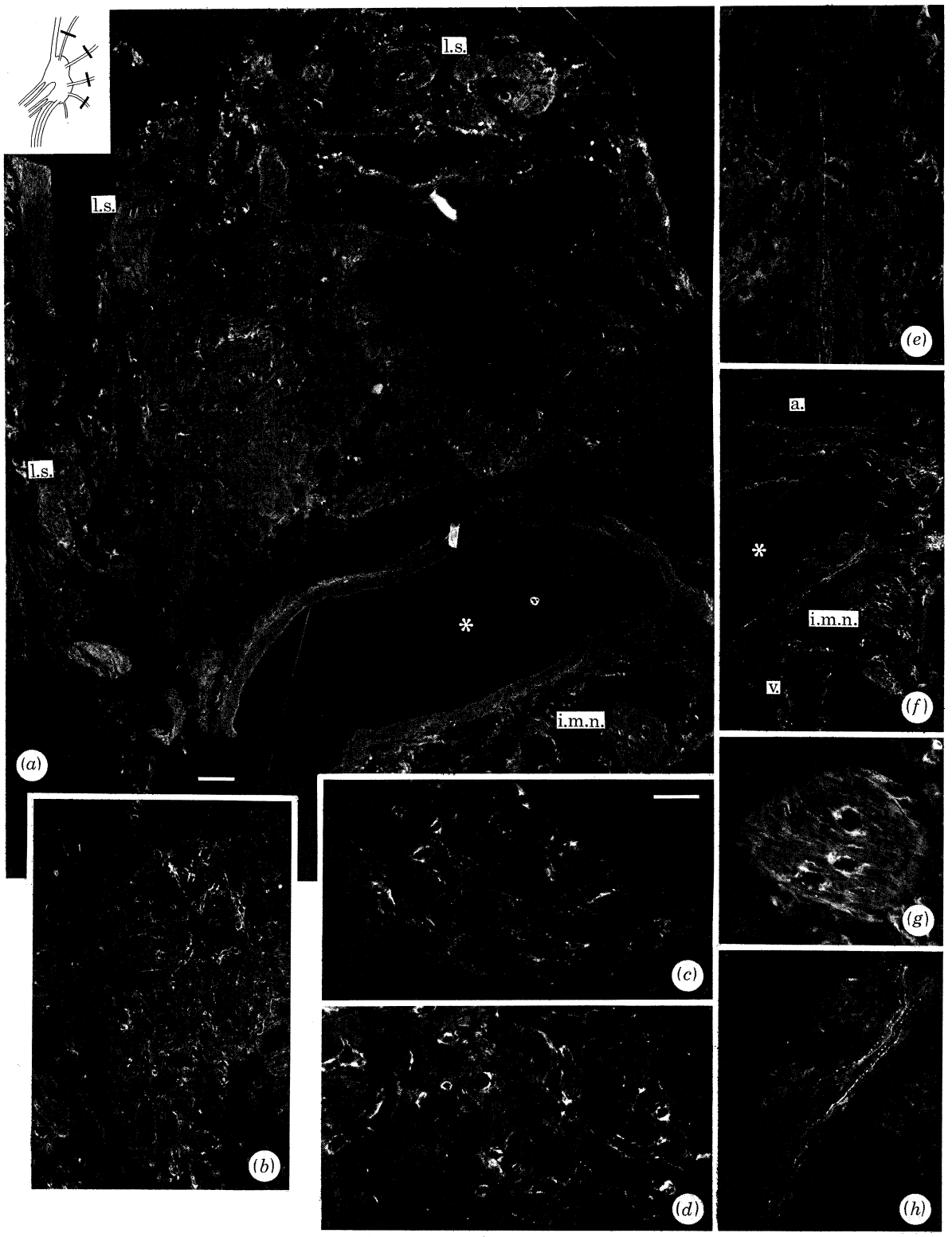

FIGURE 9. For description see opposite.

Description of plates 5–8

Figure 6. Consequences of hypogastric nerve lesions (see inset in (c)). (*a*), (*d*) Massive accumulation of s.P i. material in ganglionic stumps of hypogastric nerves, 4 d after nerve division. The free end of the nerve shown in (*a*) gives evidence of possible sprouting of some fibres, being somewhat splayed out, with s.P i. material extending into delicate cytoplasmic processes.

(*b*), (*c*) Montages of sections through the inferior mesenteric ganglion, same experiment as in (*a*) and (*d*), showing intensely s.P i. nerve fibres of increased calibre in the more proximal parts of the hypogastric nerves (h.), up to and entering the ganglion. Similar enlarged s.P i. non-varicose fibres were traceable in successive sections toward the bases of the lumbar splanchnic nerves entering the caudal lobe of the ganglion, and into two bundles (arrowed in (*c*)) passing toward the cranial lobe of the ganglion, which receives the remaining lumbar splanchnic nerves and the intermesenteric nerve.

(*e*)–(*g*) Another ganglion after a similar lesion. (*e*) Enlarged s.P i. nerve fibres entering via a hypogastric nerve (h.) and traceable into the base of a lumbar splanchnic fascicle of the same ganglionic lobe (l.s.), also into bundles which cross the ganglion. Varicose s.P i. nerve networks are scanty among the enlarged hypogastric nerve fibres but appear normal near the lumbar pole to the right. (*f*) Area at higher magnification from mid-lobe region showing enlarged non-varicose s.P i. nerve fibres (for example, arrow) dispersed in the ganglionic tissue, more numerous near the hypogastric pole (upper half of (*f*)) and local paucity of varicose s.P i. nerve networks. (*g*) Base of a lumbar splanchnic fascicle (on right) showing gross enlargement of some of its s.P i. nerve fibres (arrows), and normal-looking varicose s.P i. nerve networks in the adjacent area of ganglion (on left), together with some enlarged s.P i. nerve fibres (for example, arrow). Scales, 50 μm (*a*), (*d*), (*f*), (*g*); 100 μm (*b*), (*c*), (*e*).

Figure 7. (*a*), (*b*), (*d*) Montages of sections from inferior mesenteric ganglia, 4 d after cutting colonic nerves (see inset in (*b*)); (*a*) and (*d*) are from the same ganglion.

These show (i) accumulations in the ganglionic stumps of the divided colonic nerves (c.) and in the colonic pole of the ganglion (*, (*a*) and (*d*)), (ii) normal s.P i. in non-varicose nerve fibres in hypogastric nerve (h.), and (iii) convergence of distended s.P i. nerve fibres on the bases of the lumbar splanchnic nerves (l.s.; arrows in (*a*)) and intermesenteric nerve (i.m.n.; crossed arrows in (*b*)). Varicose s.P i. networks are seen near the entry of the hypogastric nerve and near the bases of the lumbar nerves and intermesenteric nerve but not close to the colonic pole where distended non-varicose s.P i. fibres predominate. No s.P i. non-varicose nerve fibres or networks are seen in an area of s.i.f. cells (s.) close to the lumbar pole in (*a*). (*c*) Endogenous catecholamine fluorescence seen in a semi-serial no-antibody control section of the same s.i.f. cell group. Scale, 100 μm.

Figure 8. Alterations in s.P i. nerve fibres following lesions of colonic nerves. (*a*) Normal inferior mesenteric ganglion: entry of lumbar splanchnic nerves, showing faint granular s.P i. in some non-varicose nerve fibres, and varicose s.P i. networks in adjacent ganglionic area; control for (*b*). (*b*) Corresponding region of a ganglion, 4 d after section of colonic nerves. Some of the s.P i. fibres in the lumbar splanchnic nerves and adjacent area of ganglion are grossly distended. The varicose s.P i. nerve networks in the adjacent ganglionic tissue show in places some suggestion of enlarged varicosities or branch points, but are otherwise within normal limits.

(*c*), (*d*) Regions of two different inferior mesenteric ganglia, 4 d after colonic nerve section. In (*c*) the colonic pole is to the right; in (*d*) it is to the left. In the colonic half of each figure, s.P i. varicose nerve networks are absent or much less in evidence, and s.P i. non-varicose fibres are enlarged and increased, showing a brighter than usual but still fairly granular s.P i. The line of demarcation is relatively sharp. Varicose s.P i. networks are still present near the lumbar poles. In (*c*) note a persistent basket-like formation round a neuron, and enlarged s.P i. non-varicose fibres in a lumbar-directed fascicle along the border of the ganglion (lower margin of the figure).

(*e*), (*f*) Enlarged s.P i. nerve fibres running through from colonic (left) to lumbar poles ((*f*), upward and to right) and from colonic pole toward the base of the intermesenteric nerve ((*e*), downward and to right), 4 d after cutting colonic nerves. In some places the fluorescence of the accumulations is seen to be granular; in others it is of such high intensity that it becomes confluent.

(*g*), (*h*) Apparent sprout formation from stumps of divided colonic nerves, 4 d after lesion. Cut ends are to left. Scale, 50 μm.

Figure 9. Montage and single views of sections of the inferior mesenteric ganglion which was the most heavily depleted at 4 d after section of the lumbar splanchnic nerves (see inset in (*a*)). This ganglion had its major lobe caudal to the inferior mesenteric artery. (*a*) Ganglionic montage showing major lobe of ganglion with the ganglionic stumps of many associated lumbar splanchnic nerve fascicles (l.s.), empty of s.P i., the inferior mesenteric artery (*) with evidence of persistent s.P i. innervation, and a larger nerve identified as the intermesenteric nerve (i.m.n.). (*b*) Sequential section of the same lobe. (*c*), (*d*) Sections of the minor and major lobes respectively, at higher magnification, to show the paucity of s.P i. elements in this ganglion: perivascular and interstitial connective tissue shows some non-specific fluorescence. (*e*)–(*h*) Other nerves associated with the ganglion: (*e*) longitudinal section of hypogastric nerve showing a single s.P i. nerve fibre; (*f*) semi-serial section of inferior mesenteric artery (part, *) showing the intermesenteric nerve (i.m.n., enlarged in (*g*)) containing few s.P i. nerve fibres, and a small para-arterial nerve fascicle (enlarged in (*h*)), containing a number of brightly fluorescent s.P i. varicose nerve fibres, possibly associated with the innervation of the artery. An adjacent vein (v.) and smaller artery (a.) also show points of s.P i. innervation. Scales, 100 μm (*a*), (*b*), (*f*); 50 μm (*c*)–(*e*), (*g*), (*h*).

the case of the hypogastric nerves, the ganglionic stumps of the colonic nerves in all experiments, whether at 4 d or at 6 d postoperatively, showed massive accumulations of s.P i. material, with gross distension and some distortion of nerve fibres (figure 7, plate 6), which extended back (that is, proximally) into the colonic poles of the inferior mesenteric ganglion and there eclipsed or replaced the intraganglionic varicose networks (figure 7*d*). The latter were much less in evidence than usual in the colonic pole of the ganglion, but were still seen in the caudal half of the ganglion near the entry of the hypogastric nerves and also near both lumbar poles (figure 8, plate 7). In these regions further from the entry of the colonic nerves and near the entry of the hypogastric nerves the appearance of the intraganglionic varicose s.P i. networks was still within the normal range.

Intense s.P i. and increased calibre were evident in some fibres in the lumbar splanchnic nerves, and s.P i. nerve fibres of increased calibre were traceable across from the colonic poles to the lumbar splanchnic and also into the intermesenteric nerve (figure 7). Other s.P i. nerve fibres in these nerves remained of normal appearance (figure 8*b*). The hypogastric nerves did not show any abnormalities. In intermediate regions of the ganglion, enlarged non-varicose fibres were seen without accompanying varicose networks (figure 8*c–f*). A certain amount of crossing of the enlarged non-varicose fibres was observed (figure 8*e*). In some of these regions trails of varicosities were found which appeared to be of reduced fluorescence intensity though not of reduced calibre. No change was seen in the innervation of the inferior mesenteric artery (figure 14*i*).

Lumbar splanchnic nerve lesions

The inferior mesenteric ganglion was severely but variably depleted both of non-varicose s.P i. nerve fibres and of varicose s.P i. networks. The ganglionic stumps of the cut lumbar splanchnic nerves were totally depleted of non-varicose s.P i. fibres. In the ganglion a few non-varicose nerve fibres, mostly slender, and occasional short varicose trails of s.P i. networks persisted, apparently more or fewer according to the numbers of non-varicose fibres seen in the intermesenteric nerve, and correspondingly some non-varicose fibres were seen in the colonic and hypogastric nerves. The severest depletion left the ganglion almost totally empty of s.P immunoreactivity (figure 9, plate 8). In three other ganglia some s.P-positive nerve fibres were still seen and it was possible to observe that their trend lay from the intermesenteric nerve toward the hypogastric and colonic nerves, not from the hypogastric to the colonic nerves nor from any of these toward the lumbar splanchnic nerves. Examination of all available sections in each experiment (one section in three was taken for s.P control incubations) permitted all the classes of nerves attached to the inferior mesenteric ganglion to be identified, and in ganglionic montages it was evident that the regions of the ganglion closest to the lumbar nerve stumps were the most severely depleted and in some regions totally empty of s.P i. material, whereas further away, and particularly in the bands connecting the two lobes of the ganglion round the inferior mesenteric artery, non-varicose s.P i. fibres were traceable from the intermesenteric nerve through into the colonic and hypogastric nerves (figure 10). In one case, one or possibly two colonic nerve fascicles were evidently accidentally pinched at operation. In these fascicles there was accumulation of s.P i. material in the ganglionic stumps and in some non-varicose fibres in the adjacent region of the ganglion, traceable toward the intermesenteric nerve. One small lumbar splanchnic fascicle was seen to join the intermesenteric nerve higher up, and to have escaped division at operation. In all these experiments, it was in those regions of the

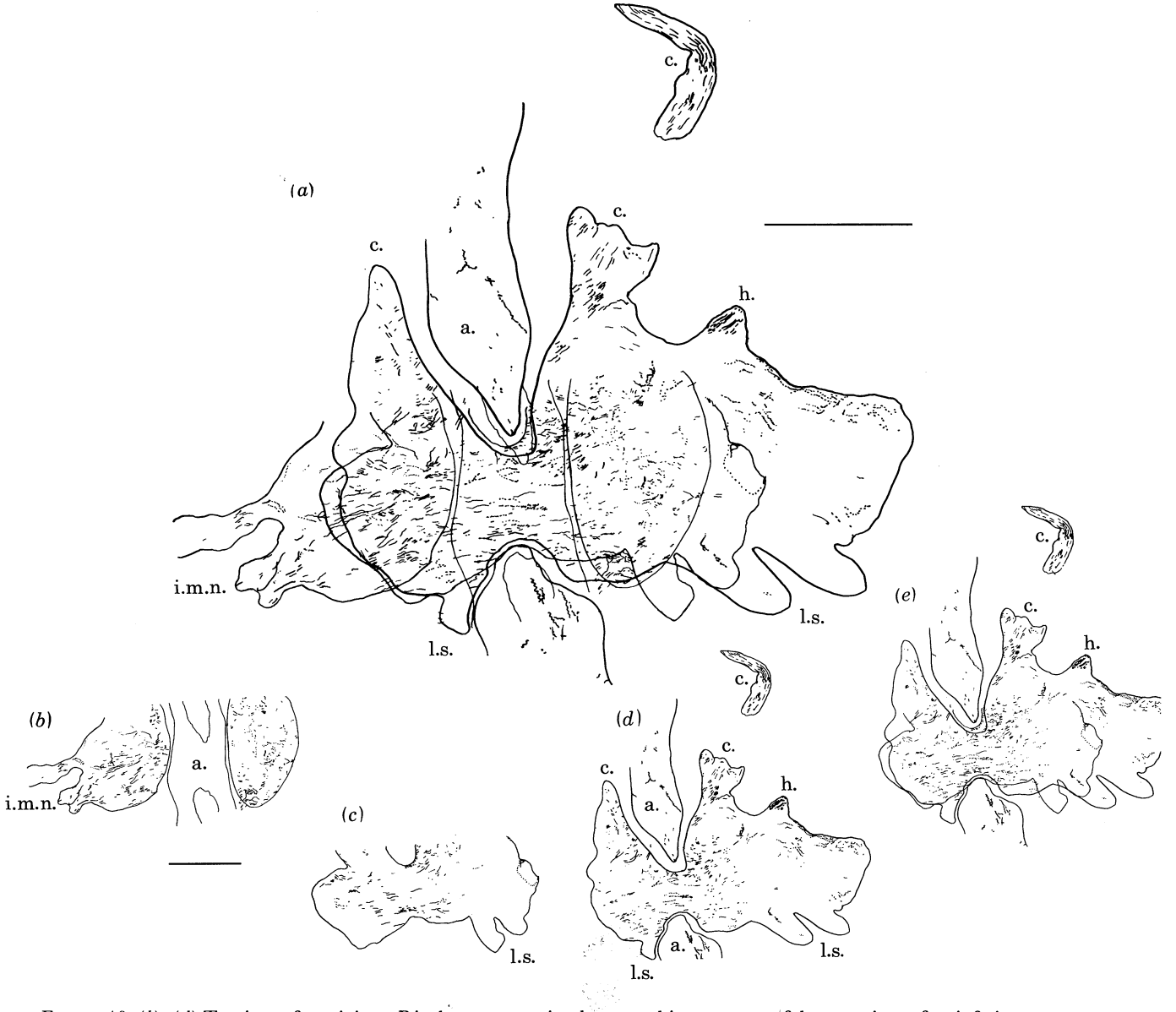

FIGURE 10. (*b*)–(*d*) Tracings of surviving s.P i. elements seen in photographic montages of three sections of an inferior mesenteric ganglion at 4 d after division of lumbar splanchnic nerves. All three sections are shown superimposed in (*a*). (*e*) The superimposition of (*c*) and (*d*), using the peri-arterial band of the ganglion as a guide. All surviving s.P i. elements have been traced; they are represented in reverse, that is, as black on white. The sections pass through both lobes of the ganglion, and (*b*) and (*d*) also pass tangentially through the wall of the inferior mesenteric artery (a.). The surviving s.P i. innervation of the arterial wall has been included in (*d*), but not in (*b*) where superimposition upon the ganglion would cause confusion. A rich s.P i. innervation persisted close to the adventitial–medial boundary. s.P i. nerve fibres are seen in the intermesenteric nerve (i.m.n.) and are traceable from this nerve across the ganglion toward and into (or from) the bases of the colonic (c.) and hypogastric nerves (h.). Other sections showed that the surviving s.P i. fibres were not uniformly distributed in the colonic and hypogastric nerves, and some colonic fascicles were not seen to receive any. A few s.P i. varicose trails are seen to persist in the ganglion and are more profuse in its cranial lobe (to the left) and toward the colonic and hypogastric poles; the lumbar splanchnic nerves (l.s.) are seen to be depleted of s.P i. fibres, and the adjacent regions of the ganglion (below and to the right) are relatively empty of s.P i. elements. The dotted outline in (*c*) demarcates a group of s.i.f. cells in the caudal lobe. Scales, 500 μm (note larger scale for (*a*)).

ganglion occupied by non-varicose s.P i. fibres that varicose s.P i. nerve networks were also present, though in much reduced quantity (figures 9*b–d*, 10). The central stumps of the lumbar splanchnic nerves showed massive accumulations of brightly fluorescent s.P immunoreactive material, and apparent s.P immunoreactive sprouts were seen from their tips (figure 11, plate 9).

S.i.f. cells after lumbar splanchnic nerve division

In one ganglion a group of 'small intensely fluorescent' cells (s.i.f. cells, Eränkö 1976) situated at the lumbar pole was found to contain a number of substance P immunoreactive cells that had a branching morphology suggestive of s.i.f. cells. These cells are illustrated in figure 12 (plate 10). A few similar cells were seen elsewhere in the ganglion. They were distinguishable from activated macrophages, which were stainable by the second antibody as a consequence of cross immunoreactivity, by the presence of short processes (figure 12*f*). (Cross reactivity tended to vary from batch to batch of the second antibody. The number of activated macrophages present near to lesions also varied, but was greater after more extensive lesions, for example, figure 13).

Lesions of lumbar splanchnic and intermesenteric nerves

After lesions of single categories of nerves it is obviously not possible to say in which direction the cell bodies of the residual nerve fibres might lie, that is, in this case, after division of lumbar splanchnic nerves, whether in the intermesenteric or colonic or hypogastric nerve territories. Combined lesions of the lumbar splanchnic and intermesenteric nerves were therefore performed. These experiments also served as controls for the spinal cord lesions in indicating the time required after nerve section for degeneration and disappearance of s.P immunoreactivity from the ganglion. This combination of lesions resulted in virtually complete depletion of s.P immunoreactivity in the inferior mesenteric ganglion in the animal which survived for 3.8 d (figure 13, plate 11). One small surviving fascicle was found entering the ganglion and a few strictly localized varicose trails of s.P i. fibres were found in the ganglion; otherwise specific s.P immunoreactivity was completely absent. All nerves associated with the ganglion were examined. All colonic nerve bundles seen were empty of s.P i. nerve fibres. In the hypogastric nerves one possible s.P i. fibre was found. The ganglionic stumps of the intermesenteric and lumbar nerves were empty of s.P i. immunoreactivity except for the minute nerve bundle described above which appeared to be innervating blood vessels. In the central stumps of the intermesenteric and lumbar nerves brilliantly fluorescent s.P i. accumulations were seen (figure 13*c*). A normal distribution of s.P i. innervation was found on the inferior mesenteric artery (figure 13 (asterisk)) and also on the aorta. In the inferior mesenteric ganglion of a guinea-pig at 46 h after cutting the lumbar splanchnic, intermesenteric and hypogastric nerves the intraganglionic s.P i. non-varicose fibres were barely in evidence and the varicose s.P i. networks were in part absent, in part dust-like and apparently fragmenting, and only in one relatively small region, toward the colonic pole, normal-looking but with a subdued immunoreactivity. Some accumulation of s.P i. material was seen in nerve fibres in the ganglionic stumps of the hypogastric nerves; here, the ends of some s.P i. fibres were expanded and showed a granular and relatively weak immunofluorescence. The inferior mesenteric artery, in contrast, showed brightly fluorescent s.P i. innervation that was normal in pattern. In another inferior mesenteric ganglion of a guinea-pig that died at approximately 2.5 d after cutting

the lumbar splanchnic and intermesenteric nerves and of which the tissues were fixed by immersion, only dust-like fragmented traces of s.P immunofluorescent networks remained; accumulations of s.P i. material however extended proximally for at least 3 mm in the intermesenteric nerve (figure 26). Brightly immunofluorescent, normal-looking networks were still found in the coeliac–superior mesenteric ganglion of this animal.

S.P i. innervation of the arteries

The distribution of s.P i. nerves on the inferior mesenteric artery was consistently found to be confined to the deep parts of the adventitia, where a wide meshed ladder-like array is formed of partly varicose, partly non-varicose but brightly fluorescent s.P i. fibres, which form a slightly coiled network (figure 14, plate 12). None of the various nerve lesions produced depletion of this network in any consistent way (figure 14*d*, *e*, *f*, *i*). Some evidence of local depletion of s.P immunoreactivity in the nerves of the artery was seen occasionally after lumbar nerve lesions, but this was not entirely consistent and was patchily localized. The periodicity of s.P i. nerves in the adventitia of the inferior mesenteric artery is rather regular and is consistently much less than that seen on the superior mesenteric artery, which Furness *et al.* (1982) found to be the most heavily innervated of all the arteries they examined. This great density of s.P i. innervation of the superior mesenteric artery was confirmed in these experiments (figure 14*c*). It was also established and confirmed that the s.P i. nerves of the great vessels are independent of the catecholamine fluorescent networks, i.e. the postganglionic sympathetic innervation of the vessels (figure 14*g*, *h*). This was possible by virtue of the formaldehyde-induced catecholamine fluorescence resulting from fixation in 4% paraformaldehyde: by changing the wavelength of the exciting illumination it was possible to compare on the same vessel the s.P i. and the catecholamine fluorescent networks.

Capsaicin experiments

In the tissues taken from animals injected with the vehicle of the capsaicin alone no difference from the normal was detected in s.P immunoreactivity in any site examined. In the tissues of the capsaicin-treated animals severe changes in s.P immunoreactivity were consistently found in certain sites.

Dorsal root ganglion cells

The s.P immunoreactivity of normal dorsal root ganglion cells is illustrated in figure 15, plate 13. Some of the neurons show moderate to intense s.P immunofluorescence. This is occasionally so intense as to obscure the view of the nucleus. The s.P i. neurons are not confined to one region of a ganglion, nor are they compactly arranged. A finely granular fluorescence is seen in the cytoplasm of the cell bodies. The single process tends to be more intensely fluorescent than the somatic cytoplasm although the fluorescence is still granular; it is seen to take the classical short, sometimes tortuous course away from the cell body before bifurcating, one branch passing into the central and one into the peripheral sensory root of the ganglion. These nerve fibres are non-varicose. The apparent calibre of the fibre and the intensity of s.P i. in the central processes are markedly less than those in the peripheral processes (figure 15*a*, *b*), and in some cases it is possible to see at the T-junction that the change in level of fluorescence intensity and in calibre is abrupt at this point between the central and peripheral processes (figure 15*c*). Neither of these is of greater calibre or fluorescence intensity than the parent

process, and sometimes both are less (figure 15*c*). Some instances have been seen of elaborate coiling of fibres round about the cell body, as described by Ramón y Cajal (1911): a particularly rich example is illustrated in figure 15*d*, *e*. It is not clear whether these fibres originate from the same or another neuron. They have not been seen round cell bodies which are not themselves immunoreactive for substance P. The s.P i. neurons are of small to medium size; their diameter ranges from approximately 25–50 μm (figure 15).

In capsaicin-treated dorsal root ganglia there was total depletion of s.P immunoreactivity apart from faint residual traces in one or two neurons (figure 16*a*, *b*, plate 14).

Spinal cord

The s.P immunoreactivity of the spinal cord in the cat and rat has been extensively described and illustrated (Hökfelt *et al.* 1975; Cuello & Kanazawa 1978; Ljungdahl *et al.* 1978), and we confirm that the distribution of s.P immunoreactivity in the guinea-pig spinal cord is similar. In the spinal cord after capsaicin treatment s.P immunoreactivity was lost from laminae I and II and was thinned in the lateral part of lamina V. Other zones appeared to be unaffected or less affected.

Small intestine

In the layers dissected from the walls of the ileum, s.P immunoreactivity was lost from the serosa and its blood vessels and from the blood vessels in the submucosa (figures 16–19,

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DESCRIPTION OF PLATES 9–11

FIGURE 11. Accumulation of s.P i. material in central ends of cut lumbar nerves, 4 d after section, with evidence of its passage into nerve sprouts. (*a*), (*b*) Serial, non-consecutive sections of the cut central end of a lumbar splanchnic nerve fascicle, showing heavy accumulation of strongly s.P i. material and evidence of sprouting from the cut ends.

(*c*) Enlargement of part of (*b*), showing detail of apparent sprouts. (*d*), (*e*) Sections successive to (*c*), showing extension of sprouts into the surrounding tissue. (*e*) Shows also two intersecting fascicles of apparently uninjured s.P i. nerve fibres, which probably represent sensory nerves in the periganglionic tissue (sub-serosal).

(*f*), (*g*), (*h*) Accumulated s.P i. material in the tip of the central stump of another lumbar splanchnic nerve fascicle, showing in (*f*), sheath-like tissue surrounding the stump and in (*g*), (*h*), apparent invasion of the sheath and surrounding tissue by slender sprouts with expanded tips, from the s.P i. nerve fibres of the stump. Scales, 50 μm.

FIGURE 12. Groups of s.i.f. cells in control ganglia and after lumbar splanchnic nerve lesions.

(*a*), (*b*) Two sections of a group of s.i.f. cells at the lumbar pole of an inferior mesenteric ganglion, 4 d after section of lumbar splanchnic nerves. Dotted outlines are distal stumps of lumbar splanchnic nerves, empty of s.P immunoreactive material. Very few s.P i. nerve fibres remain in the ganglion. Some of the s.i.f. cells are s.P positive. (*e*) Enlargement of part of (*b*), showing s.P i. processes of the s.i.f. cells (arrows).

(*f*) False positive reaction of macrophages in the periganglionic tissues of the same ganglion.

(*c*) Group of s.i.f. cells in a control inferior mesenteric ganglion showing (i) absence of s.P i. among the s.i.f. cells (note that the faint fluorescence of the s.i.f. cells is non-specific here); (ii) s.P i. fibres in the adjacent part of the ganglion; (iii) a small fascicle of s.P i. fibres, possibly accompanying a blood vessel, obliquely cut in the centre of the s.i.f. cell mass.

(*d*) Group of s.i.f. cells illuminated by light of a shorter wavelength appropriate to elicit catecholamine fluorescence, in a control section incubated without antibody to s.P. (Colonic nerve lesion, same ganglion as in figure 7*a*). Scales, 100 μm (*a*), (*b*); 50 μm (*c*), (*d*)–(*f*).

FIGURE 13. Montages of three sections of an inferior mesenteric ganglion, 3.5 d after section of lumbar splanchnic and intermesenteric nerves (see inset in (*a*)). Non-specific fluorescence is seen in many associated macrophages. The inferior mesenteric ganglion (g.) and associated nerves (c., colonic, h., hypogastric) are empty of specific s.P immunofluorescence, except for a few fibres entering from a small fascicle at one lumbar pole (arrows, (*a*), (*c*)). In (*c*), brilliantly s.P i. accumulations are seen in the proximal stump of the intermesenteric nerve (i.m.n.) and the proximal tips of several lumbar fascicles (l.s.). S.P i. innervation persists on the inferior mesenteric artery(*) in its deeper adventitia, seen well in (*b*) and (*c*). Scale, 100 μm.

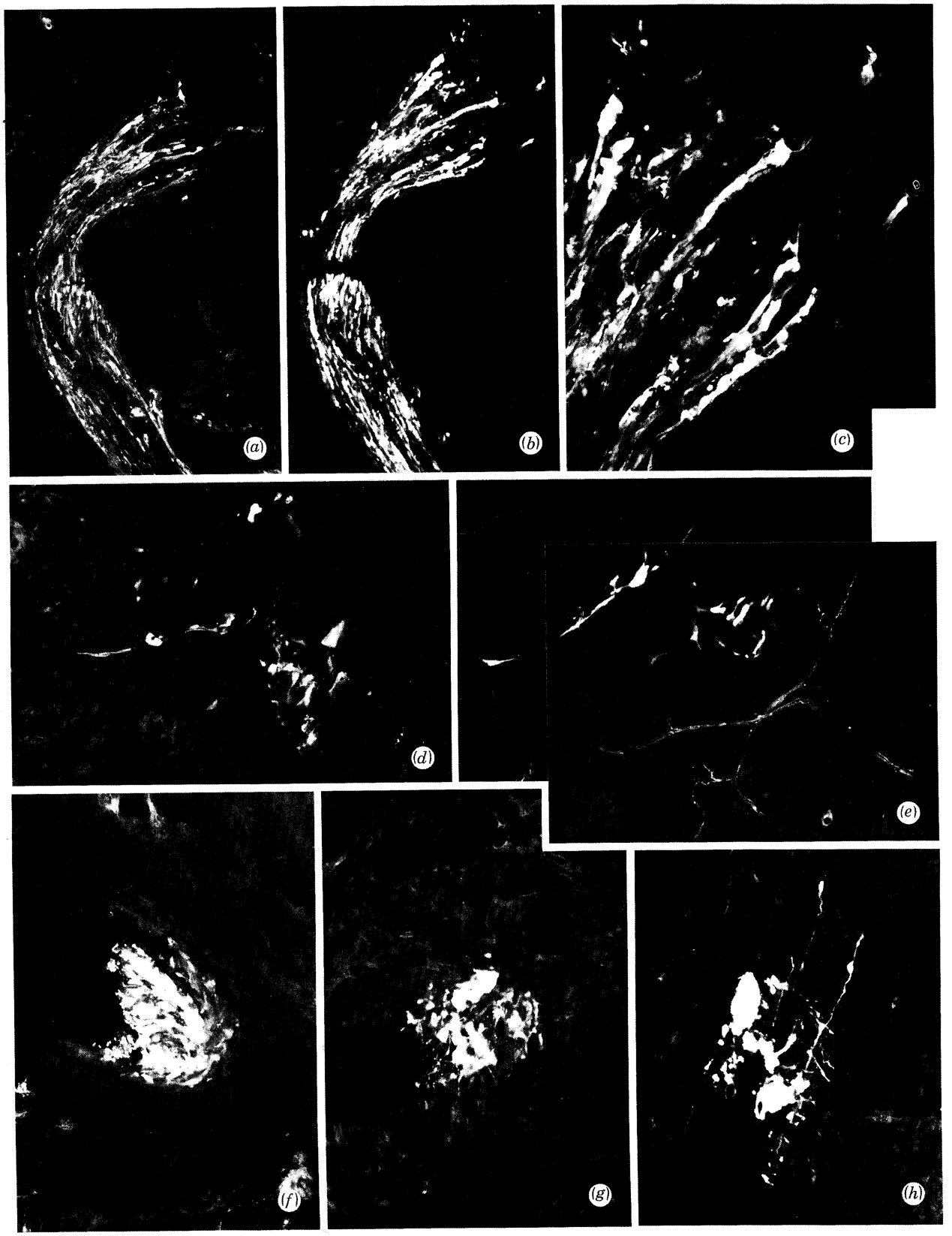

FIGURE 11. For description see opposite.

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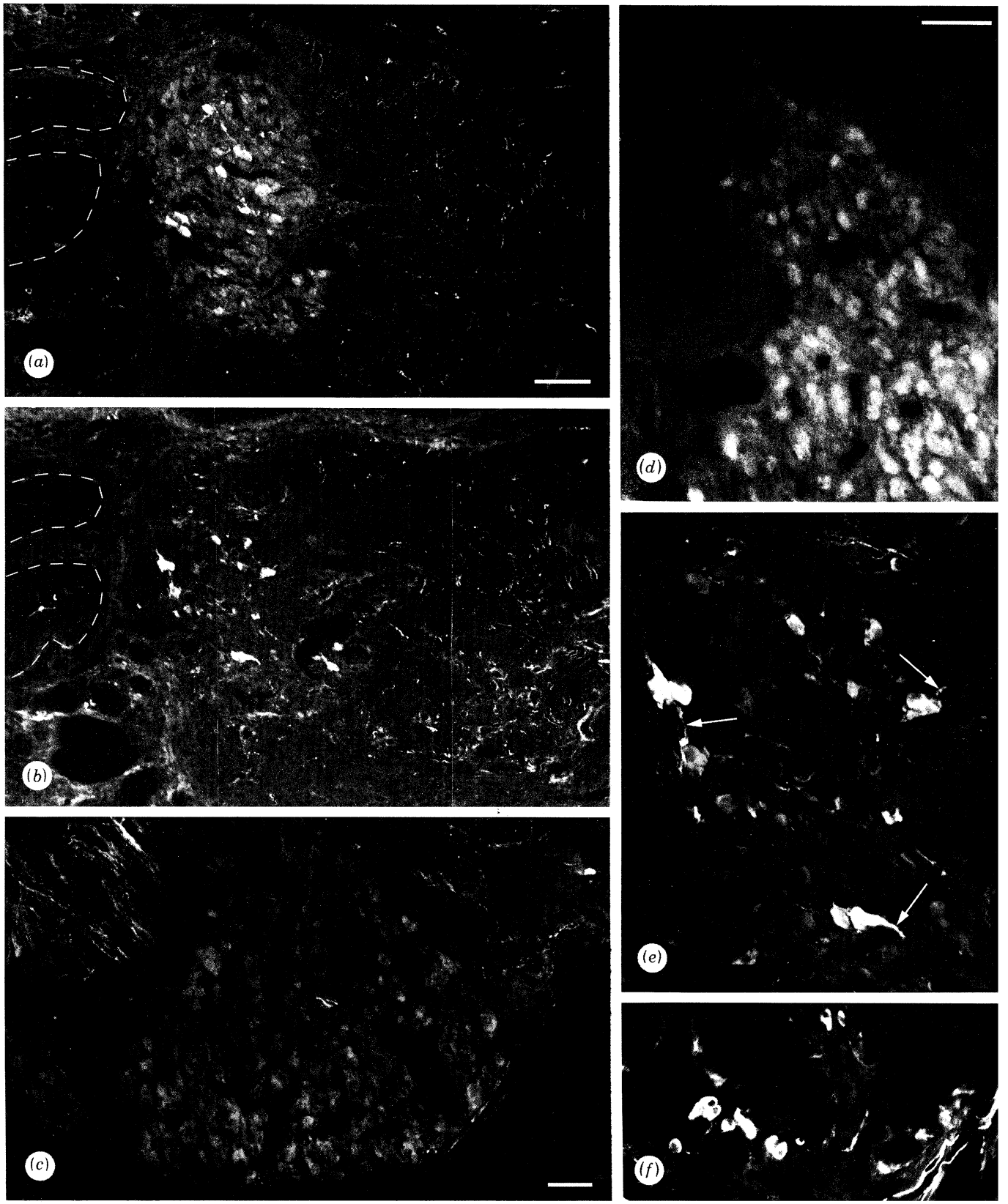

FIGURE 12. For description see p. 262.

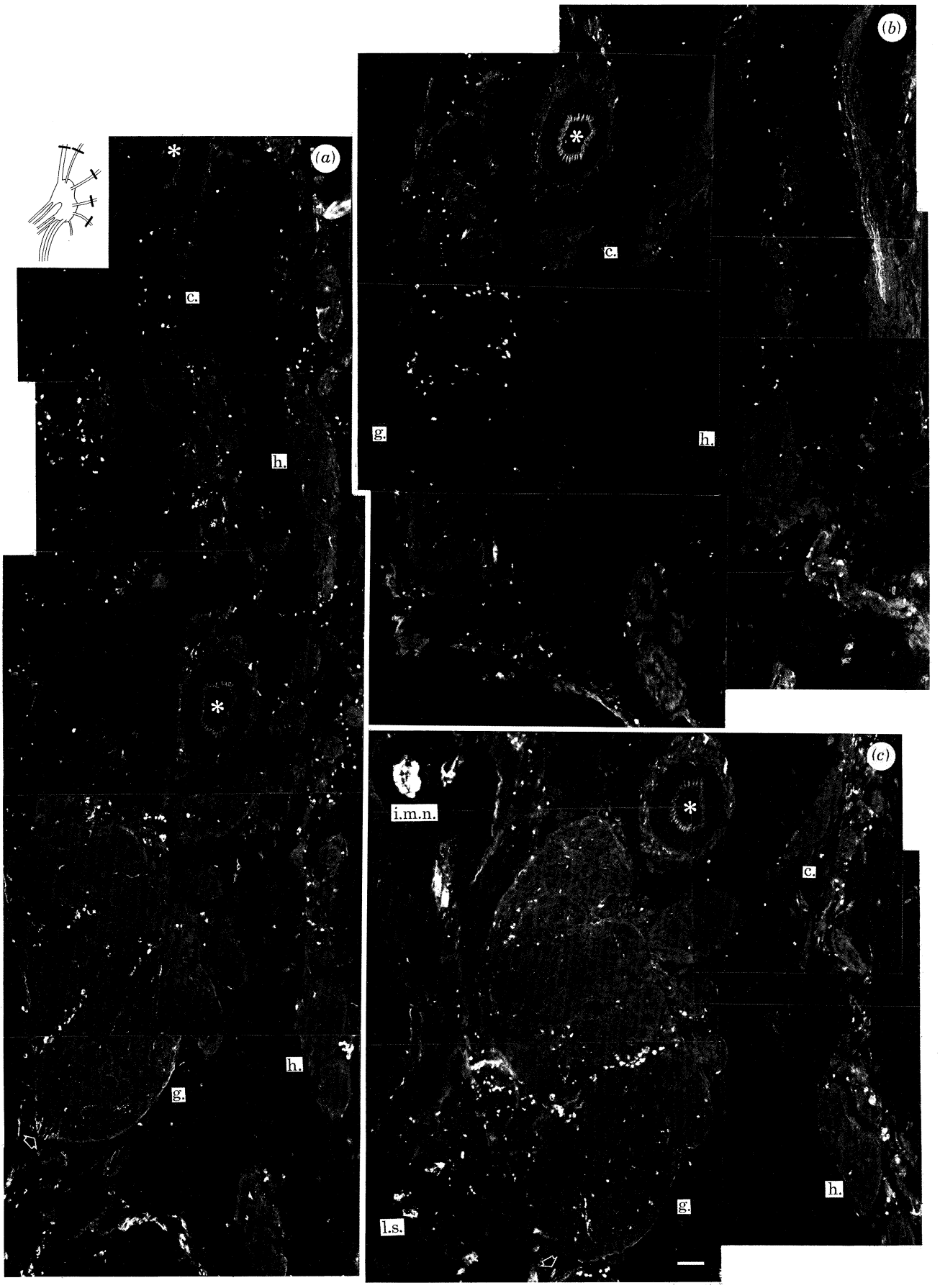

FIGURE 13. For description see p. 262.

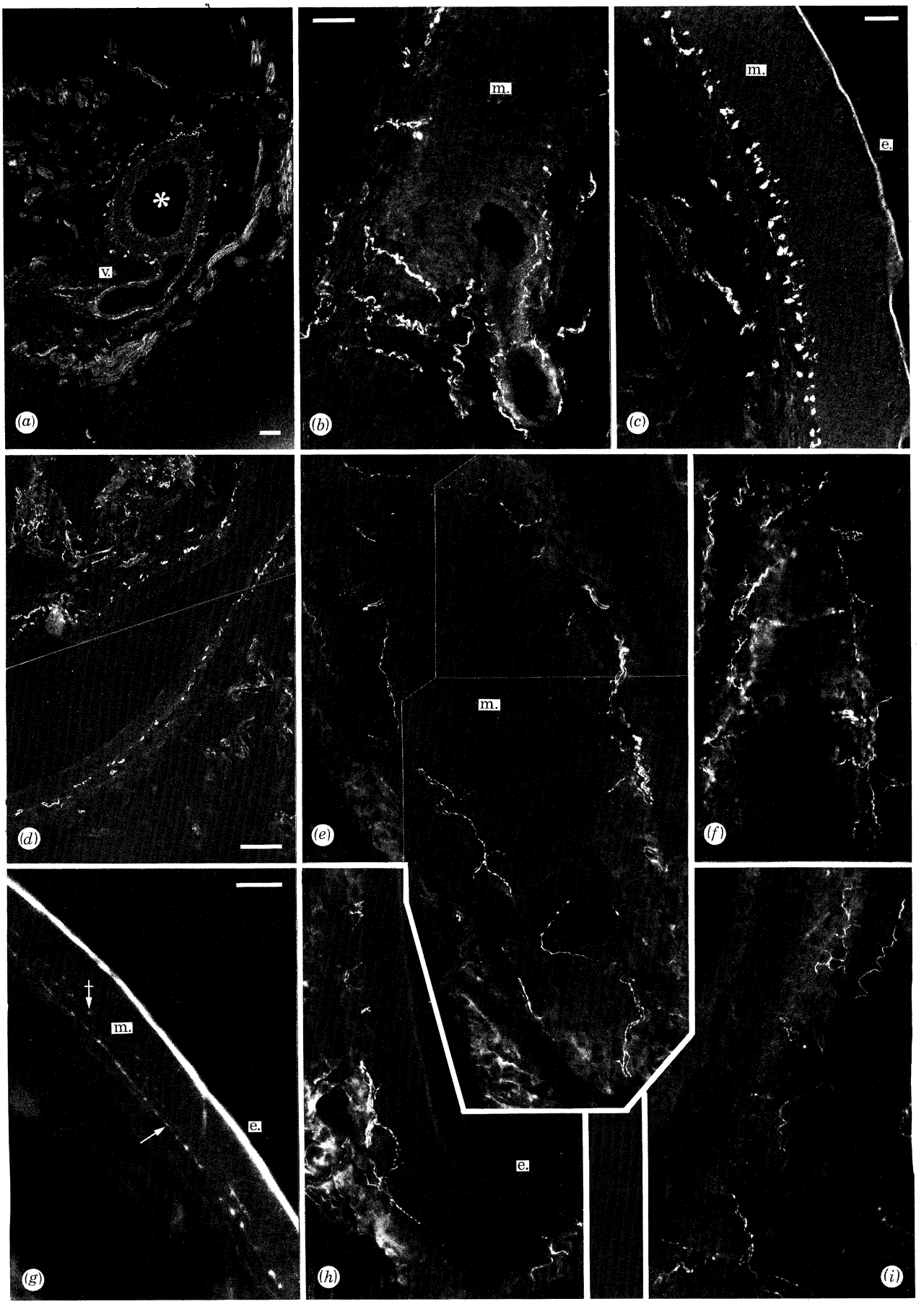

FIGURE 14. For description see p. 263.

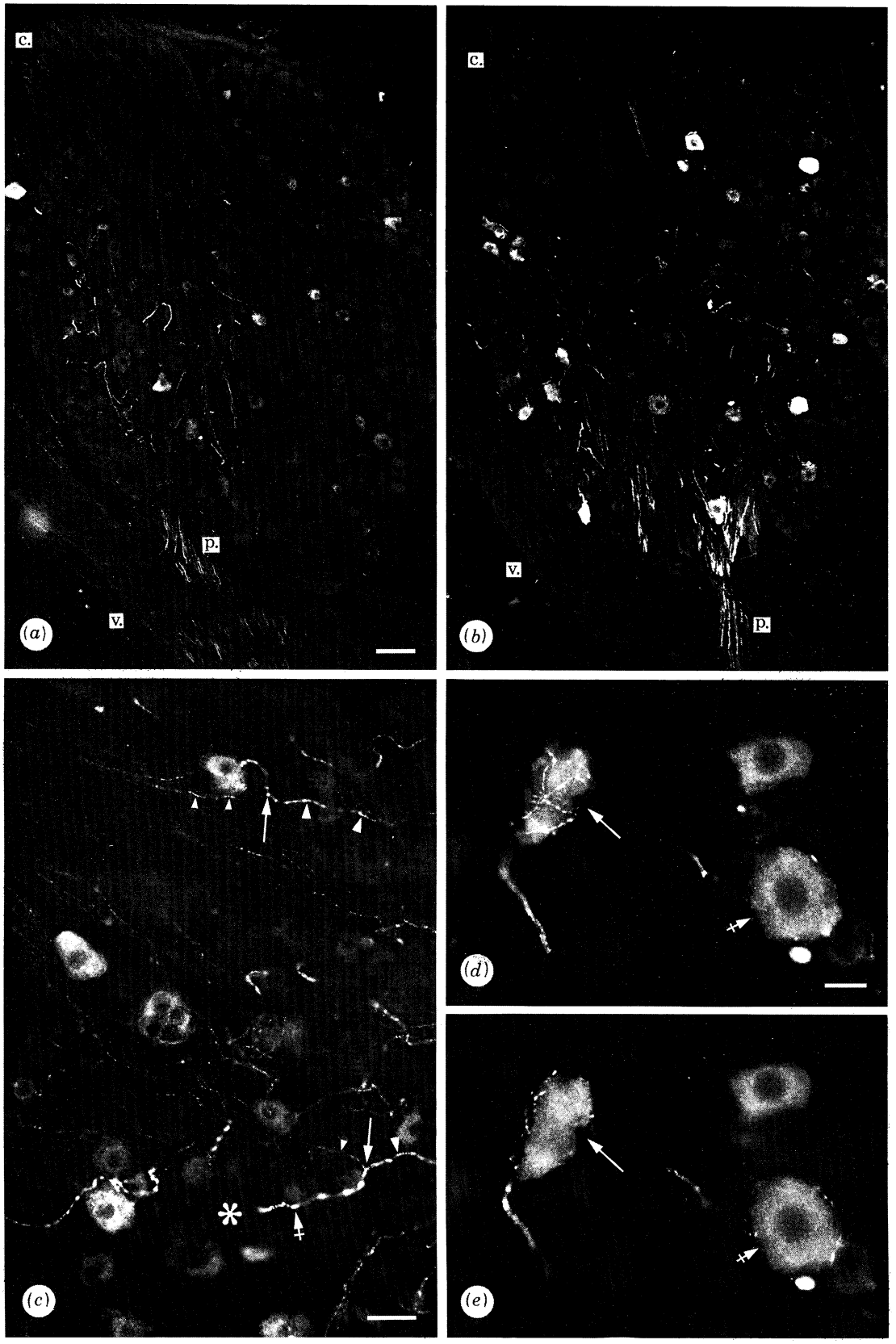

FIGURE 15. For description see p. 263.

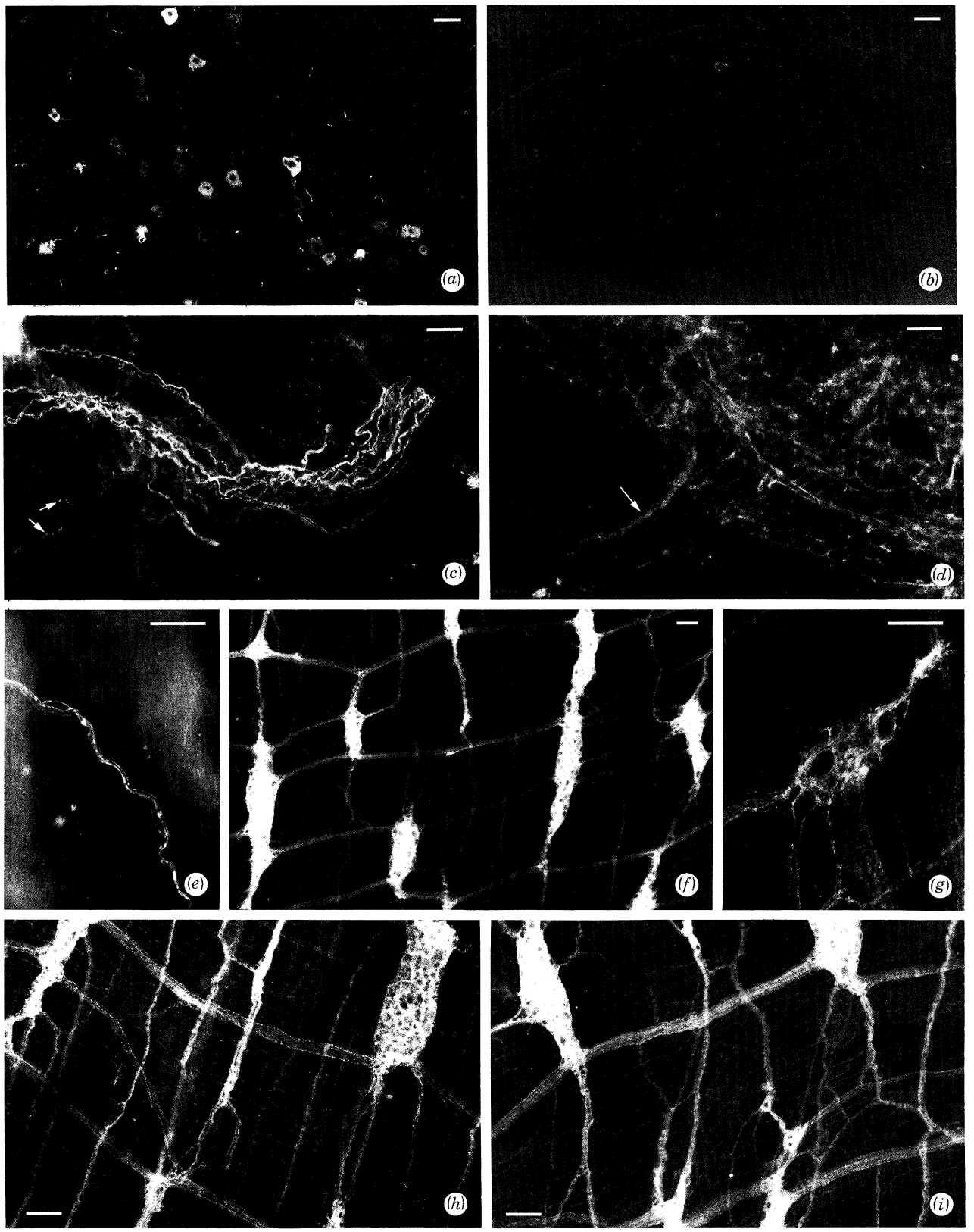

FIGURE 16. For description see opposite.

FIGURE 14. Innervation of the great arteries of the gut and of associated vessels, in control guinea-pigs and after various nerve lesions: m., tunica media; e., internal elastic lamina.

(*a*) Inferior mesenteric artery(*) and vein (v.) in transverse section, showing s.P i. nerve fibres and nerve bundles in the adventitia and at the adventitial–medial junction, with a wide approximately regular circumferential spacing of about 100 μm on the artery. At the wavelength used for observation of f.i.t.c. fluorescence the tissue fluorescence of the internal elastic lamina is relatively weak or absent. (*b*) Inferior mesenteric artery, showing at lower left a horizontal ladder-like spacing of the s.P i. nerve fibres at about 50 μm intervals on the artery, where the section has become oblique, and at the same level the origin of a well-innervated branch from the vessel. The arterial s.P i. nerve fibres have a coiled or wavy course, seen also in (*i*), and they are associated with the adventitia at various depths. The branch at its origin is innervated at least as richly as the parent artery. (*a*), (*b*) Are from a vehicle-treated capsaicin control guinea-pig.

(*c*) Superior mesenteric artery of a normal guinea-pig. The s.P i. innervation of this vessel is strongly immunoreactive and is very rich (cf. Costa *et al.* 1982) but, as in the inferior mesenteric artery, it is concentrated close to the adventitial–medial junction and does not penetrate deeply into the tunica media.

(*d*), (*e*), (*f*), (*i*) The innervation of the inferior mesenteric artery after various nerve lesions: (*d*) 4 d after section of inferior mesenteric nerve; (*e*) 4 d after section of lumbar splanchnic nerves; (*f*) 4 d after section of hypogastric nerves; (*i*) 6 d after section of colonic nerves. None of these lesions has produced significant changes in the s.P i. innervation of the inferior mesenteric artery in the regions shown. (*d*) The great regularity of spacing of the s.P i. nerve bundles is seen in a long oblique section of the artery. (*e*) An almost tangential section showing how single fibres and small bundles spread and ramify in the deeper adventitia, suggesting a rectilinear network; note that some segments of the fibres are non-varicose or nearly so, whereas others show distinct varicosities. It is not known whether this might represent the difference between proximal and distal, or receptor and effector regions. View (*f*) is again nearly tangential and longitudinal; it shows well the ladder-like appearance referred to earlier.

(*g*), (*h*). These figures permit comparison of the s.P i. innervation with the adrenergic innervation of the artery. (*g*) Inferior mesenteric artery in s.P control section incubated without specific anti-s.P antibody, viewed at a wavelength suitable to elicit catecholamine fluorescence (note the bright tissue fluorescence of the internal elastic lamina). Points of formaldehyde-induced catecholamine fluorescence are closely and rather regularly spaced along the adventitial–medial border (for example, arrow) and are scattered also at greater intervals within the outer third of the tunica media (for example crossed arrow); hypogastric nerve lesion 4 d previously. (*h*) Inferior mesenteric artery incubated and viewed to show specific s.P immunofluorescence (note the subdued endogenous fluorescence of the internal elastic lamina at this wavelength). s.P i. nerve fibres occur singly or in small bundles in the deeper part of the adventitia and do not encroach upon the tunica media in the manner of the adrenergic fibres. Scales, 100 μm (*a*), (*c*), (*d*), 50 μm (*b*), (*e*)–(*i*).

FIGURE 15. Dorsal root ganglia of normal and control guinea pigs. (*a*), (*b*) Sections of a normal dorsal root ganglion showing, above and to the left, the central sensory root (c.) and, below, the peripheral sensory root (p.), joining and mixing with the ventral root (v.) to form the mixed spinal nerve. The s.P i fibres in the central sensory root appear of finer calibre and have a weaker overall s.P immunofluorescence than those entering the peripheral sensory root. No s.P immunofluorescence is seen in the ventral root, apart from one or two very fine superficial fibres which appear to be in its sheath or associated with its blood vessels. The s.P i. neurons are scattered, form a minority and are of small or medium size. Only a few large neurons are present at this level and none of these is s.P immunoreactive. (*c*) Control dorsal root ganglion from an animal in which lumbar splanchnic and intermesenteric nerves were cut, ganglion from a level above the lesion, showing several T-branches (for example, arrows) at which the central process (small arrowheads), passing upward and to the left, is clearly seen to be of smaller calibre and, or, to contain less s.P i. material than the peripheral process (large arrowheads, passing rightward toward the mixed spinal nerve). The asterisk indicates presumed location of cell body, not included within the section. The single parent process of this neuron (crossed arrow) is at least as thick and as strongly immunofluorescent as the peripheral process, and may be more so, that is, carrying more s.P i. than either of the daughter processes. All processes tend to appear beaded, especially the more slender ones: this could be due to clumping of immunofluorescent material. The cell bodies have a distinctly grainy immunofluorescence.

(*d*) A normal s.P immunoreactive neuron elaborately enwrapped by a spiral net composed of varicose s.P i. filaments, possibly derived from its own single process which leaves it below and to the left and turns sharply downward. (*e*) The same neuron in a different plane of focus. Another s.P i. neuron shows suggestions of a similar or less elaborate arrangement (crossed arrows (*d*), (*e*)). Scales, 100 μm (*a*), (*b*); 50 μm (*c*); 20 μm (*d*), (*e*).

FIGURE 16. (*a*), (*b*) Dorsal root ganglia of capsaicin-treated and vehicle-treated control guinea-pigs.

(*a*) Control dorsal root ganglion. Brilliant s.P immunofluorescence is seen in scattered neurons of various sizes; others show a weaker reaction and many are s.P i. negative. (*b*) Capsaicin-treated dorsal root ganglion. Only one or possibly two neurons show a weak reaction. Most sections showed no s.P immunoreactivity.

(*c*)–(*i*) Serosal and myenteric s.P i. innervation of the ileum, in capsaicin-treated and vehicle-treated control guinea-pigs. Tissue spread preparations.

(*c*) Control guinea-pig, ileal neurovascular bundle close to point of entry to ileum, showing s.P i. nerve fibres in perivascular networks and paravascular nerve bundles, also in adjacent serosa (arrows); (*e*) serosa of control ileum showing a fine nerve fascicle running independently of main vessels. (*d*) Ileal neurovascular bundle, close to point of entry to ileum, with adjacent serosa, showing total absence of specific s.P immunofluorescence after capsaicin treatment. The outlines of accompanying fat cells fill the background along the major vessels and in the upper right quadrant; a substantial unlabelled nerve bundle curves through the field from lower left to upper centre (arrow). (*f*), (*h*), (*i*) Myenteric (Auerbach's) plexus in ileum of two capsaicin-treated guinea-pigs (*f*), (*i*) and in control ileum (*h*). (*g*) At higher magnification, a single ganglion of the myenteric plexus from a capsaicin-treated guinea-pig. No obvious differences from the control are seen after capsaicin treatment. Scales, all 100 μm except (*e*), (*g*) (50 μm).

plates 14–17). In the submucosa there was loss of communicating s.P i. branches to the submucous plexus from the peri- and paravascular nerves and, in conjunction with this change, the few large s.P i. varicosities normally present (Costa *et al.* 1980) were lost from the submucous ganglia of Meissner's plexus, but otherwise the submucous plexus showed no change in s.P immunoreactivity (figures 17*e–k* and 18*a–c*). The myenteric plexus and the plexus within the circular muscle, also the deep muscular plexus at the inner aspect of the circular muscle, showed no change (figures 16*d–g* and 17*a–d*); and persistence of s.P immunoreactivity was seen also in the delicate nerve strands running within the muscularis mucosae (figure 18*a*). Spread preparations of submucosa were also treated with antibody to dopamine β-hydroxylase. It was confirmed that nerve networks immunoreactive to dopamine β-hydroxylase, that is, postganglionic sympathetic nerve fibres, were still present on the blood vessels of the submucosa and in the ganglia and networks of the submucous plexus, in the capsaicin-treated just as in the normal material (figure 18*d–h*). No change was found after capsaicin in the delicate nerve networks associated with the epithelium, including both crypts and villi (figure 19).

Prevertebral ganglia

In the inferior mesenteric and coeliac–superior mesenteric ganglia of capsaicin-treated animals no substance P immunoreactivity remained apart from occasional short trails of

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Description of plates 15–17

Figure 17. Comparison of s.P i. innervation of ileal circular muscle and submucosa in capsaicin-treated and vehicle-treated control guinea-pigs.

The plexus in the circular muscle (*a*), (*c*) and the deep muscle plexus at its inner boundary (*b*), (*d*) show no detectable differences after capsaicin ((*a*), (*b*), control; (*c*), (*d*), capsaicin-treated). In the submucosa of control animals, slightly coiled or wavy peri- and paravascular s.P i. nerves accompany and innervate the blood vessels (*(*e*), (*g*)) and communicate with the submucous plexus (*e*). The ganglia and strands of the submucous plexus are permeated by finely beaded or varicose s.P i. nerve fibres ((*e*), (*g*), (*j*)), among which a few larger varicosities are seen ((*e*), (*g*), (*j*)). Only occasional neurons of the plexuses in these preparations prepared after perfusion fixation show faint s.P i. These are often superficially located in the ganglia ((*g*), (*j*), arrows). After capsaicin treatment the s.P i. innervation of the blood vessels disappears (*(*f*), (*h*), (*i*), (*k*)) and so do the largest s.P i. varicosities of the submucous plexus (*f*), (*k*) but the finely varicose or beaded networks of the submucous ganglia and connecting strands of the plexus persist (*f*), (*h*), (*k*); weak s.P i. is still seen in occasional neurons ((*k*), arrows). Figures (*h*) and (*i*) are different focal planes of the same field, with focus on the submucous plexus and the blood vessels (*) respectively; (*f*), (*h*) and (*i*), and (*k*), are from different capsaicin-treated guinea-pigs, with their respective controls ((*e*), (*g*), and (*j*)). Scales 100 μm (*a*), (*c*), (*e*), (*f*); 50 μm (*b*), (*d*), (*g*)–(*k*).

Figure 18. Differential effects of capsaicin treatment on s.P and DβH immunoreactivity. (*a*)–(*c*) Submucosa of ileum from a capsaicin-treated guinea-pig. The same microscope field is photographed in three focal planes, showing: (*a*), a submucosal blood vessel (*) devoid of s.P i. innervation, but persistent, single, finely beaded s.P i. fibres running parallel with, or within, the muscularis mucosae (arrows, lower part of figure); (*b*), in the submucous ganglia, a persistent dense s.P i. network of finely beaded fibres, but lacking large s.P i. varicosities, and the same branching blood vessel, devoid of s.P i. innervation; (*c*), the persistent dense s.P i. network of the deep plexus of the circular muscle; note that its principal alignment is approximately at right angles to the fibres in the muscularis mucosae. (*d*)–(*h*), DβH immunoreactivity in the ileal submucous plexus of a vehicle-treated control guinea pig (*g*), (*h*) and two capsaicin-treated guinea-pigs (*d*), (*e*), (*f*). Figures (*e*) and (*f*) show the same field in different focal planes. Finely varicose or beaded networks of DβH-immunoreactive fibres permeate the connecting strands and ganglia of the submucous plexus (in which no DβH-positive neurons are seen) and also closely accompany the blood vessels in the submucosa (*), outlining them with an almost continuous coating of varicosities. Scale, 50 μm.

Figure 19. S.P i. nerves of the ileal epithelium in vehicle-treated control (*a*), (*c*), (*e*), (*g*) and capsaicin-treated guinea-pigs (*b*), (*d*), (*f*), (*h*), (*i*), showing finely beaded or granular s.P i. immunoreactivity in delicate nerve fibres which form a pericryptal net in the deep part of the mucosa ((*a*), (*b*), (*d*); c., crypt); this becomes continuous, ((*c*)), with a close-meshed sub-epithelial reticulum in the villi, lying immediately beneath the bases of the columnar epithelial cells (*e*), (*f*), (*g*)–(*i*). No essential difference is seen between the s.P i. epithelial nerve networks in control and capsaicin-treated animals. Scale, 50 μm.

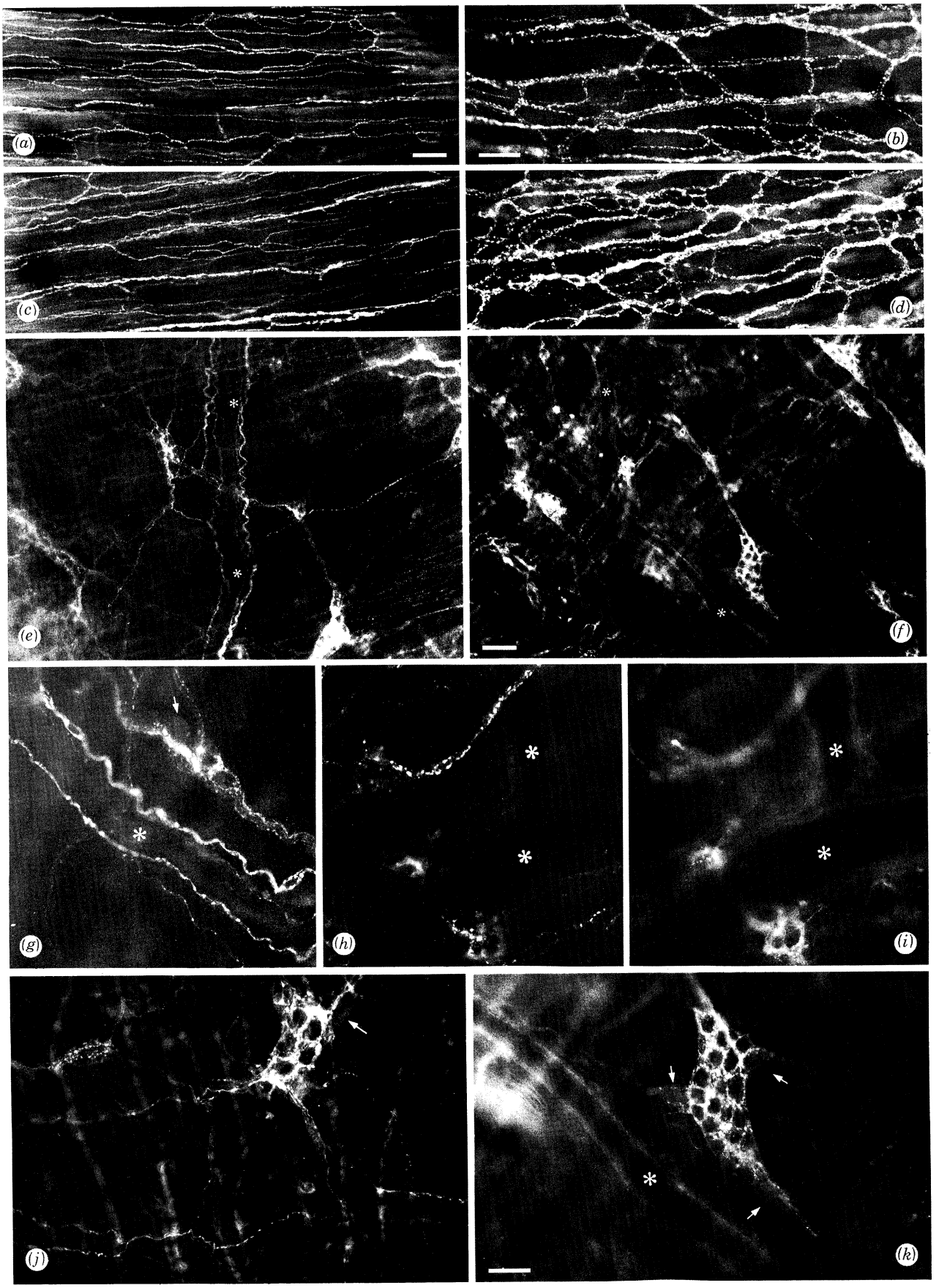

FIGURE 17. For description see opposite.

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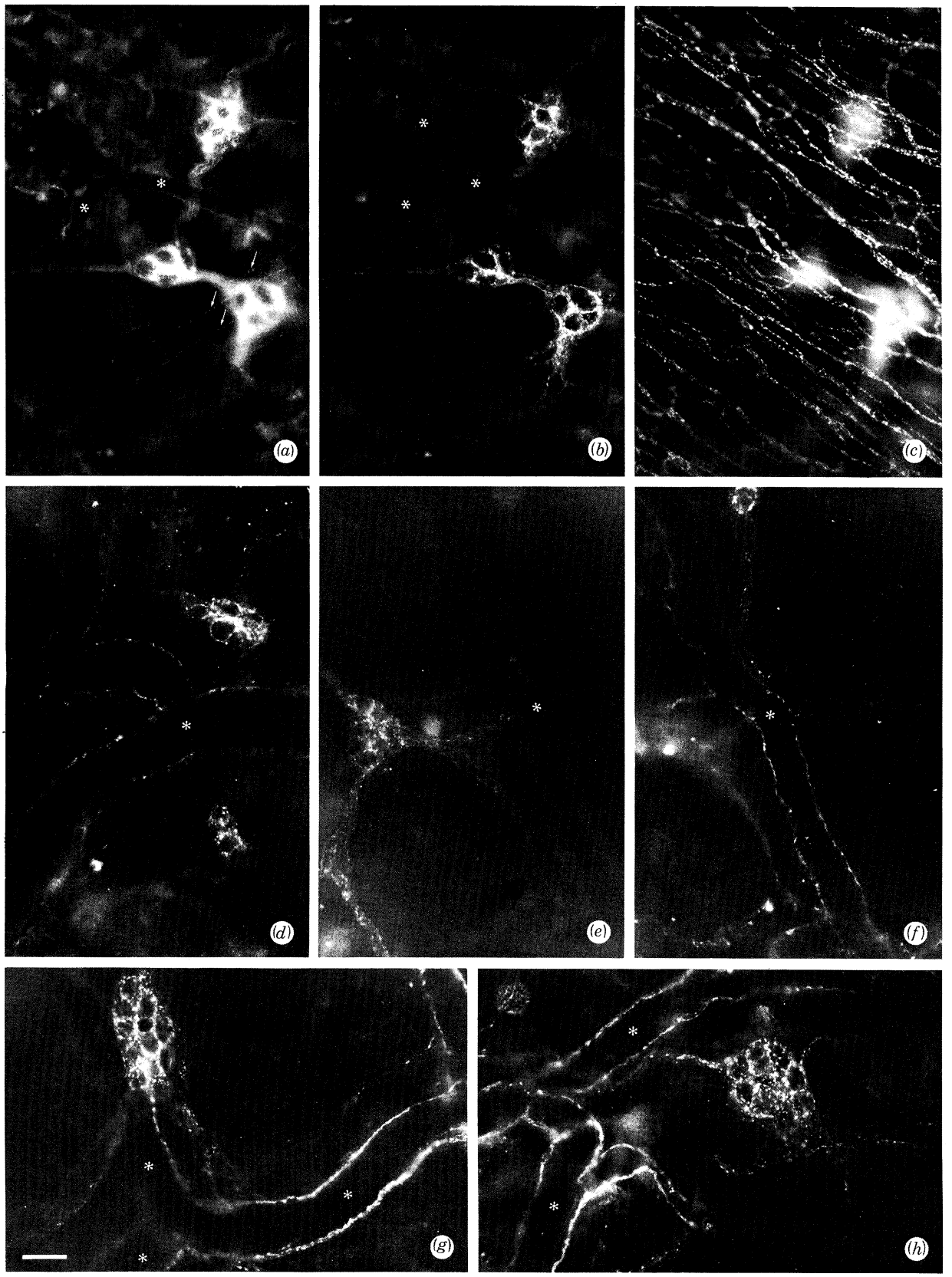

FIGURE 18. For description see p. 264.

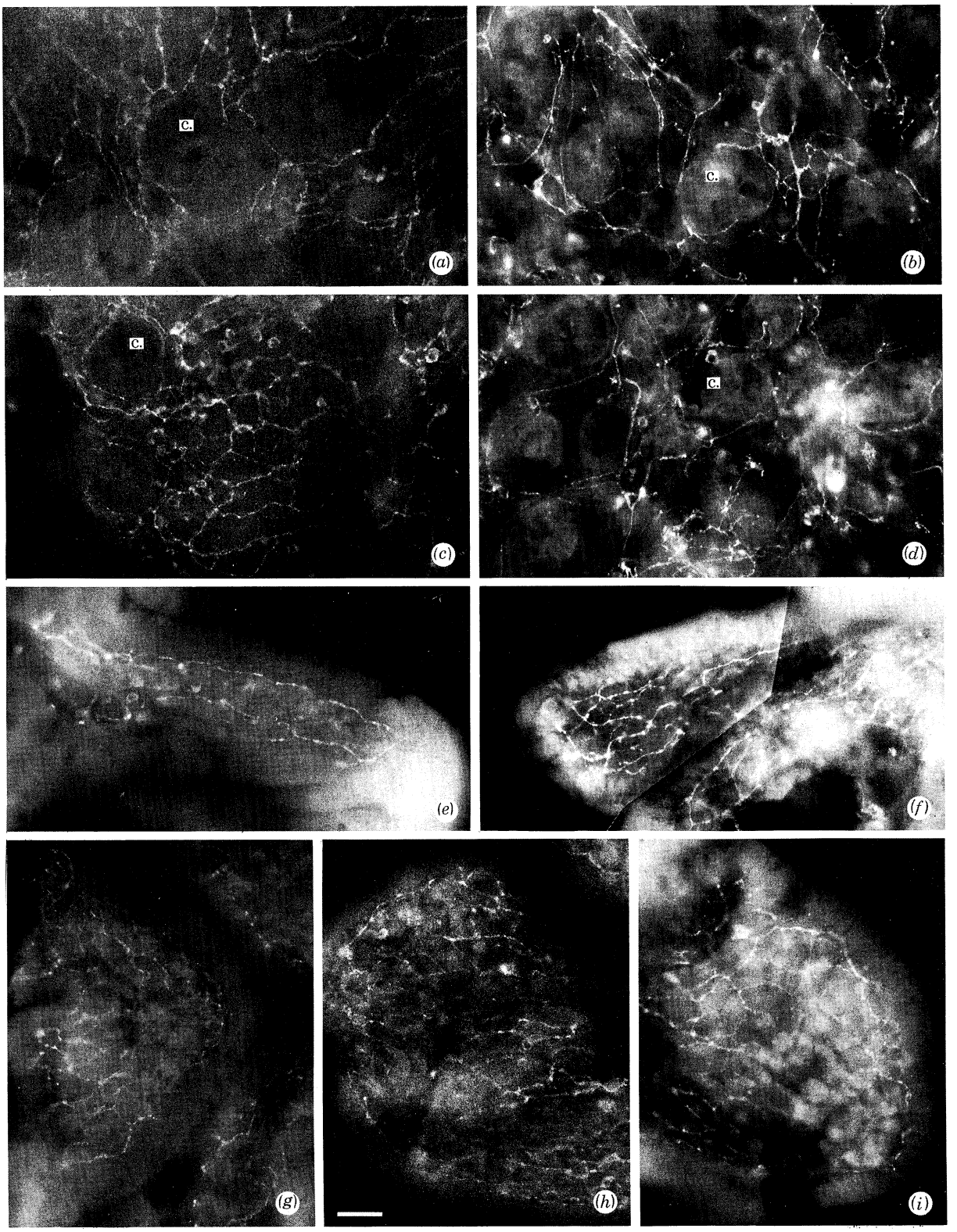

FIGURE 19. For description see p. 264.

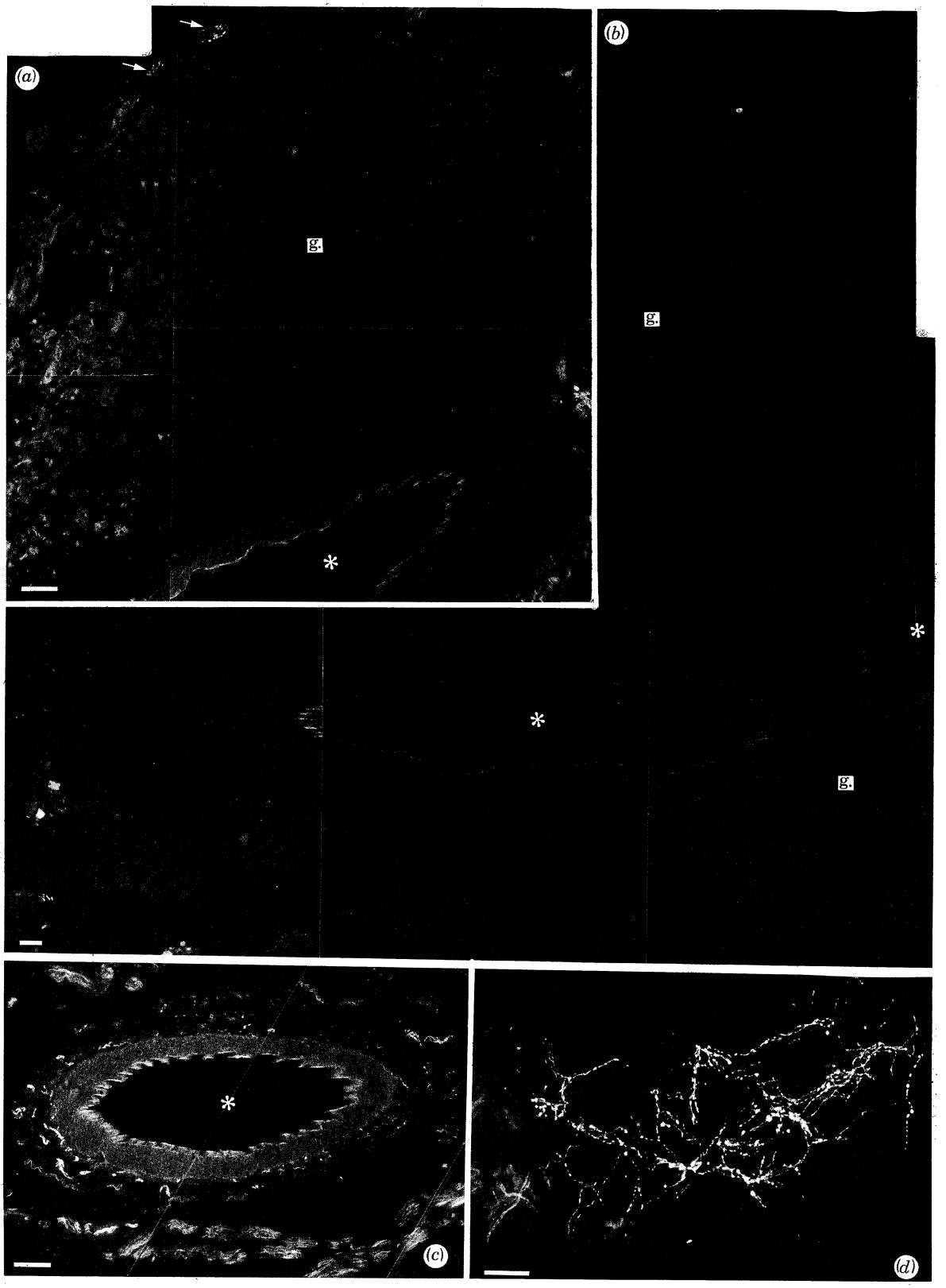

FIGURE 20. For description see p. 265.

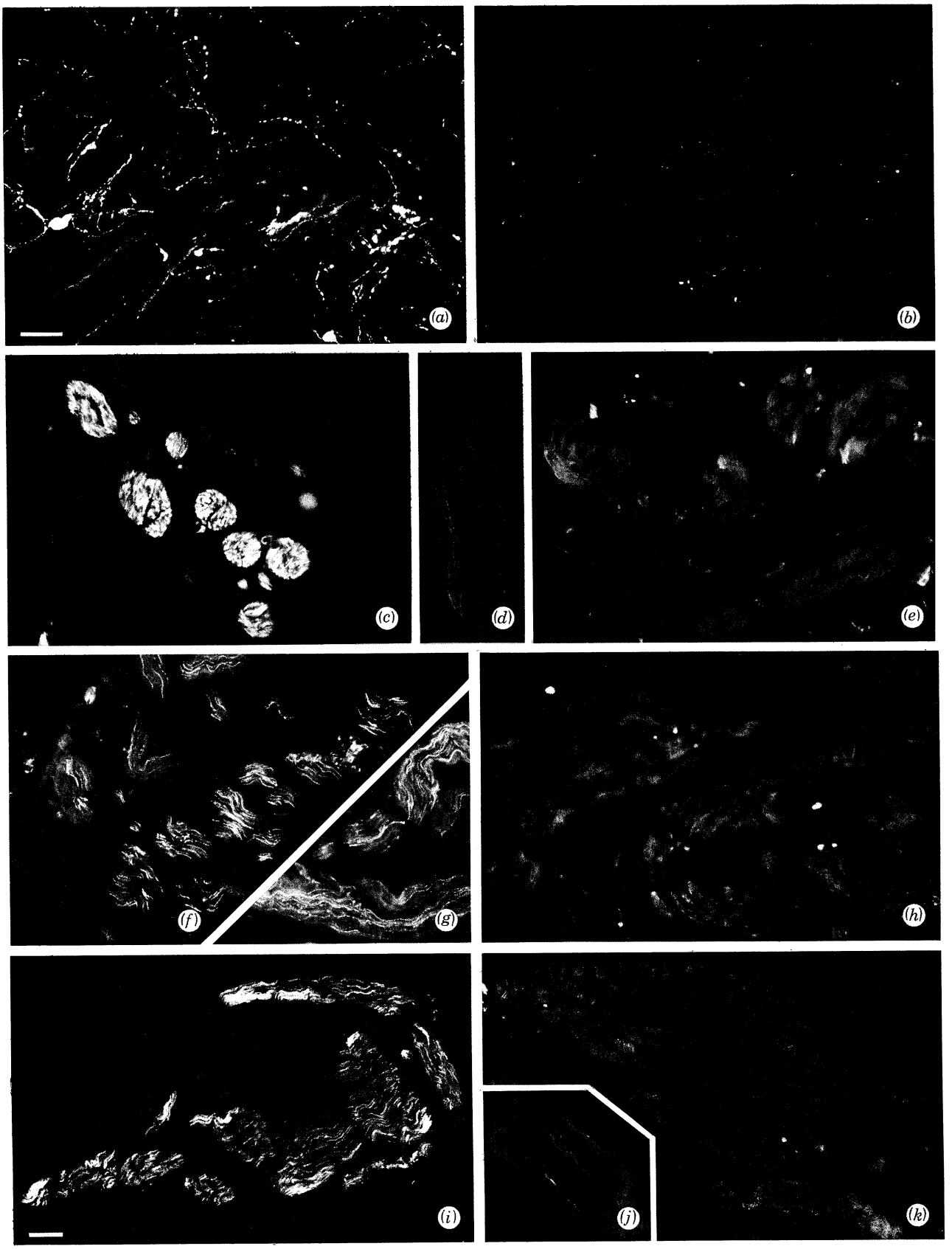

FIGURE 21. For description see p. 265.

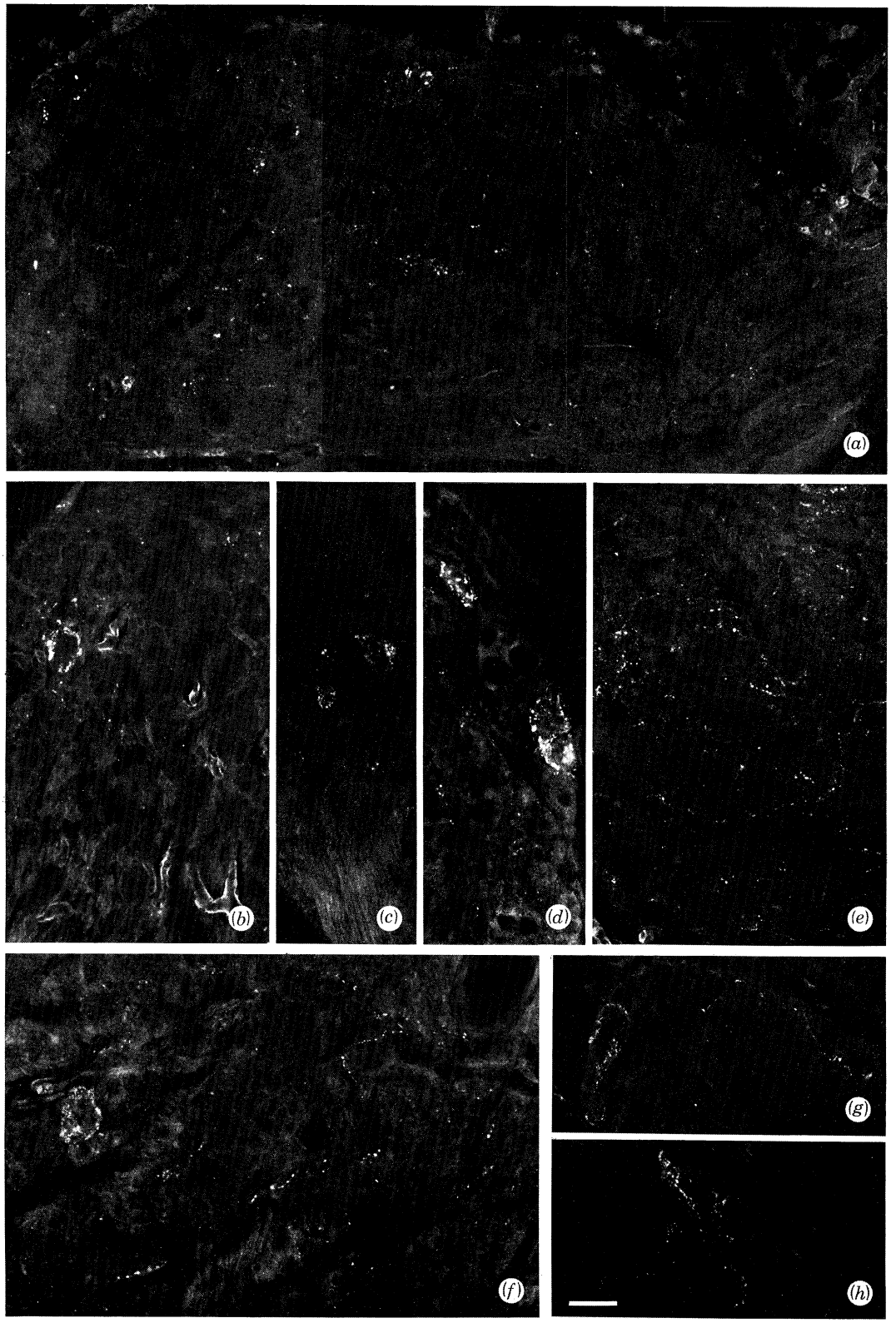

FIGURE 22. For description see opposite.

apparently very fine varicose fibres and a very few fine basket-like structures round isolated neurons (figure 20, 21*b*, 22, plates 18–20). Such structures were not conspicuously seen in normal ganglia. The intensity of their s.P i. was very faint, and they were certainly not sufficient to account for all the previous networks (figures 20*d*, 21*a*). Of the nerves associated with the inferior mesenteric ganglion, the intermesenteric and lumbar splanchnic nerves were all completely empty of s.P i.; in the colonic nerve one or two possible non-varicose fibres were seen in one animal and the same applied to the hypogastric nerves (figures 21*c–k*). It is conceivable that these very few fibres could have accounted for the few surviving varicose trails and baskets in the inferior mesenteric ganglia.

Spinal cord lesions

At dissection of the animals it was confirmed that dorsal root ganglia had not been encroached upon by the lesion. The central ends of the dorsal roots were seen projecting into the empty vertebral canal, and the dorsal root ganglia themselves were found to be intact. It was also confirmed that the level of the spinal cord section was either through the T7 segment or between T7 and T8. Dorsal root ganglia from levels below the lesion were compared with dorsal root ganglia from higher levels. There was no evidence of degeneration or disorganization in ganglia from below the level of the lesion; persistence of substance P immunoreactivity at varying intensity within the normal range was found in neurons in these ganglia, and accumulation of substance P immunoreactive material was seen in the ends of the severed dorsal root fibres, even as early as at 24 h after spinal cord removal (figure 23). Non-varicose substance P immunoreactive nerve fibres were still seen in the central and peripheral parts of the dorsal roots (figure 23, plate 21).

In the various mesenteries of the gut, substance P immunoreactive nerve fibres and nerve networks were seen accompanying the vessels, both in paravascular nerves and perivascular networks, and were distributed also independently of blood vessels as solitary fibres and bundles in the omenta and mesenteries examined at all levels; all the s.P i. nerve elements were indistinguishable in appearance from those in control animals even at 4 and 5.5 d after the

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Description of plates 18–20

Figure 20. General views of inferior mesenteric ganglia from guinea-pigs treated with capsaicin and control sections from guinea-pigs injected with the vehicle used for capsaicin administration.

(*a*), (*b*) Montages of ganglia (g.) from two capsaicin-treated guinea-pigs, showing virtually total depletion of s.P i. material from the ganglion and its associated nerves (remnants are indicated by arrows), and absence of s.P i. innervation of inferior mesenteric artery (*).

(*c*), (*d*) Vehicle-injected control guinea-pigs. (*c*) S.P i. innervation of inferior mesenteric artery (*). (*d*) S.P immunofluorescence in inferior mesenteric ganglion. Scales, 100 μm (*a*), (*b*), (*c*); 50 μm (*d*).

Figure 21. The inferior mesenteric ganglion and associated nerves after capsaicin treatment (right and centre), with vehicle-treated control material (left) for comparison. These show (i) the virtual disappearance from the ganglion of s.P i. non-varicose nerve fibres and varicose nerve networks ((*b*); compare with (*a*)); (ii) the loss of s.P i. fibres from the lumbar splanchnic nerves ((*e*) transverse section, (*d*) longitudinal section, compare with (*c*)); (ii) absence of s.P i. nerve fibres in the colonic nerves ((*h*); compare with (*f*) and (*g*)); and (iv) absence of s.P i. nerve fibres in the hypogastric nerves (major bundles in (*k*), compare with corresponding bundles in (*i*)). In one animal two persistent s.P i. nerve fibres were found in one hypogastric nerve (*j*) and one or two possible s.P i. fibres in the colonic nerves (not shown). Scales, 50 μm (*a*)–(*h*), (*j*); 100 μm (*i*), (*k*).

Figure 22. Residual s.P i. material in inferior mesenteric ganglion following capsaicin treatment. (*a*) Montage of one half of ganglion, colonic pole to right; (*b*)–(*d*), (*f*)–(*h*) fine basket-like pericellular formations or networks; (*e*), (*f*), (*h*), trails of varicosities. These and figure 21*b* were the only such traces found in three capsaicin-treated ganglia. Figure (*d*) is an enlargement from part of figure 20*a*. Scale, 50 μm.

spinal cord lesions (figure 24, plate 22). In experiments in which the intermesenteric nerve and lumbar splanchnic nerves had been divided it was confirmed that after 3.8 d survival there was extreme depletion, disorganization and virtually complete disappearance of s.P immunoreactive nerve fibres and nerve nets from the mesentery below the level of the lesion, that is, in the mesentery of the distal colon. This was in sharp contrast with the bright fluorescence observed in nerve bundles in the mesenteries of the duodenum and ileum of the same animal (figure 25, plate 23). Thus, both the perivascular s.P i. networks and paravascular s.P i. nerve bundles were lost from the serosal and mesenteric blood vessels after capsaicin and, at hindgut level, after lumbar splanchnic and intermesenteric denervation, but not after spinal cord removal.

In the inferior mesenteric ganglion, at all survival intervals after the spinal cord lesions, the networks of s.P immunofluorescent varicose fibres and the non-varicose bundles survived apparently intact, showing bright s.P immunofluorescence (figures 26 and 27, plates 24 and 25). No obvious difference was detected from the normal networks at 4 d postoperatively (figure 27), although shorter intervals sufficed to produce almost total depletion of s.P immunoreactivity in the inferior mesenteric ganglion and associated nerves after combined section of intermesenteric and lumbar splanchnic nerves, as described earlier (figures 13 and 26).

In the L3 dorsal root ganglion central to the lesion of intermesenteric and lumbar nerves the intensity of s.P immunoreactivity in neurons appeared in general to be at least as high as in ganglia from the same animal at more cranial levels (cervical, upper and mid-to-lower thoracic), and the proportion of neurons seen to be immunoreactive was as high as, or slightly higher than, that at more cranial levels (figure 27*h*, *i*). Particularly high levels of immunoreactivity, sufficient to obscure the nucleus, were observed in rather more neurons than usual at 3.8 d postoperatively, and this suggested some accumulation of s.P i. material in the cell body. There was certainly no obvious diminution in the content of s.P immunoreactive material in the cell bodies at a level which must have included some injured neurons.

Discussion

This study has provided a fairly complete picture of the pattern of distribution of the sensory s.P i. neurons that innervate the alimentary tract in the guinea pig. The findings of this and related studies are summarized diagrammatically in figure 28. These studies have revealed an unexpected complexity of these sensory neurons, in that they are shown as providing, probably by collateral branches, nerve terminal networks in the prevertebral ganglia that are likely to

Description of plate 21

Figure 23. Dorsal root ganglia caudal to spinal cord removal.

(*a*)–(*e*) Dorsal root ganglion 4 d after removal of spinal cord from a higher level (T7–8) downward. (*f*), (*g*) dorsal root ganglion from a level caudal to removal of spinal cord 24 h previously. (*a*) Montage showing dorsal root ganglion with (above) accumulated s.P i. material in divided central end of dorsal root, and (below) peripheral end of dorsal root and mixed spinal nerve in continuity. (*b*)–(*e*) Enlargements of areas from (*a*) showing, (*b*), accumulation of s.P i. material in nerve fibres in central stump of divided dorsal root; (*c*), (*d*), s.P i. in intact, normal-looking dorsal root ganglion neurons and in intraganglionic nerve fibres; (*e*), s.P i. in nerve fibres in the mixed spinal nerve. (*f*) Accumulation of s.P i. material in central stump of divided dorsal root, 24 h postoperatively. (*g*) s.P i. in neurons of the same dorsal root ganglion as in (*f*) and in nerve fibres in the attached peripheral sensory root. Scales, 100 μm (*a*), (*f*), (*g*); 50 μm (*c*)–(*e*).

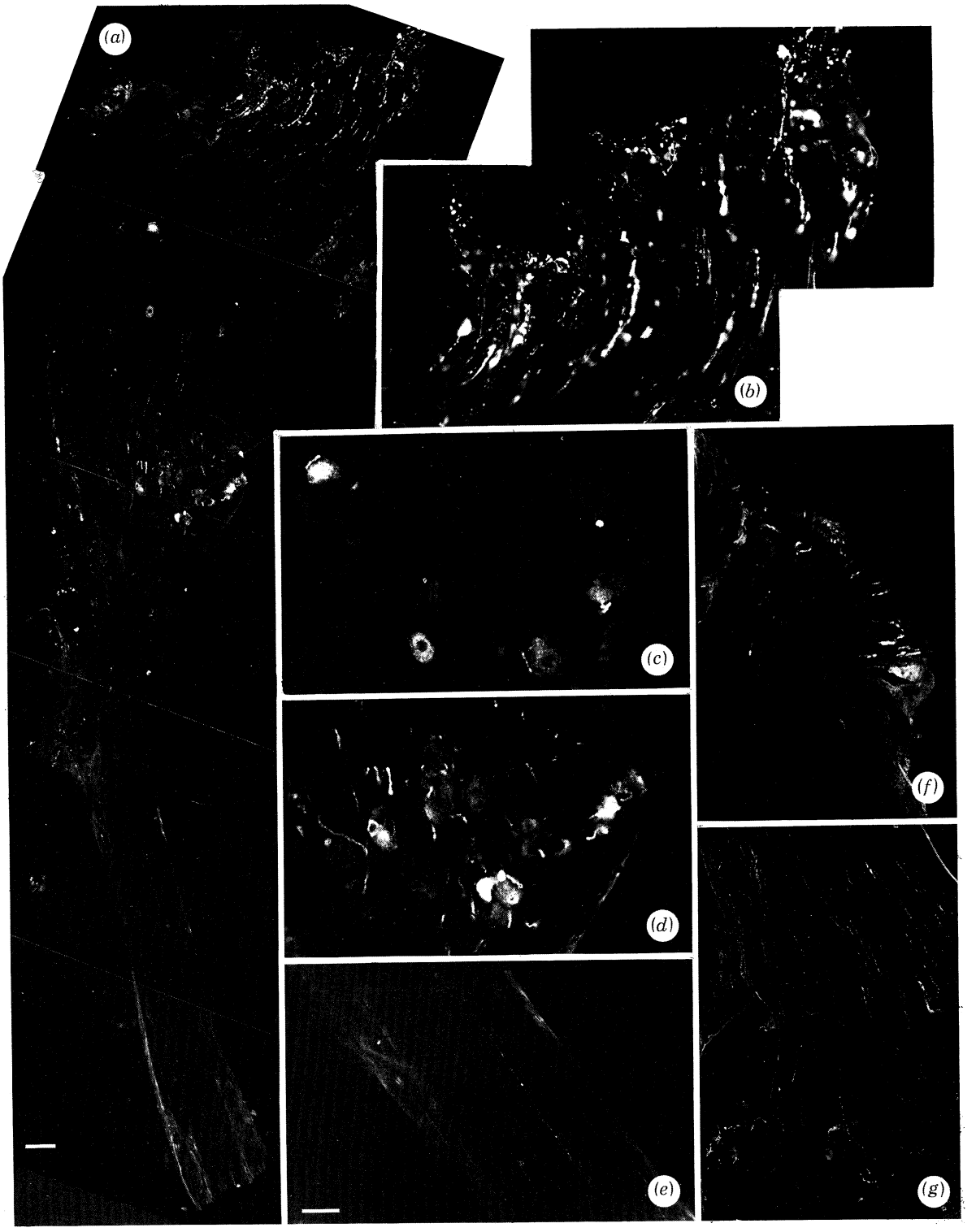

FIGURE 23. For description see opposite.

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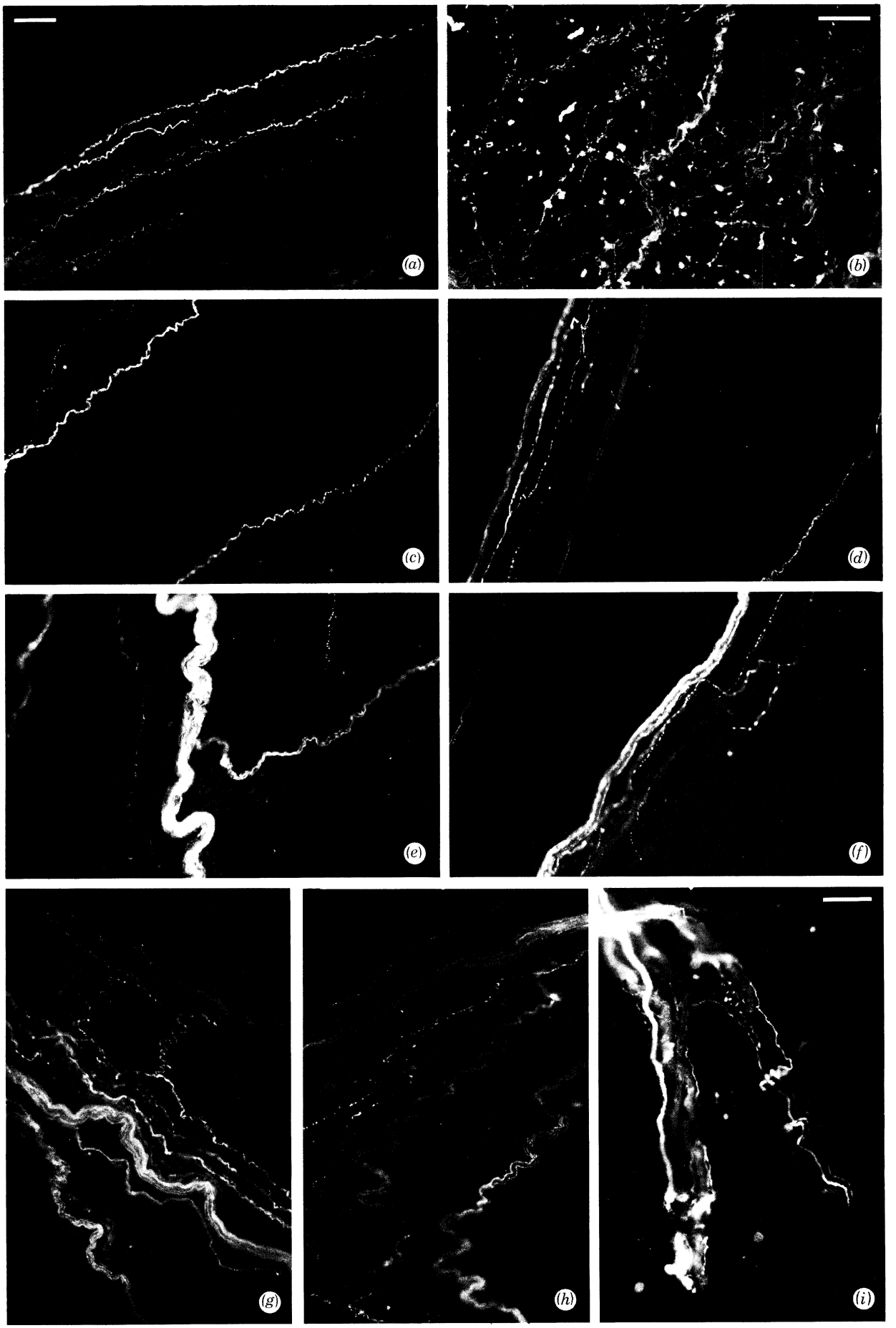

FIGURE 24. For description see opposite.

Description of plates 22–24

Figure 24. Omentum and mesenteries of guinea-pigs at 4 d and 5.5 d after spinal cord ablation below T7, and in control guinea-pig. Figures (*a*)–(*d*), 4 d after the spinal cord lesion: (*a*) greater omentum, showing slender paravascular s.P i. nerves giving rise to delicate perivascular network; (*b*) profuse s.P i. networks and diffuse paravascular bundles on great vessels in mesentery of ileum; (*c*) solitary s.P i. bundles and individual s.P i. nerve fibres in mesentery of ileum; (*d*) paravascular s.P i. bundles and long-meshed perivascular net, also solitary fascicle containing s.P i. fibres, in mesentery of distal colon. (*e*)–(*i*) Mesentery of distal colon in control guinea-pig ((*e*), (*g*)) and at 4 d ((*f*), (*h*)) and 5.5 d (*i*) after the spinal cord lesion. Figures (*e*) and (*f*), (*g*) and (*h*) show comparable smaller and larger neurovascular bundles, respectively; the distribution and appearance of the s.P i. peri- and paravascular nerves do not differ in any significant respect. Intensely s.P i. nerves are still present at 5.5 d after the spinal cord lesion ((*i*)). Scales, 100 μm (*a*), (*c*)–(*h*); 50 μm (*b*); 200 μm (*i*).

Figure 25. Disappearance of para- and perivascular s.P i. nerves from the mesentery of the distal colon following division of lumbar splanchnic and intermesenteric nerves. (*a*), (*c*), (*f*) Mesentery of distal colon of control guinea-pig showing (i) perivascular nerves in intimate association with vessels, (ii) various sizes of paravascular nerve bundles, (iii) delicate s.P i. bundles or single s.P i. nerve fibres detaching themselves from the perivascular nerves (*a*), (*c*), (*f*) or sometimes from paravascular nerves (*c*) and running independently in the mesentery; (*f*) shows one such nerve fibre apparently ending blindly (arrow).

(*b*), (*d*), (*g*) Mesentery of distal colon of guinea-pig 3.8 d after division of lumbar splanchnic and intermesenteric nerves. S.P i. material has totally or almost totally disappeared from both peri- and paravascular nerves: note large empty paravascular nerve crossing vessel obliquely in (*b*) (arrows). Figures (*e*) and (*h*) show normal s.P i. in a free nerve fibre (*e*) and nerve bundle (*h*) in the mesentery of the jejunum of the same guinea-pig, that is, from above the level of the nerve lesions. Scales, 100 μm (*a*)–(*d*), (*f*), (*g*); 50 μm (*e*), (*h*).

Figure 26. Montages of sections of an inferior mesenteric ganglion after spinal cord lesion, with normal and denervated ganglia for comparison. (*a*), (*c*) Inferior mesenteric ganglion from a guinea-pig 4 d after removal of the spinal cord below T7. Brightly s.P i. non-varicose nerve fibres and varicose networks show a normal pattern and distribution in the ganglion. (*b*) Control ganglion perfused and processed at the same time. (*d*), (*e*), (*f*) An inferior mesenteric ganglion 2.5 d after division of lumbar splanchnic and intermesenteric nerves; here, the intraganglionic s.P i. elements are fragmented and already much reduced. Accumulations of s.P i. material in the proximal tip of one of the severed nerves are seen in (*d*) (arrow, lower left), and s.P i. accumulations in the intermesenteric nerve of the same animal are shown in (*g*), which shows the tip of the nerve, and (*h*), which is a continuous montage extending backward from close to the tip of the slightly curved nerve (above) to a region in which the accumulations are less massive (lower edge of figure): this indicates that gross accumulations of s.P i. material in the intermesenteric nerve extended proximally for at least 3.5 mm from the nerve tip in this animal. This may be compared with the 4 d accumulations in the central processes of the dorsal root ganglion cells (figure 23). Scales, 100 μm (*a*)–(*f*), (*h*); 50 μm (*g*).

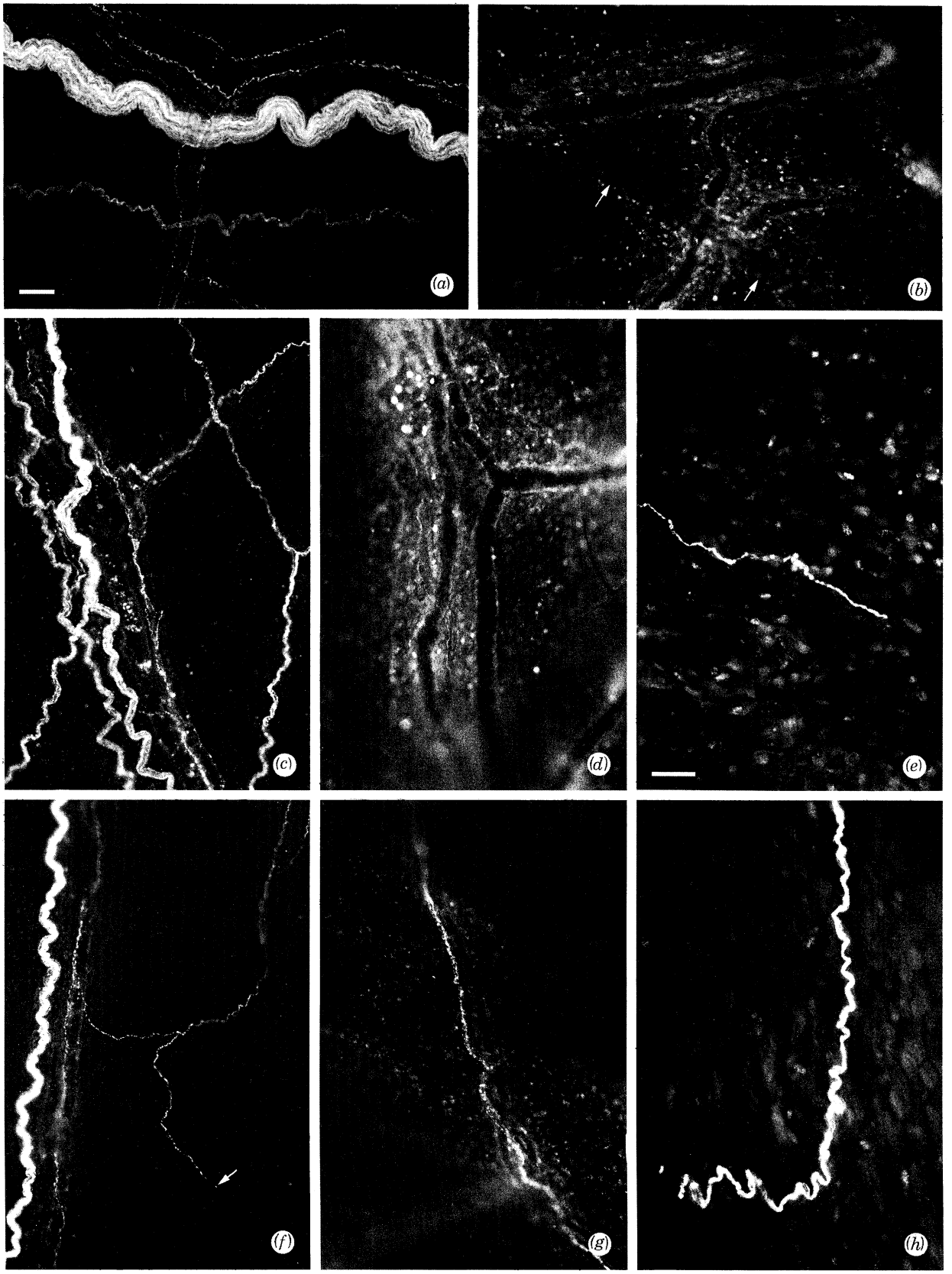

FIGURE 25. For description see opposite plate 22.

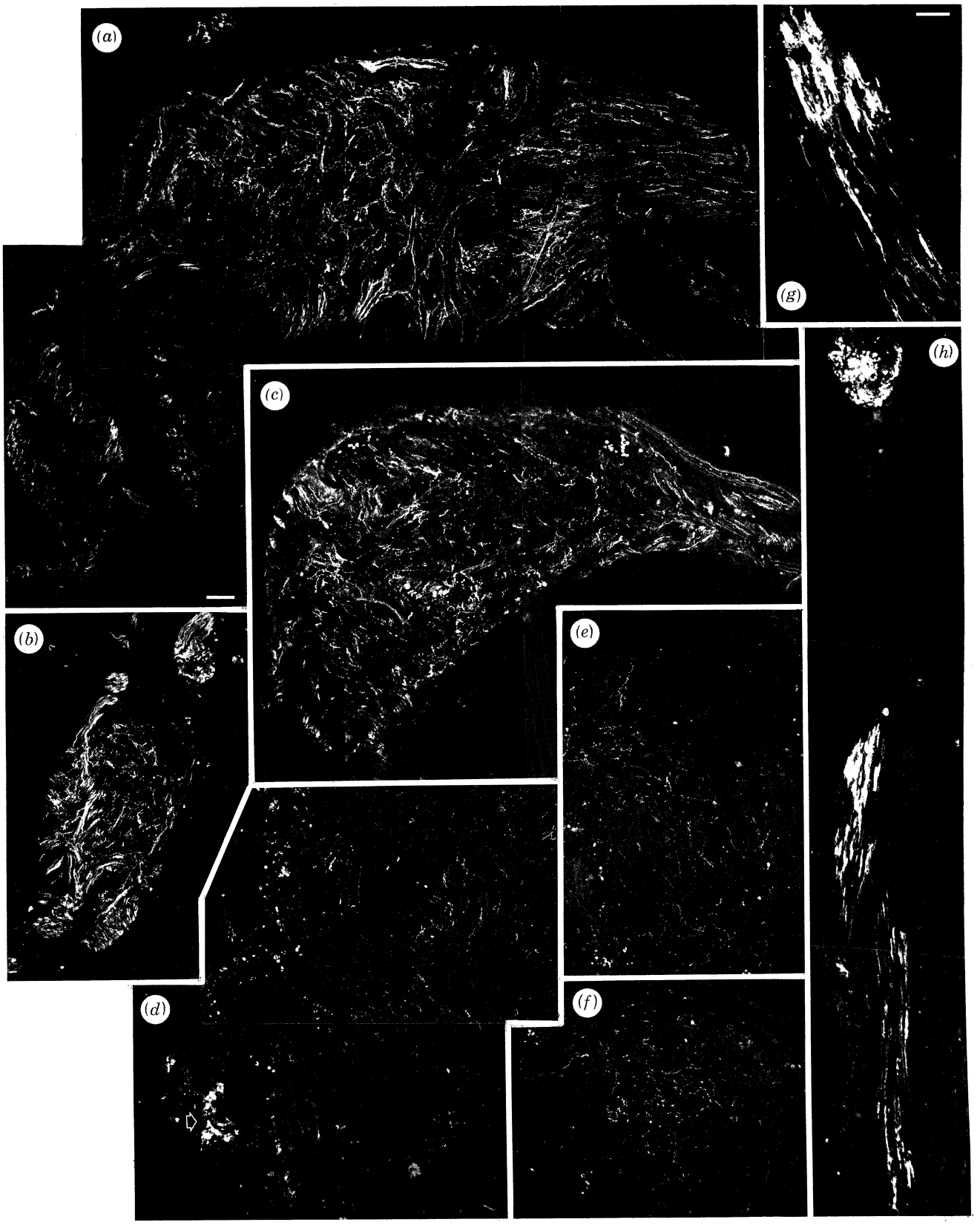

FIGURE 26. For description see opposite plate 22.

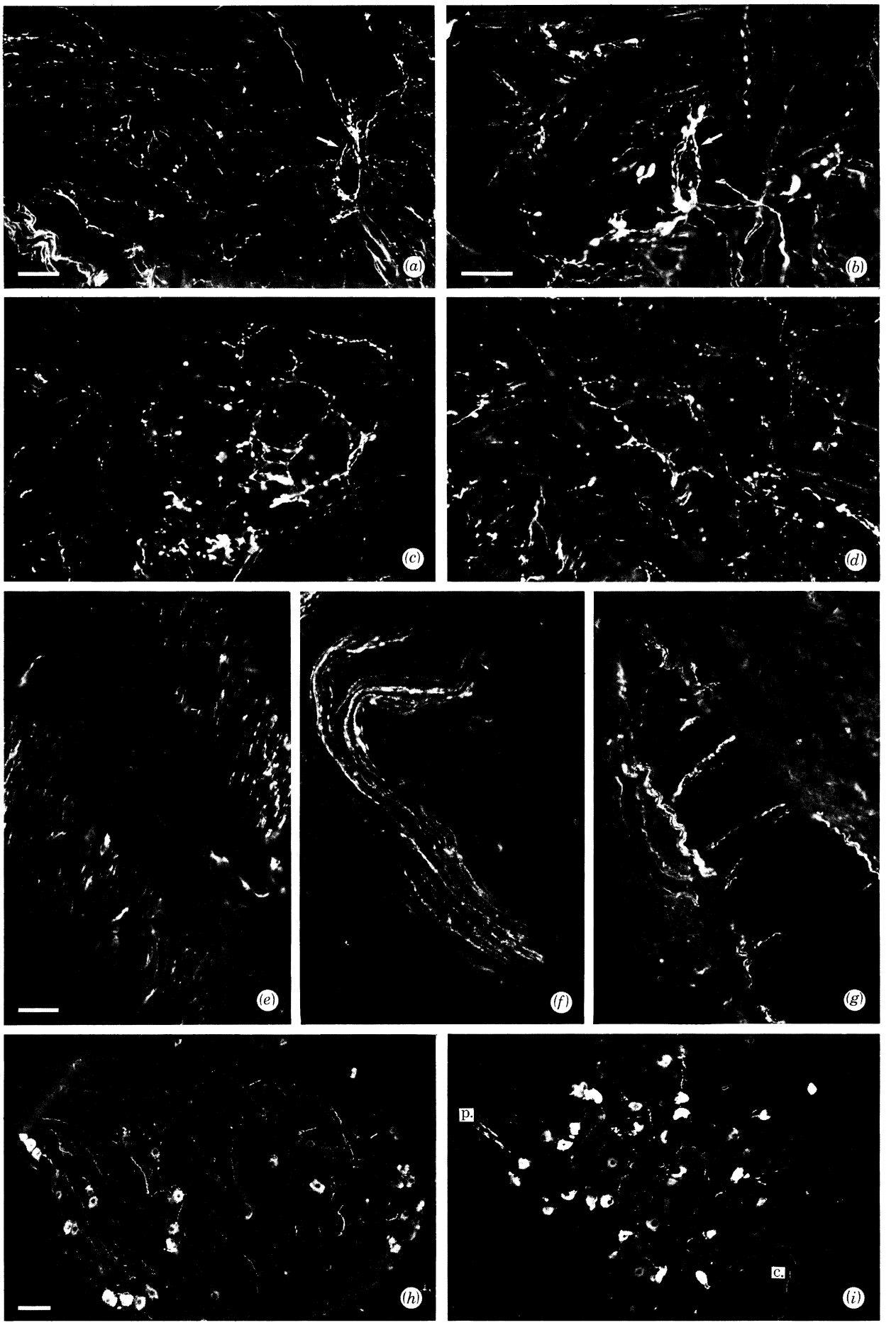

FIGURE 27. For description see opposite.

contribute significantly to the reflex control of activity and vasomotor function in the alimentary tract. Thus, lesions of the various nerves associated with the inferior mesenteric ganglion demonstrate that the great majority of s.P i. nerve fibres and networks in this ganglion are neither intrinsic in origin nor derived from the gastrointestinal tract, since they are largely preserved following division of the colonic and hypogastric nerves and are virtually entirely depleted following the combined section of the lumbar splanchnic and intermesenteric nerves. In normal guinea-pigs there are many more s.P i. nerve fibres in the lumbar splanchnic nerves, taken together, than in the intermesenteric nerve. Separate division of lumbar splanchnic nerves and intermesenteric nerve indicates that most of the s.P i. non-varicose nerve fibres and varicose networks of the inferior mesenteric ganglion are derived from fibres reaching the ganglion through the lumbar splanchnic nerves. Relatively few arrive along the intermesenteric nerve, as cutting the lumbar splanchnic nerves leaves the inferior mesenteric ganglion almost empty. But the remaining nerve fibres are traceable, by superimposition of montages, from the intermesenteric nerve through to the hypogastric and colonic nerves. This is in line with the findings of Konishi *et al.* (1979) on the reduction of substance P levels in the inferior mesenteric ganglion following lumbar splanchnic nerve lesions. The variable extent of the depletion may depend on the extent to which additional lumbar splanchnic fascicles join the intermesenteric nerve cranial to its entry into the inferior mesenteric ganglion (Costa & Furness 1973). Cutting the intermesenteric nerves produces a barely noticeable change in the intraganglionic nerve fibres and networks. Depletion is obvious only in the immediate neighbourhood of the intermesenteric nerve, where in normal ganglia s.P i. non-varicose fibres are seen spreading out into the ganglion; but the results of lumbar splanchnic nerve lesions indicate that varicose networks derived from the intermesenteric nerve are regionally present in both lobes of the ganglion. Application of horseradish peroxidase to the inferior mesenteric ganglia has been shown to label neurons in dorsal root ganglia up to the T13 level (Elfvin & Dalsgaard 1977) and some of these (at L2–L3 level) are s.P immunoreactive (Dalsgaard *et al.* 1982). The highest lumbar splanchnic nerve fascicle, carrying s.P i. nerve fibres, may join the intermesenteric nerve cranial to the inferior mesenteric ganglion. Enlarged s.P i. nerve fibres have however in the present experiments been seen in the intermesenteric nerve near its exit from the superior mesenteric ganglion, 4 d after it has been divided between the two ganglia. The intermesenteric nerve probably therefore carries s.P i. fibres that have passed through the coeliac–superior mesenteric ganglion and may have given

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FIGURE 27. Preservation of s.P i. nerve fibres and nerve networks of the inferior mesenteric ganglion at 4 d after spinal cord ablation below T7. (*a*) Inferior mesenteric ganglion of normal guinea-pig; (*b*)–(*d*), normal appearance of intraganglionic s.P i. non-varicose nerve fibres and varicose nerve networks after spinal cord ablation; note the similarity of the basket-like perineuronal fomations in (*a*) and (*b*) (arrows). (*e*), (*f*) Persistence of many s.P i. non-varicose fibres in lumbar splanchnic nerve bundles, seen in oblique section in (*e*) and in longitudinal section in (*f*); note in (*f*) the grainy texture of the fluorescence. (*g*) Persisting dense, ladder-like s.P i. innervation of the inferior mesenteric artery, close to the inferior mesenteric ganglion.

(*h*), (*i*) Comparison of L3 dorsal root ganglion at 3.8 d after section of lumbar splanchnic and intermesenteric nerves (*i*) with a dorsal root ganglion from a higher level (cervico-thoracic) in the same guinea-pig (*h*). In the L3 ganglion the proportion of strongly s.P i. neurons is at least as high as in the uninjured ganglion, and in a number of these neurons the immunofluorescence is sufficiently intense to obscure the nucleus. Distended s.P i. fibres are seen in part of the peripheral sensory root (p.). The central sensory root (c.) contains s.P i. fibres of normal appearance. Scales, 50 μm (*a*), (*e*)–(*g*); 50 μm (*b*)–(*d*); 100 μm (*h*), (*i*).

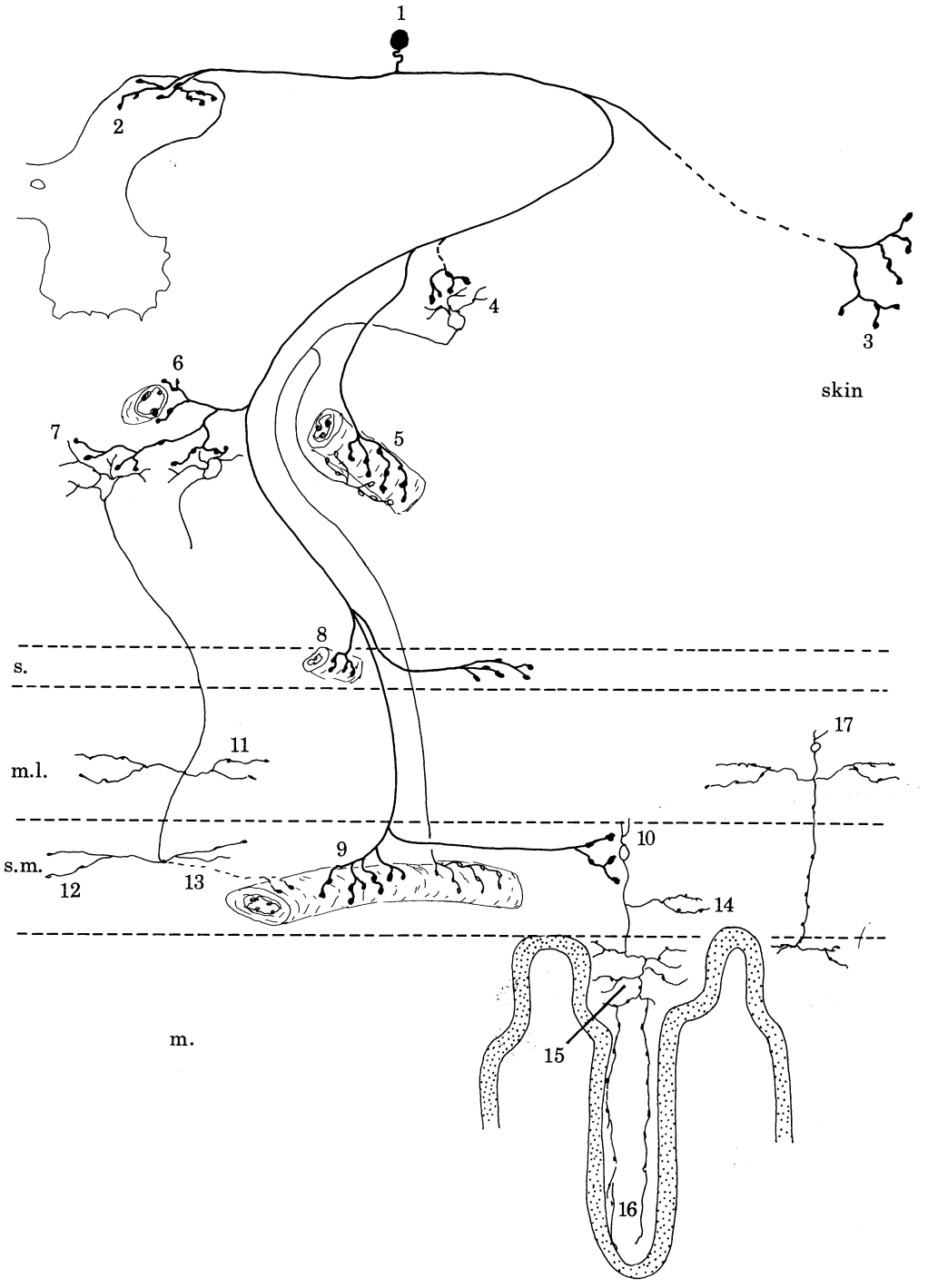

FIGURE 28. Schematic diagram (not to scale) to summarize conclusions on the distribution of substance P immunoreactive neurons innervating the alimentary tract and its blood vessels, based on present and earlier evidence. Tentative or putative nerve connections are indicated by broken lines. Postganglionic sympathetic projections are omitted except where strictly relevant. The branching peripheral process of a sensory s.P i. neuron in a dorsal root ganglion (1, this neuron type is here represented as a single cell) transmits afferent impulses from its terminals, either in somatic territory (for example, skin, 3; including blood vessels, not here indicated in detail), or in visceral territory (5,6,8,9,10). These impulses travel along the central process into laminae I and II of the spinal cord (2). The pathway of peripheral processes returning from the visceral territory passes, via

off collateral networks there also, which could be involved in regulating reflex interactions between widely different levels of the alimentary tract.

All these nerve divisions lead to s.P i. material accumulating in the central ends of the nerves concerned, that is in the cranial stump of the intermesenteric nerve, the proximal stumps of the lumbar splanchnic nerves and the ganglionic stumps of the colonic and hypogastric nerves, and this is consistent with continuing proximo-distal transport of s.Pi. material in mixed severed nerves (Hökfelt *et al.* 1977; Gamse *et al.* 1979; Brimijoin *et al.* 1980; Gilbert *et al.* 1980; Harmar & Keen 1982). Accumulation of radio-immunoassayable substance P has also been found in severed hypogastric nerves by Konishi *et al.* (1979). The evidence for sprouting from the ends of these severed nerves and fascicles in the present experiment underlines the potential for recovery in this system. The rate of loss of s.P immunofluorescence from nerve fibres distal to a lesion is not particularly rapid. After combined lesions of the lumbar splanchnic nerves and intermesenteric nerves the networks in the inferior mesenteric ganglion were relatively little altered at 46 h postoperatively though they were greatly diminished and fragmented after 2–3 d and had essentially disappeared by 3.8 d postoperatively.

The results of capsaicin administration strongly reinforce the sensory nature of the intraganglionic s.P i. elements, since it has been fully substantiated experimentally that this substance highly selectively depletes the peptide content of a subpopulation of primary sensory neurons (Jançsó *et al.* 1977; Jessell *et al.* 1978; Gamse *et al.* 1980; Lawson & Nickels 1980; Jançsó & Király 1981; Jançsó *et al.* 1981; Priestley *et al.* 1982). The present experiments have confirmed the selective depletion of s.P i. material from dorsal root ganglion neurons and from laminae 1 and 2 of the spinal cord. In the periphery, s.P immunofluorescence was found to be dramatically reduced throughout the entire prevertebral ganglion complex (coeliac–superior mesenteric ganglion and inferior mesenteric ganglion). This is in agreement with the radioimmunoassay analysis of similar material by Gamse *et al.* (1981). In the alimentary tract, capsaicin treatment does not significantly diminish the level of radioimmunoassayable substance P in the wall (Holzer *et al.* 1980). The enteric nerve plexuses are richly supplied with s.P i. neurons and nerve networks (Costa *et al.* 1980; Jessen *et al.* 1980; Schultzberg *et al.* 1980; Costa *et al.* 1981). The present experiments have shown that in the wall of the ileum the s.P i.

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rami communicantes, through the paravertebral sympathetic ganglia (4), where s.P i. collaterals may be given off; the postganglionic territory of these ganglia includes the major blood vessels of the region, which receive also an s.P i. innervation at this level (5,6). The s.P i. visceral sensory fibres also pass, via splanchnic nerves, through the prevertebral ganglia (6,7), where it is argued in this paper that collaterals of the s.P i. sensory fibres form the basis of the intraganglionic varicose s.P i. nerve networks (7). The postganglionic territory of the prevertebral ganglia includes the myenteric and submucous plexuses of the alimentary tract (11,12) and to some extent the intramural blood vessels (13). The levels of demarcation or overlap between the vascular territories of prevertebral and paravertebral ganglia are incompletely defined: at rectal level the submucosal blood vessels receive noradrenergic innervation via the pelvic plexuses, not from the inferior mesenteric ganglion (Furness & Costa 1973). To the extent that the enteric nerve plexuses and the enteric blood vessels are controlled by separate ganglia of the sympathetic system, sensory s.P i. collateral branches of the same or parallel sensory neurons will be required in each, for the fullest possible regulation of sympathetic control over visceral activity. The s.P i. enteric sensory fibres which pass through the prevertebral ganglia supply s.P i. nerve networks to the enteric blood vessels, including mesenteric and serosal (8) vessels and the distributing vessels in the submucosa (9), and contribute large boutons to the submucous plexus (10). Intrinsic s.P i. neurons in the myenteric (17) and submucous plexuses (10) provide dense s.P i. meshworks of fine calibre locally throughout the plexuses and in the muscle coats (14, 17), and in the mucosa form pericryptal (15) and subepithelial (villous, 16) s.P i. networks, including s.P i. nerve strands in the muscularis mucosae. The significance of the sensory collateral arrangements is discussed further in the text. s., Serosa; m.l., muscular layer; s.m., submucosa; m., mucosa.

of the enteric nerve plexuses is largely unaffected by capsaicin but that there is a clear-cut loss of s.P i. from peri- and paravascular nerves in the submucosa and of large s.P i. varicosities in the submucous plexus which seem, or are seen, to be derived from offsets of the perivascular nerve plexuses. Dopamine β-hydroxylase immunoreactivity in the submucous plexus and around the blood vessels survives unchanged, indicating that s.P immunoreactivity is independent of the postganglionic sympathetic nerve fibres. S.P i. is also lost from peri- and paravascular nerves and from isolated nerve fascicles in the serosa. The capsaicin-sensitive component of the gut innervation is therefore assumed to be derived, like the capsaicin-sensitive elements in the prevertebral ganglia, from spinal sensory neurons. This is in line with the observations by Costa *et al.* (1980, 1981) that extrinsic denervation of gut segments removes a similar population of s.P i. nerve fibres, and that these are independent of autonomic adrenergic innervation (Furness & Costa 1980). In the prevertebral ganglia only the merest traces of s.P i. remained after capsaicin treatment, in the form of very occasional faintly immunoreactive short varicose trails and a few basket-like formations round individual neurons. All the nerves associated with the ganglia were empty of s.P i. nerve fibres, except for one or two possible fibres found in the colonic and hypogastric nerves. The s.P i. neurons of the enteric plexuses thus cannot be regarded as providing any major proportion of the s.P i. networks of the ganglia, but might account for the minor residual elements persisting after capsaicin; alternatively, the latter might be traces remaining in s.P i. elements of dorsal root ganglion origin which have degenerated more slowly than others or are relatively more resistant to capsaicin.

Lesions of the spinal cord were performed to explore the remaining possibility that intraspinal neurons, demonstrable in small numbers in the intermediate zone after colchicine treatment (Ljungdahl *et al.* 1978), might contribute to the intraganglionic s.P i. networks in the inferior mesenteric ganglion, since the possible effect of capsaicin on these neurons is not known. The level of the lesion, T7–8, was well above the level (T13) to which horseradish peroxidase has been found to be transported retrogradely from the inferior mesenteric ganglion (Dalsgaard & Elfvin 1979). It was selected as the level that has been observed clinically in cases of human cord transection to spare a sufficient residuum of central control over vasomotor and thermoregulatory capacity (Guttmann & Whitteridge 1947). Control experiments involving simultaneous section of the lumbar splanchnic nerves and intermesenteric nerves indicated that the longer of the survivals obtained after spinal cord lesions (4 d, 5.5 d), should have been long enough to permit a significant degree of disintegration and degeneration of intraganglionic networks and s.P i. fibres, even allowing for the greater distance (of not more than 10–11 mm at the most) between the spinal cord and the lumbar splanchnic nerves at the point of severance (which was as close to the vertebral column as possible); indeed at 3.8 d following the control nerve lesions there was virtually total loss of peri- and paravascular s.P i. nerves from the mesentery of the distal colon, some 2–3 cm distal to the lesions. The peri- and paravascular s.P i. nerves of the distal colon, however, remained indistinguishable from controls at 4 and 5.5 d following spinal cord lesions. The finding that there was no discernible loss, either of non-varicose or of varicose s.P i. nerve elements, in the inferior mesenteric ganglion following the spinal cord lesions would safely indicate that intraspinal neurons cannot contribute to these to any great extent. It should be noted that the paravertebral lumbar sympathetic ganglia intervene between the spinal cord and the inferior mesenteric ganglion and were not directly injured by the spinal cord lesions. These ganglia however were not seen to contain s.P i. neurons, though

they are traversed by many s.P i. non-varicose nerve fibres and also contain s.P i. networks. They are unlikely to contain neurons supplying s.P i. elements to the inferior mesenteric ganglion. But treatment with colchicine would be required to exclude this remote possibility.

Unexpectedly, in one ganglion subjected to section of lumbar splanchnic nerves, substance P immunoreactivity was found in some s.i.f. cells. This recalls the situation reported by Kessler *et al.* (1981) in principal neurons of the superior cervical ganglion in the rat following decentralization in the adult or explantation into tissue culture of neonatal ganglia. In our case we find that the expression of s.P i. is restricted to a number of s.i.f. cells and is not seen in principal neurons. Our observations might imply that the onset of the expression of s.P i. may occur earlier in certain s.i.f. cells than in principal neurons, as the present experiments used survival intervals of 4 d, not 12 d as in the experiment of Kessler *et al.* (1981). Moreover, the s.i.f. cells of guinea-pig prevertebral ganglion contain noradrenaline or even adrenaline (Elfvin *et al.* 1975), not dopamine as in the rat superior cervical ganglion (Björklund *et al.* 1970). The present finding also relates to a situation in which decentralization was incomplete and involved in addition loss of s.P i. elements, which are not so profuse in the rat, particularly in the superior cervical ganglion (Hökfelt *et al.* 1977). The relative influences of preganglionic innervation and of sensory collateral innervation are not evident at this stage in the two experimental situations. It is more difficult still to relate the present findings to the results of the tissue culture experiments of Kessler *et al.* (1981) on neonatal rat superior cervical ganglia in the presence of 100 ng ml^{-1} of nerve growth factor. Here the commitment of cells is almost certainly not so irreversible as in the adult (Soinila & Eränkö 1982). It may also be noted that substance P is secreted by some cells of human phaeochromocytoma. These interesting observations are being explored further.

The observations on dorsal root ganglia have lent support to the finding of Harmar & Keen (1982) that more s.P i. material enters the peripheral than the central process of a sensory neuron. Peripheral branches of dorsal root ganglion cells were observed to be apparently of greater calibre and much more strongly s.P immunoreactive than central processes, both as populations of fibres in the respective root bundles and in single instances in which the T-junction was clearly shown. This is consistent with the observations of Ramón y Cajal (1911) on the relative dimensions of these processes, using the Golgi technique. Moreover, the accumulations in central ends of divided dorsal roots after 4 d were much less extensive, involved less immunoreactive material and extended less far backward towards the cell body than those seen at 4 d after cutting the peripheral branches of dorsal root ganglion neurons, whether in lumbar splanchnic, intermesenteric, hypogastric or colonic nerves. It also seems unlikely from these experiments that the output of s.P i. material into either the peripheral or the central process diminishes appreciably during the first 4 d post-section, unless an early reduction is rapidly compensated for. The 4 d accumulations in the central processes were considerably more than those seen at 24 h. The finding of s.P i. loops or spirals round the cell bodies of some s.P i. dorsal root ganglion cells, like the pericellular arborizations described by Ramón y Cajal (1911), raises the possibility that some of these neurons have autoreceptors that could mediate a feedback regulation; this would be likely to be relatively long-term, perhaps metabolic or trophic or inductive, since the soma is not directly involved in the centripetal passage of the nerve impulse, though it may be invaded by it. Katz & Karten (1980) have observed s.P i. pericellular arborizations round neurons of the nodose ganglion in pigeons and rabbits, but these neurons were 'primarily unstained'; and they formed the view that the

arborizations arose cranially to the nodose ganglion, possibly from neurons of the jugular ganglion.

The seeming paradox of a greater output of putative transmitter or neuromodulator material into the peripheral than the central process of these sensory neurons is to be correlated with the much greater peripheral territory and with the evidence that substance P may be released from peripheral branches (Olgart *et al.* 1977; Gazelius *et al.* 1981). Substance P is known to be a potent vasoactive agent and it is suspected that substance P-containing sensory nerve fibres may play an important role in antidromic vasodilatation via the axon reflex (Lembeck & Holzer 1974; Lembeck & Gamse 1982; Couture & Cuello 1984). In addition to this the peptide has demonstrable synaptic effects in the inferior mesenteric ganglion (Konishi *et al.* 1979, 1980). Preliminary electron microscopic observations have indicated that some of the substance P immunoreactive varicosities in the inferior mesenteric ganglion (Baker *et al.* 1980; Matthews & Cuello 1982), and also in the coeliac–superior mesenteric ganglion (Kondo & Yui 1981), form actual synapses upon postganglionic neurons. It may be postulated that the intraganglionic s.P i. nerve varicosities and synaptic endings arise from collateral branches of sensory nerve fibres from the alimentary tract and its blood vessels, traversing the ganglia. This hypothesis is an attractive one, as it would give a clear-cut physiological function, of axon reflex type, for the s.P i. varicose networks in the ganglia. Thus, substance P released synaptically from intraganglionic collateral branches of these primary sensory neurons could modify the activity of the sympathetic neurons via a short loop of an axon reflex type. The s.P i. sensory neurons are likely to be nociceptor, and the effect would be to enhance adrenergic inhibition of gut activity in advance of, and, or, in support of, or even independently of, the recruitment of central nervous circuits. This is not yet directly proven, but it is lent support by the following pieces of indirect evidence. First, the s.P i. fibres entering the ganglion in the lumbar splanchnic nerves appear to be of greater calibre and more strongly immunofluorescent than those leaving in the hypogastric and colonic nerves, yet the same fibres are traceable across the ganglion from the one set of nerves to the others in nerve lesion experiments. Since the s.P i. nerve fibres in the lumbar nerves are not of markedly varying calibre, it seems unlikely that there are larger, more strongly immunoreactive fibres that provide the intraganglionic networks and smaller, less immunoreactive fibres which innervate the intestine. What seems more likely is that the fibres branch, and that each daughter fibre beyond the point of branching has less immunoreactive material than the parent fibre, as may be seen to occur at the T-junction points of branching in the dorsal root ganglion. Second, there is the paradoxical finding of a regional reduction of intraganglionic s.P i. networks in ganglionic zones containing many distended non-varicose s.P i. fibres after colonic or hypogastric nerve lesions. The capsaicin experiments have shown that the intraganglionic s.P i. networks cannot be attributed on any scale to enteric neurons, thus depletion of the s.P i. networks in conjunction with s.P i. accumulation in distended non-varicose fibres indicates some interdependence of these two elements, of a trophic or metabolic kind. Collateral terminal networks arising from a fibre that has been severed distally might become depleted of transmitter by a combination of factors, for example, through injury discharges arising at the cut end, with a reduction in the output from the cell body, which may occur in the longer term. No consistent evidence was found for the converse, that is, for distension of varicosities by possible accumulation of s.P i. material diverted from the damaged fibres; a few large s.P i. varicosities were found in these ganglia, but they were not grossly outside the normal range in size or number. For these reasons it seems very probable that the

s.P i. varicose networks in the ganglion arise directly from s.P i. sensory fibres passing through the ganglion, and not independently of them. It may be noted here that the nerve lesion experiments indicate that the s.P i. nerve on the stem of the inferior mesenteric artery arise for the most part centrally to the inferior mesenteric ganglion, possibly, like the sympathetic innervation of the aorta and its main branches, from s.P i. fibres traversing the ganglia of the paravertebral sympathetic chains and leaving them proximal to the inferior mesenteric ganglion. The lumbar paravertebral ganglia of these guinea-pigs were found also to contain varicose s.P i. nerve networks and could be a site for coordination of vascular reflexes, in line with the generalized vasomotor activity of the sympathetic chain. This could involve sensory inputs from vessels supplying the alimentary tract, and could be well served or supplemented by collaterals from sensory nerve fibres crossing through these ganglia, in addition to any reflex effects which these fibres might generate centrally.

Complex reflex interactions are demonstrable between the gut and the prevertebral ganglia, and these have been shown to involve ganglionic excitation attributable to cholinergic neurons in the intestinal nerve plexuses (Crowcroft *et al.* 1971; Szurszewski & Weems 1976; Kreulen & Szurszewski 1979). Most of the extraganglionic inputs to the inferior mesenteric ganglion have been demonstrated to operate by way of the integration of subthreshold excitatory inputs to the postganglionic neurons (Weems & Szurszewski 1978). The s.P i. intraganglionic networks, if they are a system of collateral branches from the primary sensory neurons of the gut and its associated structures, could augment these reflexes, or increase the response to central activation, acting upon the postganglionic neurons in a modulatory sense by producing slow depolarization to raise the excitability of the neurons to above the firing threshold for other subthreshold inputs. The present and earlier results (Hökfelt *et al.* 1977; Konishi *et al.* 1979, 1980; Baker *et al.* 1980; Costa *et al.* 1980; Dalsgaard *et al.* 1982; Matthews & Cuello 1982) indicate the possibility of substance P mediated intraganglionic modulation, operating through a short loop system of collaterals from peripheral branches of the primary sensory neurons of the spinal ganglion with sensory, possibly nociceptive, terminals in the gut. Such an arrangement need not be confined to the s.P i. pathway, and would supplement, rather than replace, centrally generated reflexes. Further evidence, to be presented, indicates that such a loop may operate through morphologically specialized synapses, and that classical synapses may be formed by peripheral branches of mammalian primary sensory neurons. The extent to which this is involved in this intraganglionic system is currently being explored.

Questions of possible interaction of s.P i. networks with other peptidergic nerve endings, for example, enkephalinergic endings (Schultzberg *et al.* 1979; Konishi *et al.* 1980), or of co-existence with other neuroactive substances as in the central nervous system (Chan-Palay *et al.* 1978; Hökfelt *et al.* 1978; Bowker *et al.* 1981), also remain to be resolved.

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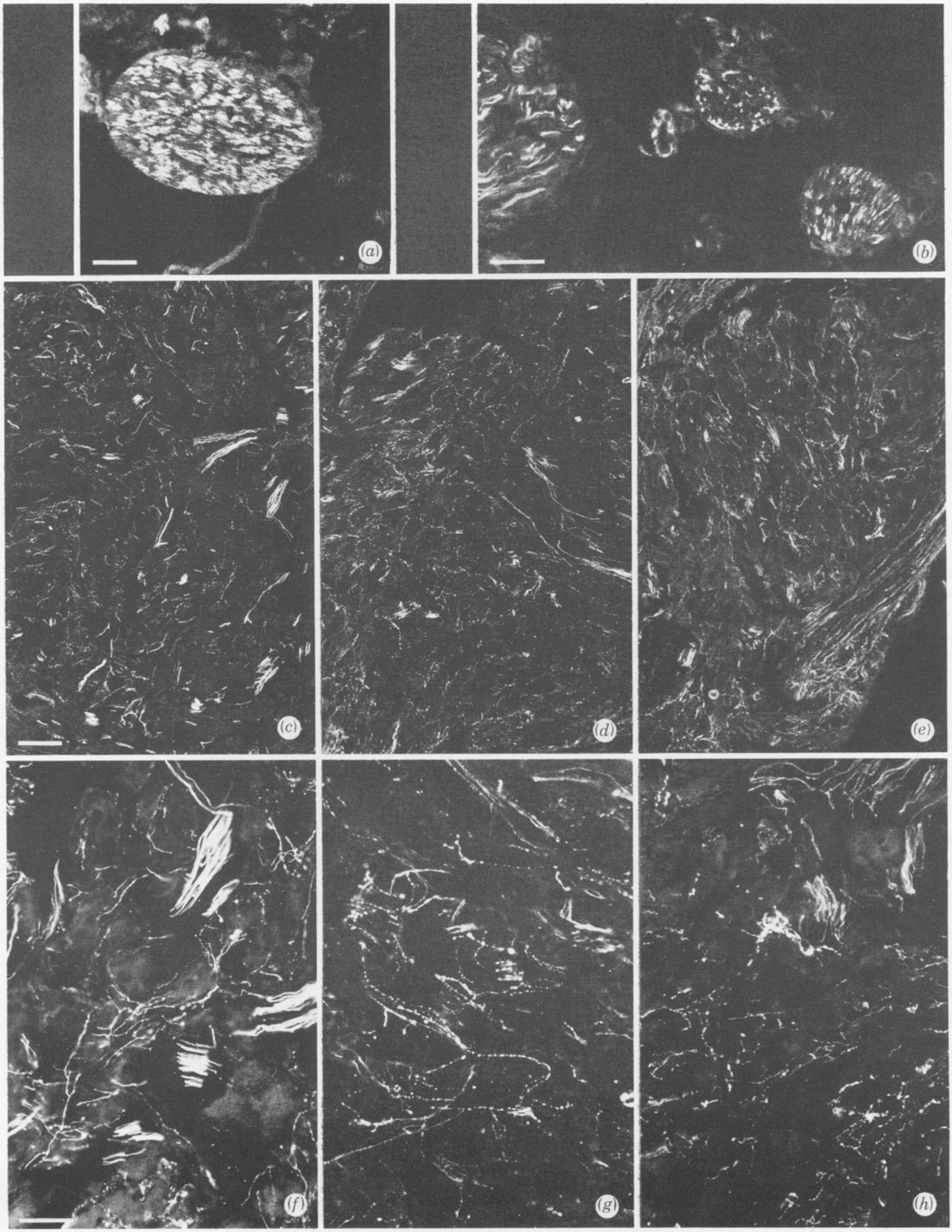


FIGURE 2. For description see opposite.

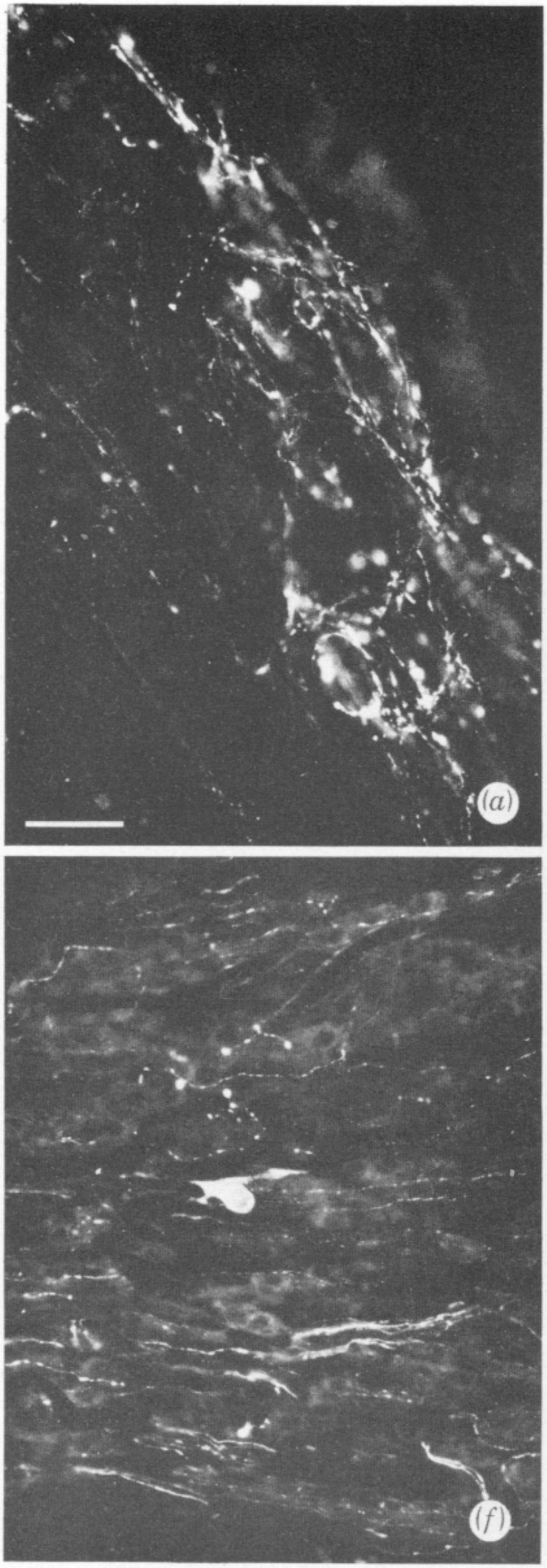


FIGURE 3. For description see p. 256.

(a)

(b)

(c)

(d)

c.

c.

h.

l.s.

l.s.

(e)

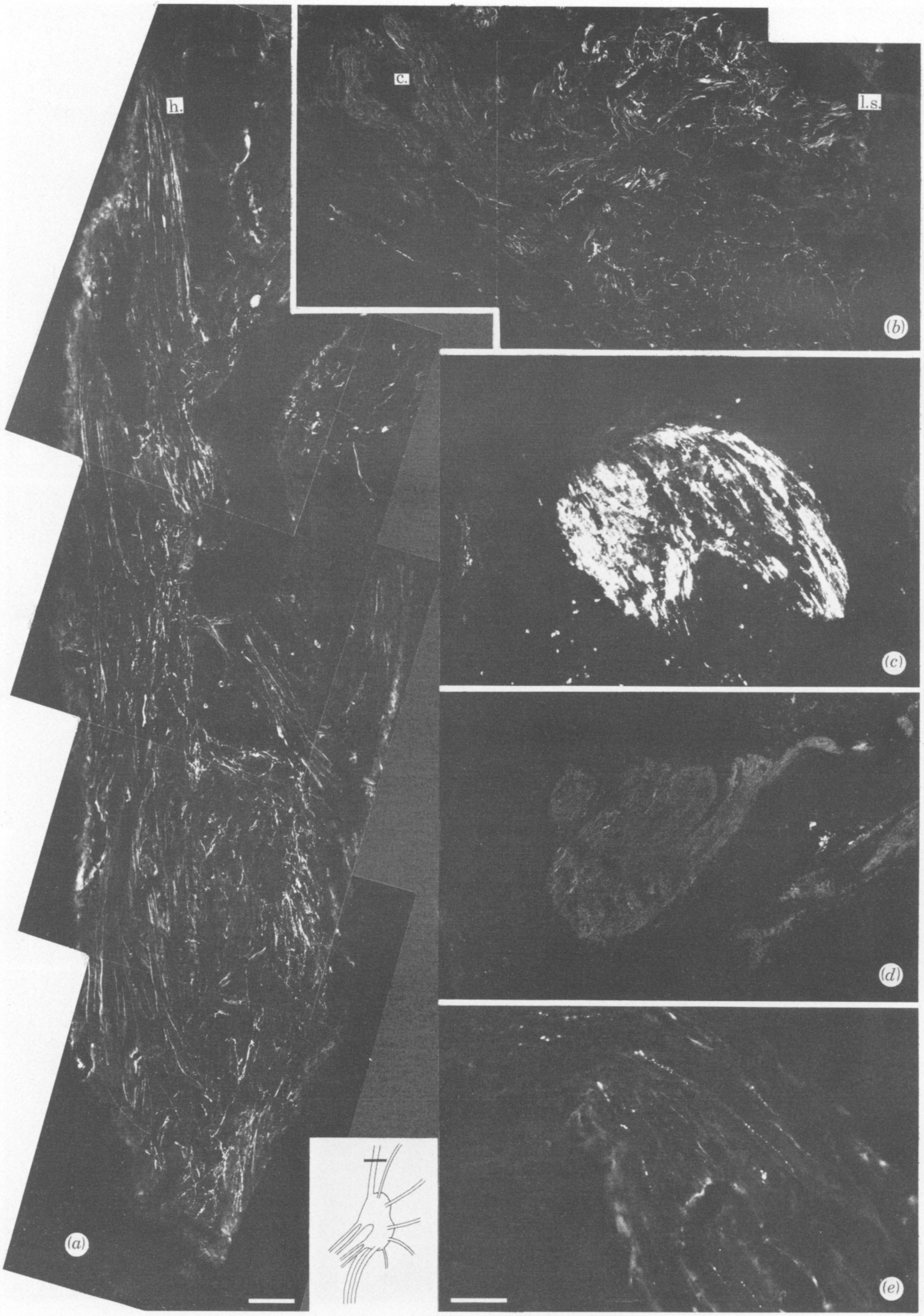
(f)

(g)

(h)

(i)

FIGURE 4. For description see p. 256.



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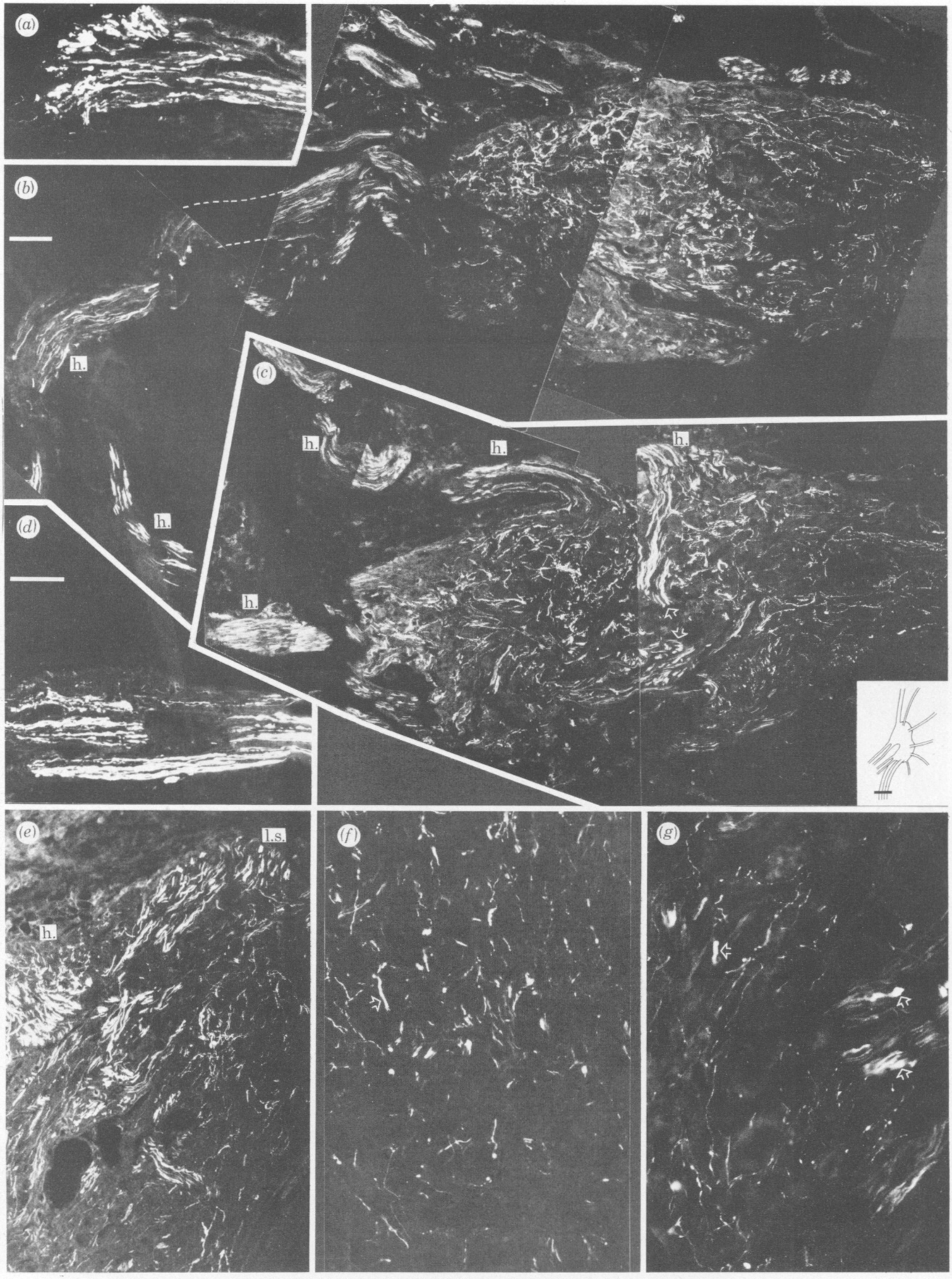

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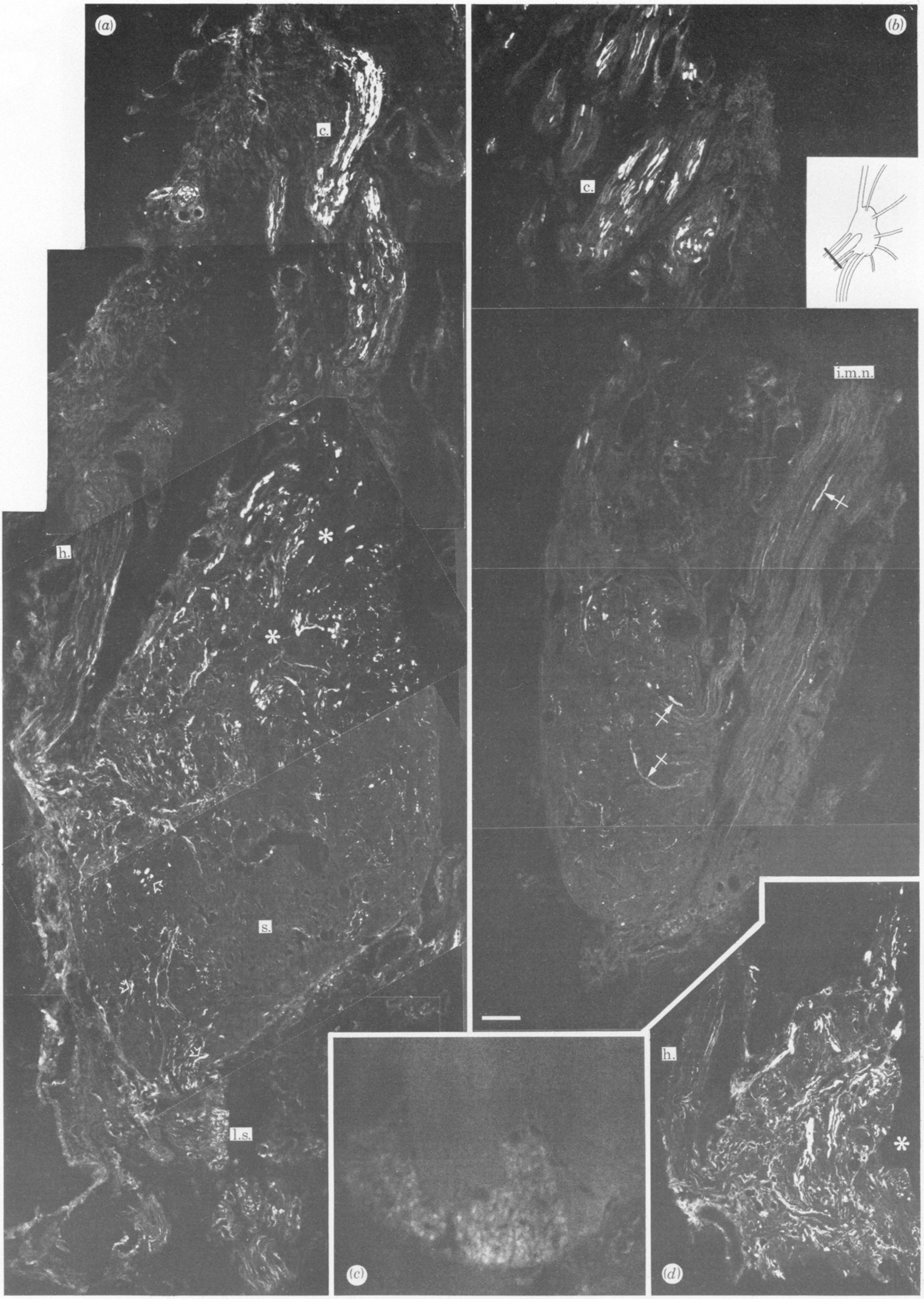

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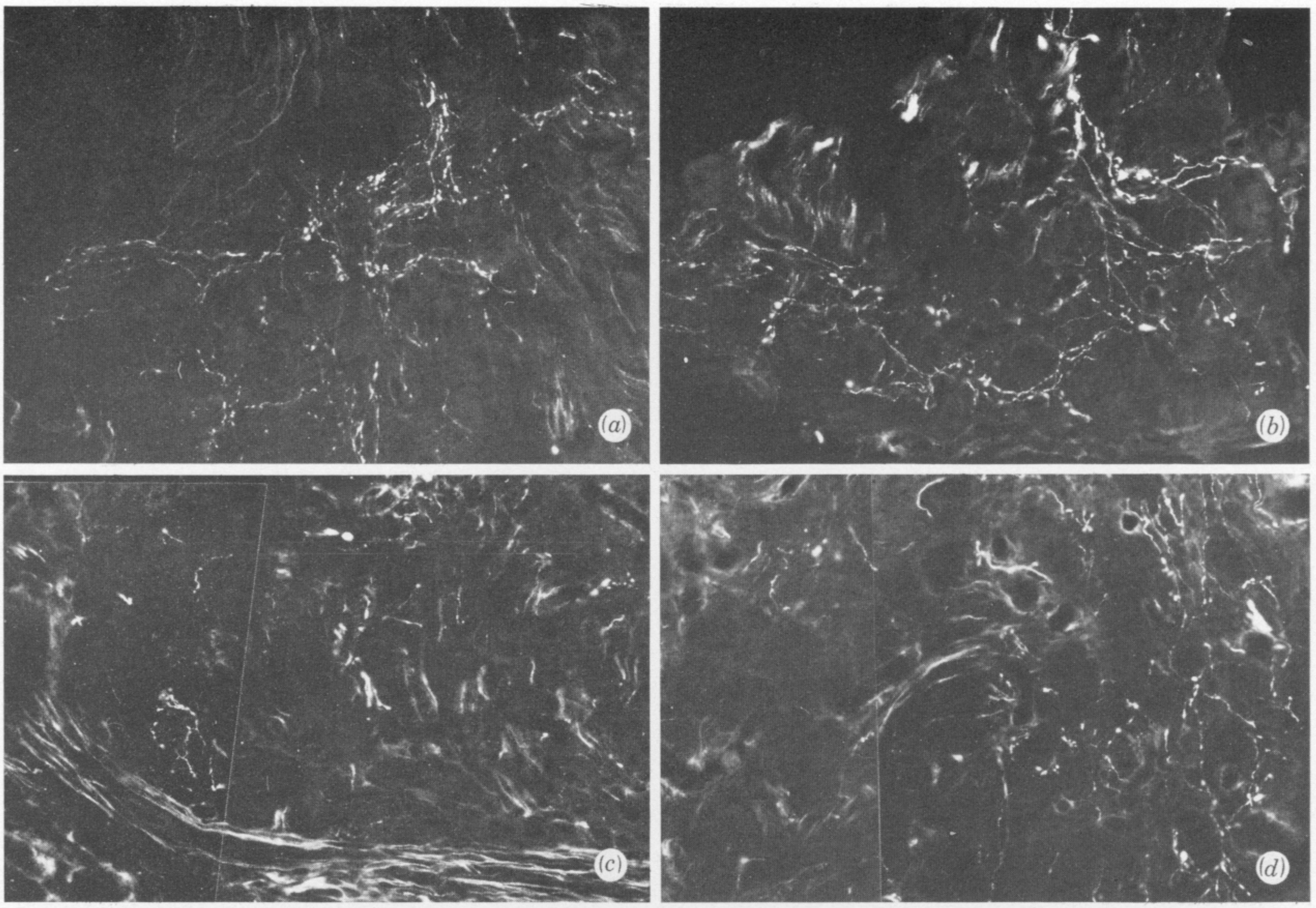

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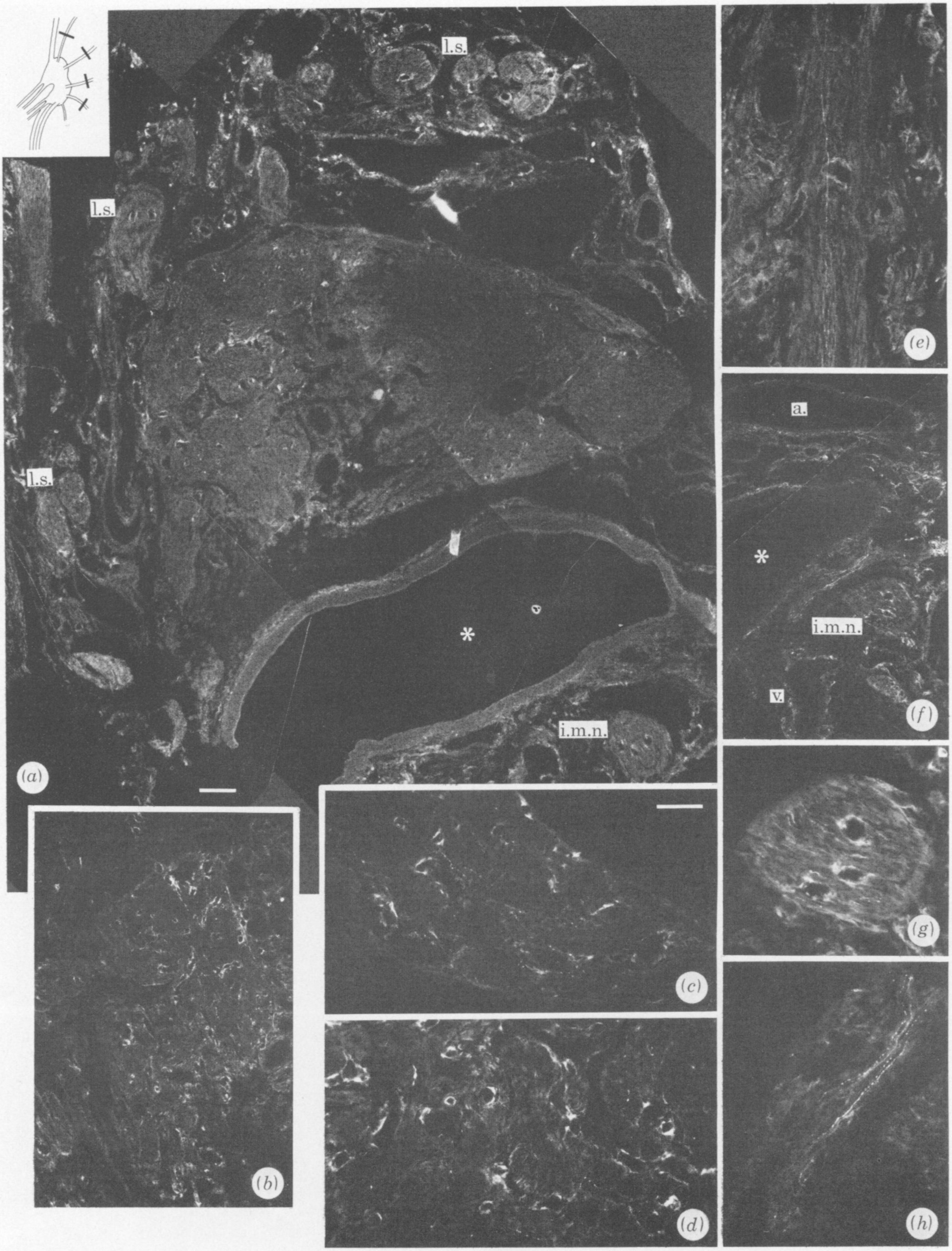

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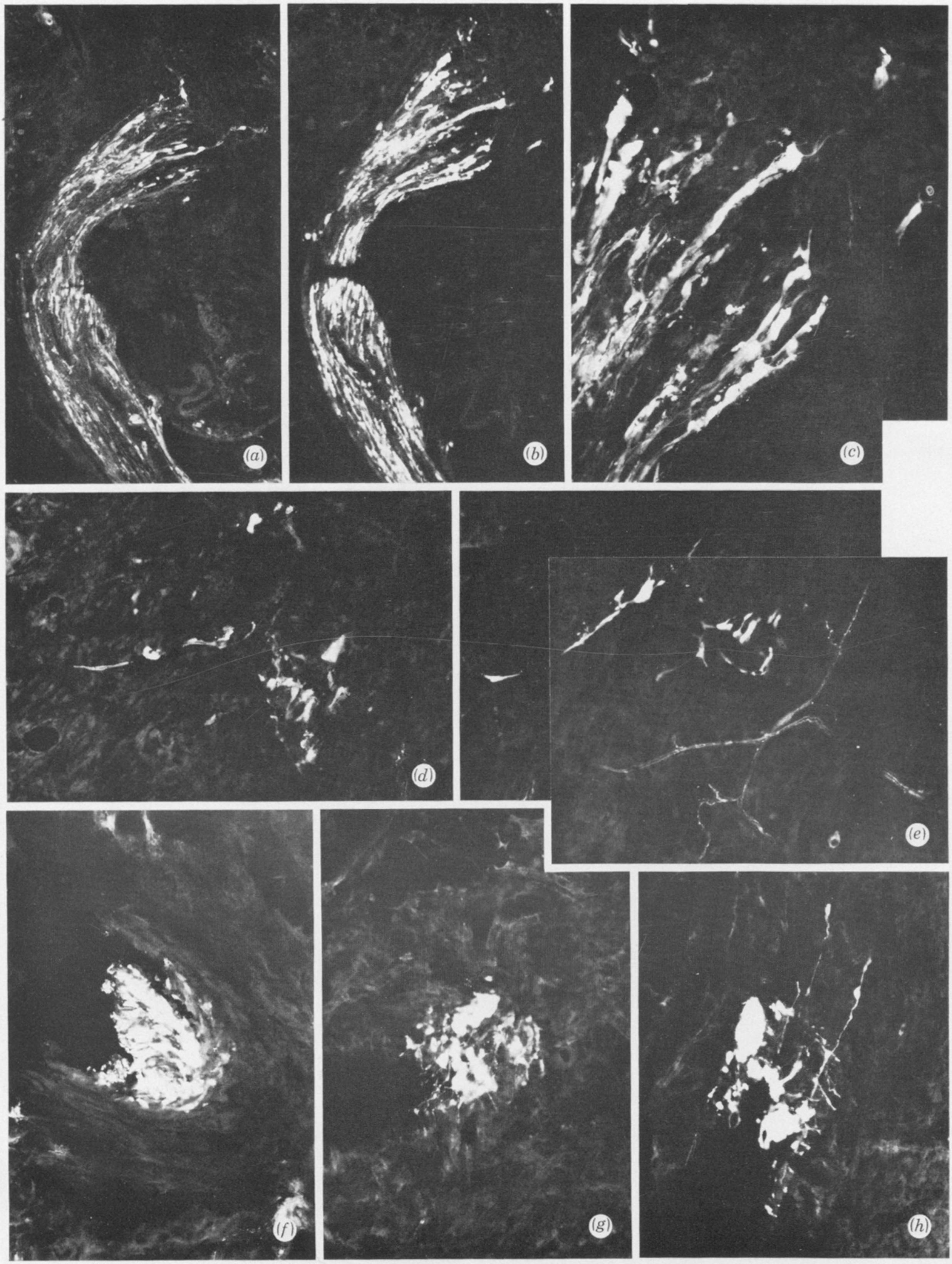
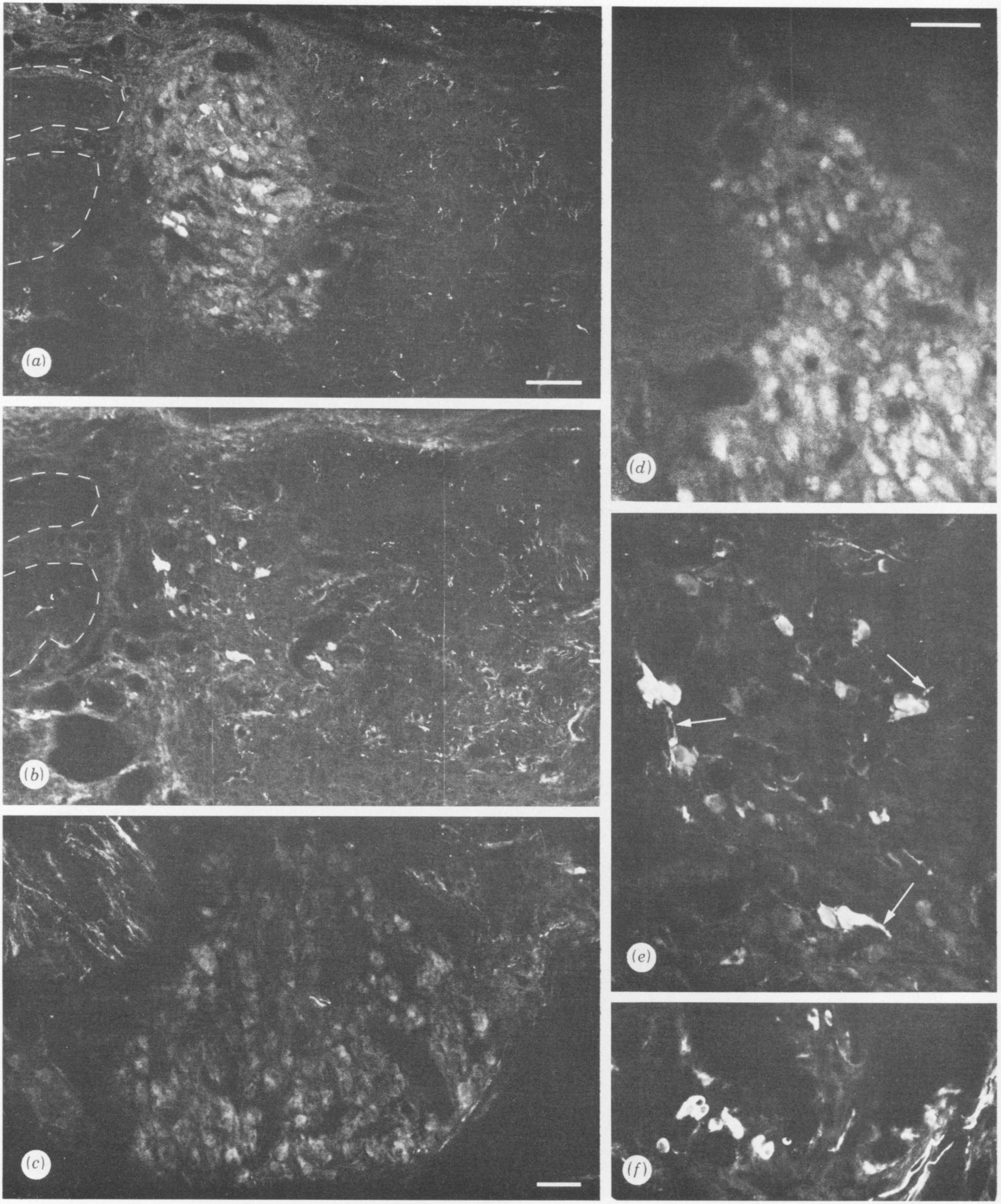


FIGURE 11. For description see opposite.

(*Facing p.* 262)



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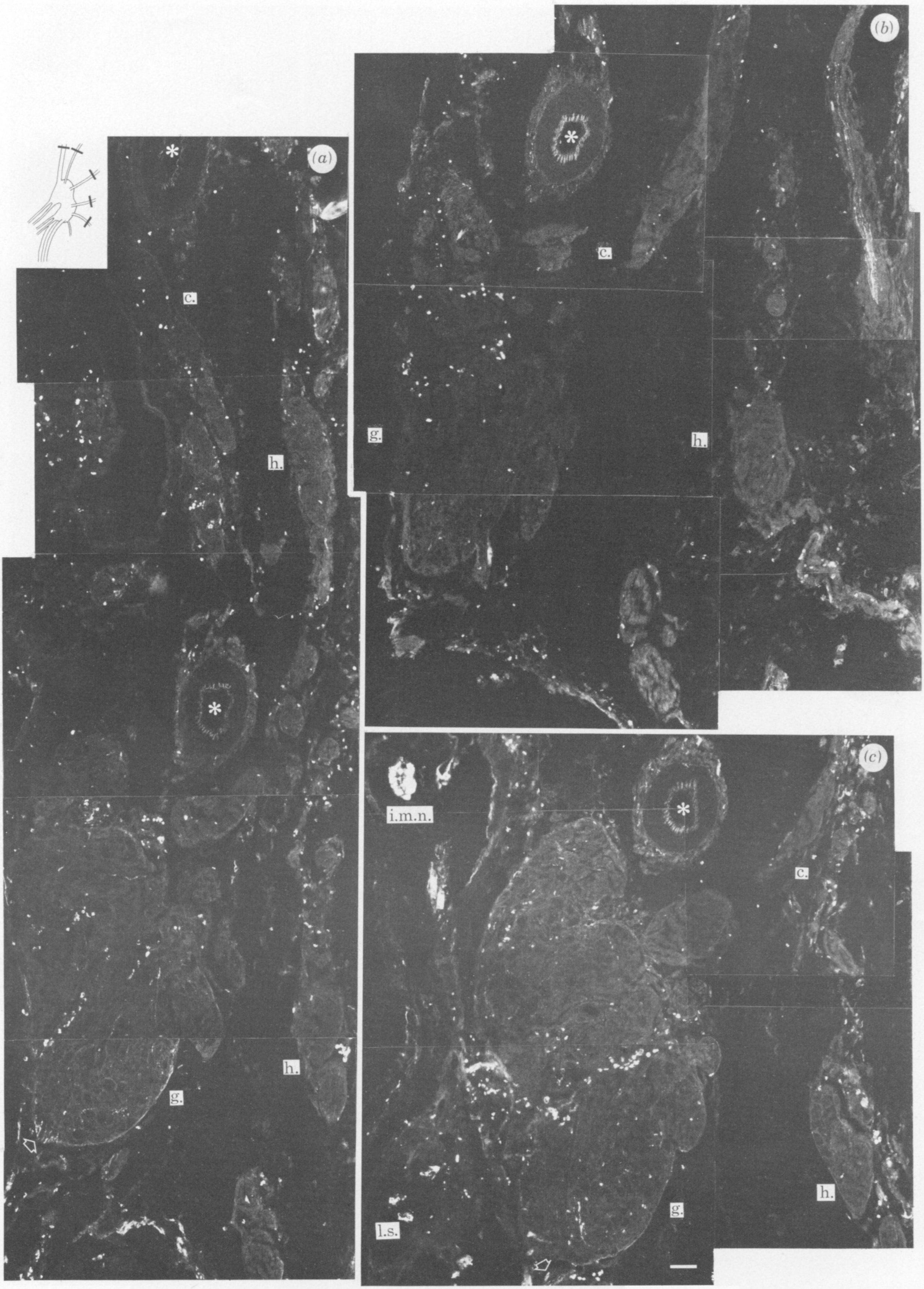


FIGURE 13. For description see p. 262.

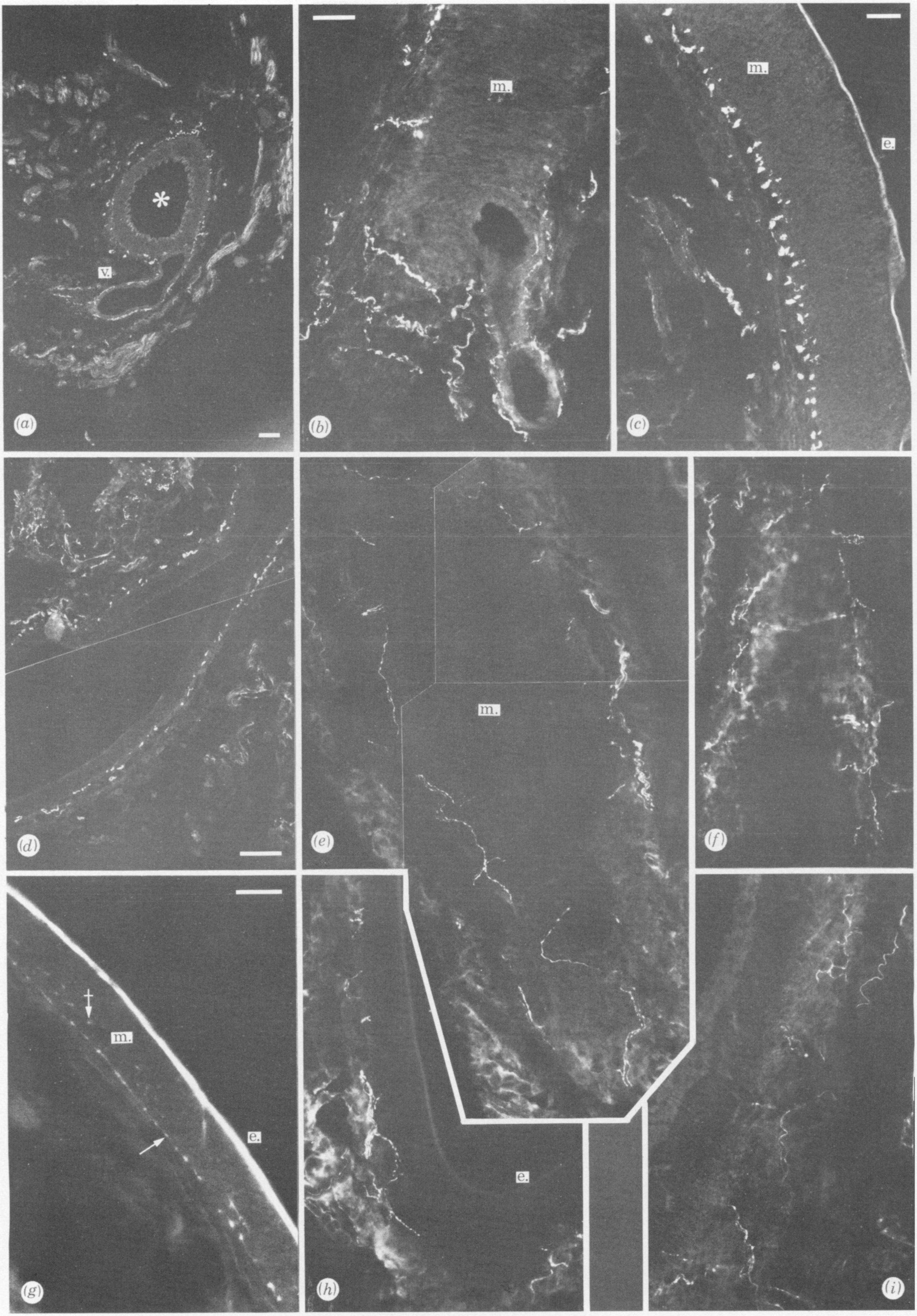
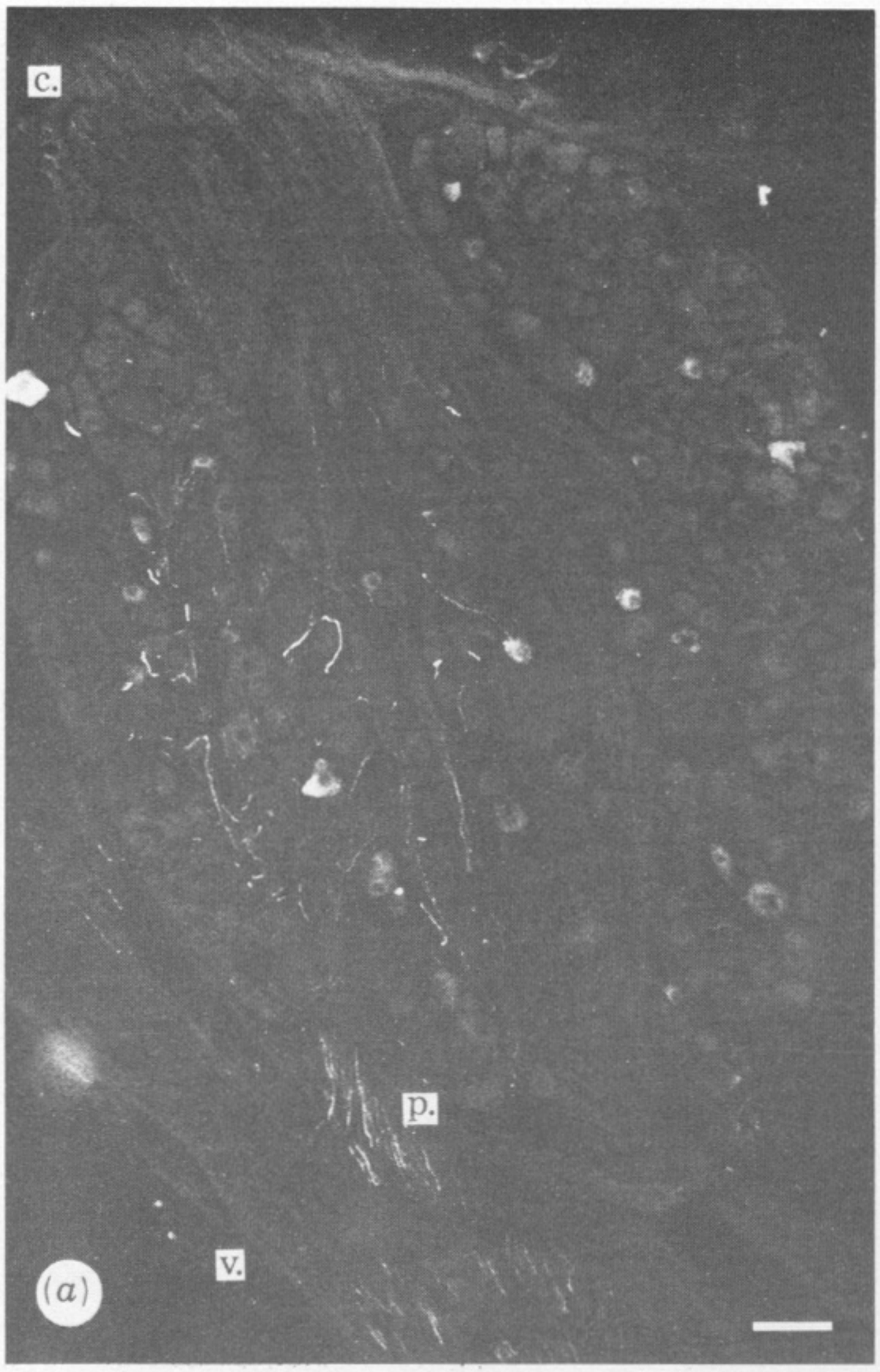
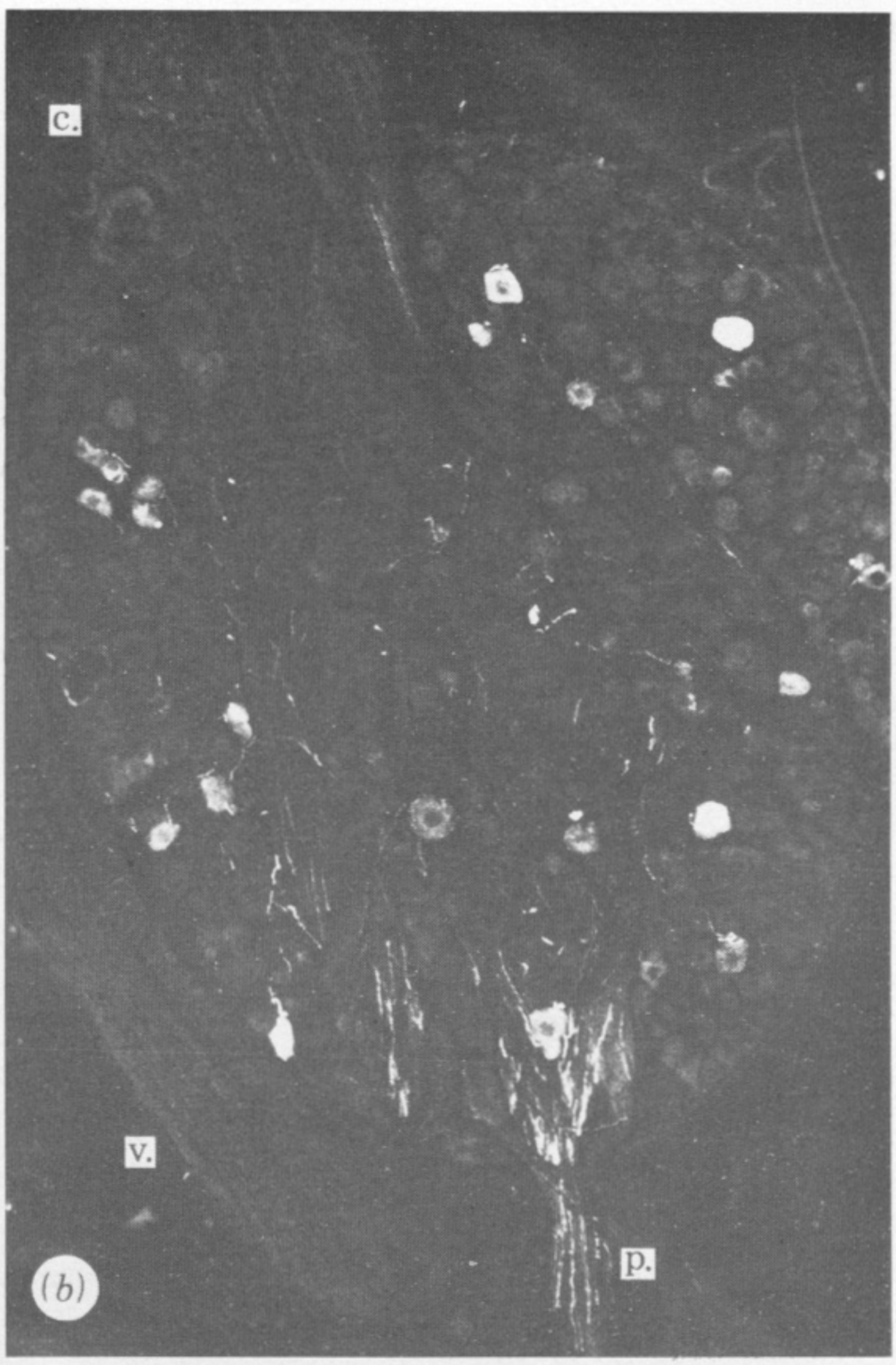
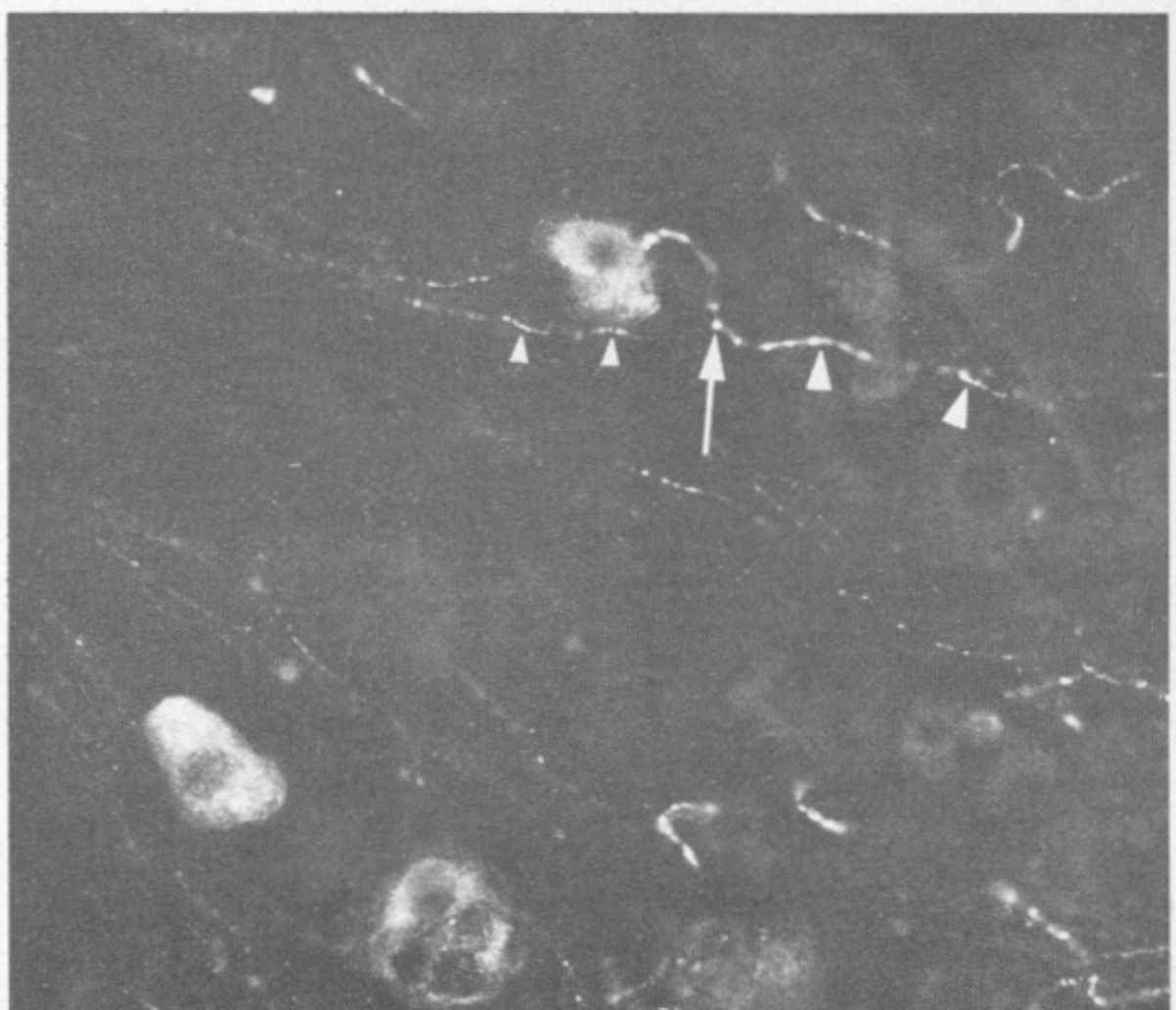
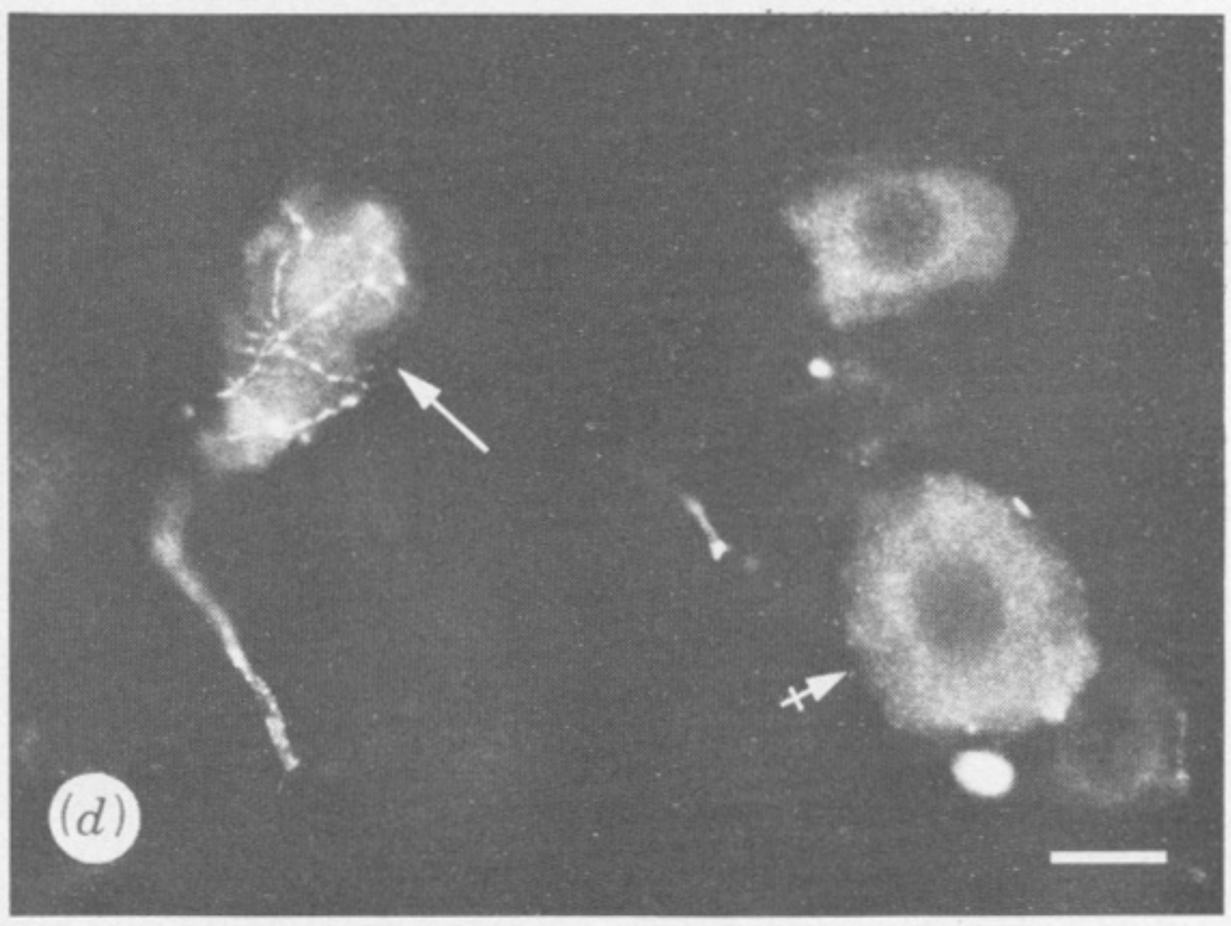


FIGURE 14. For description see p. 263.









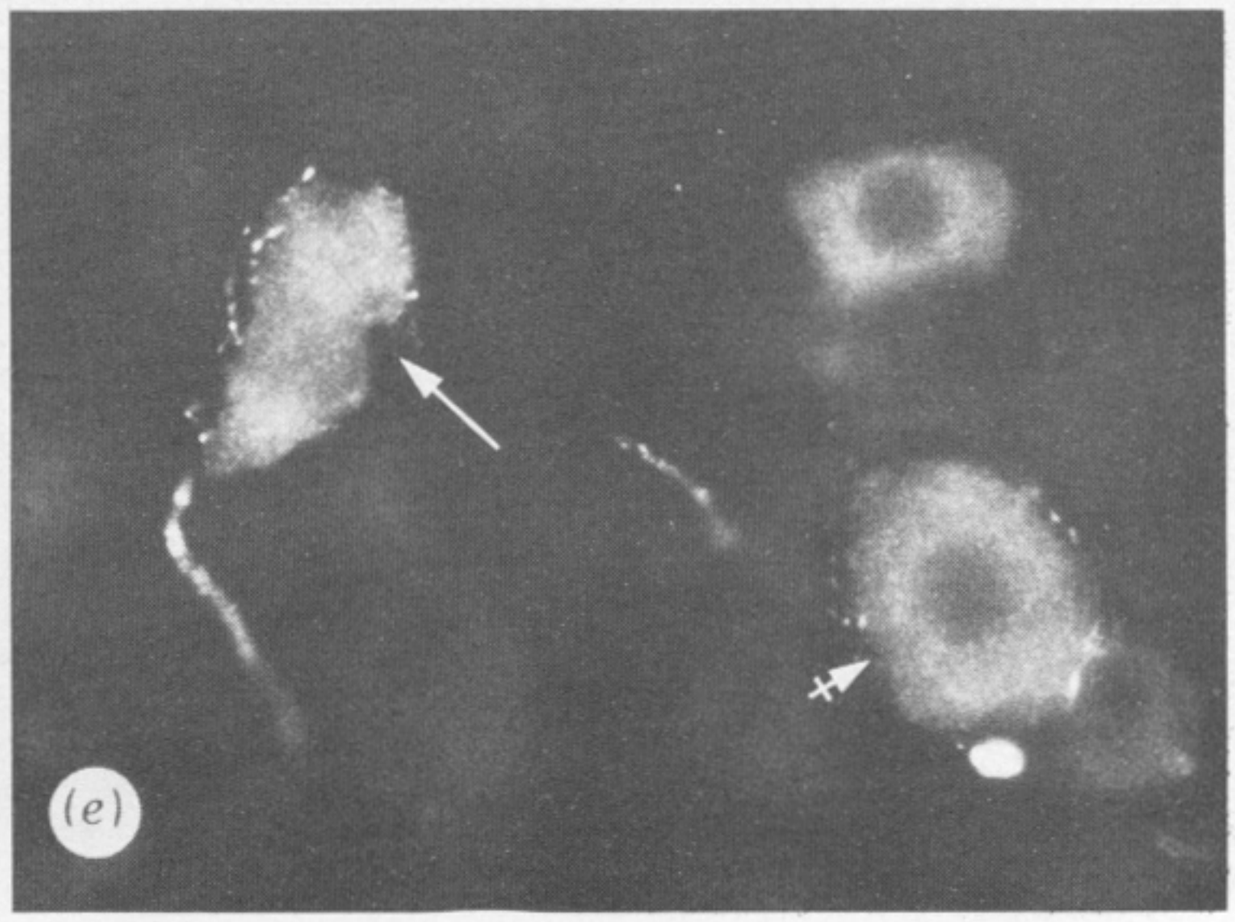
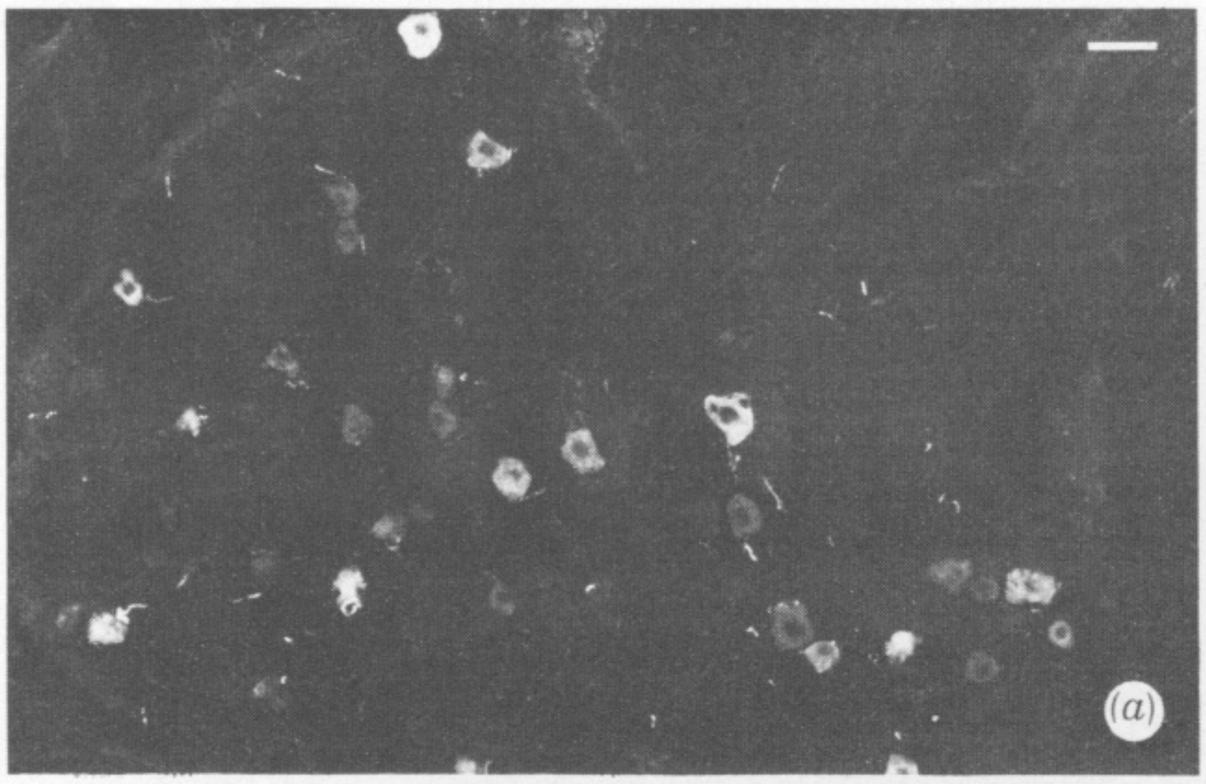
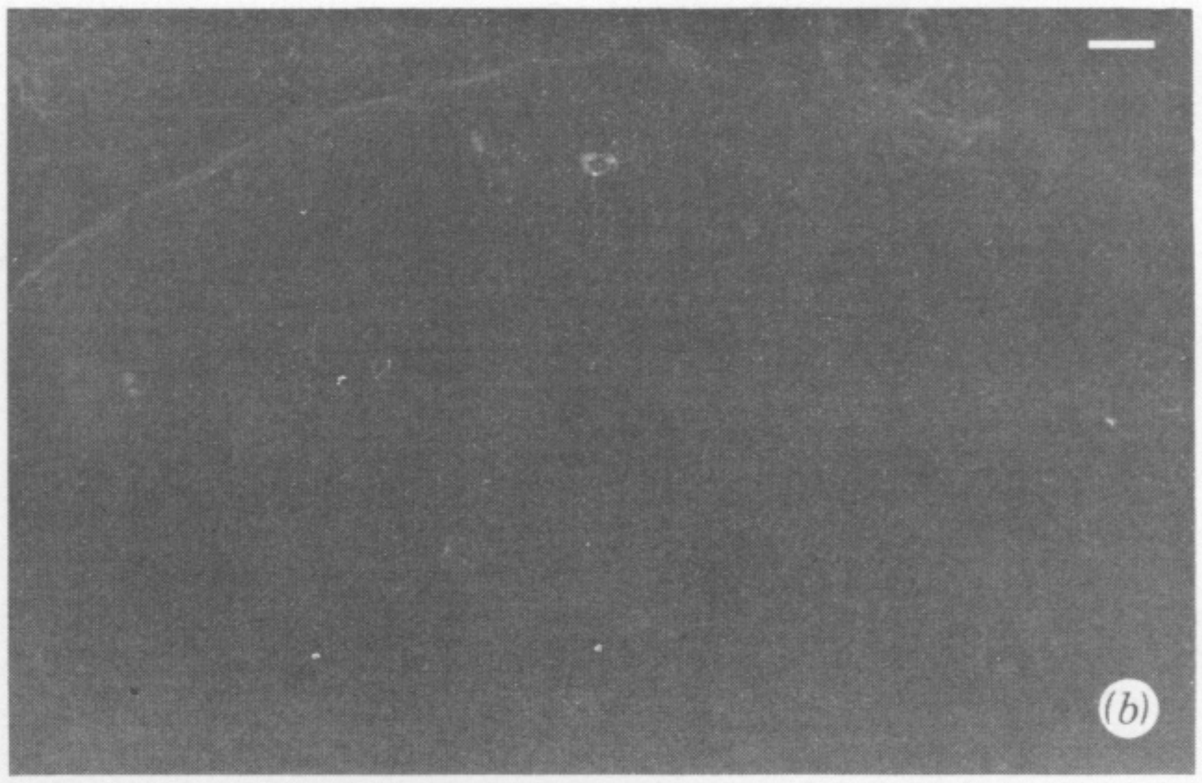
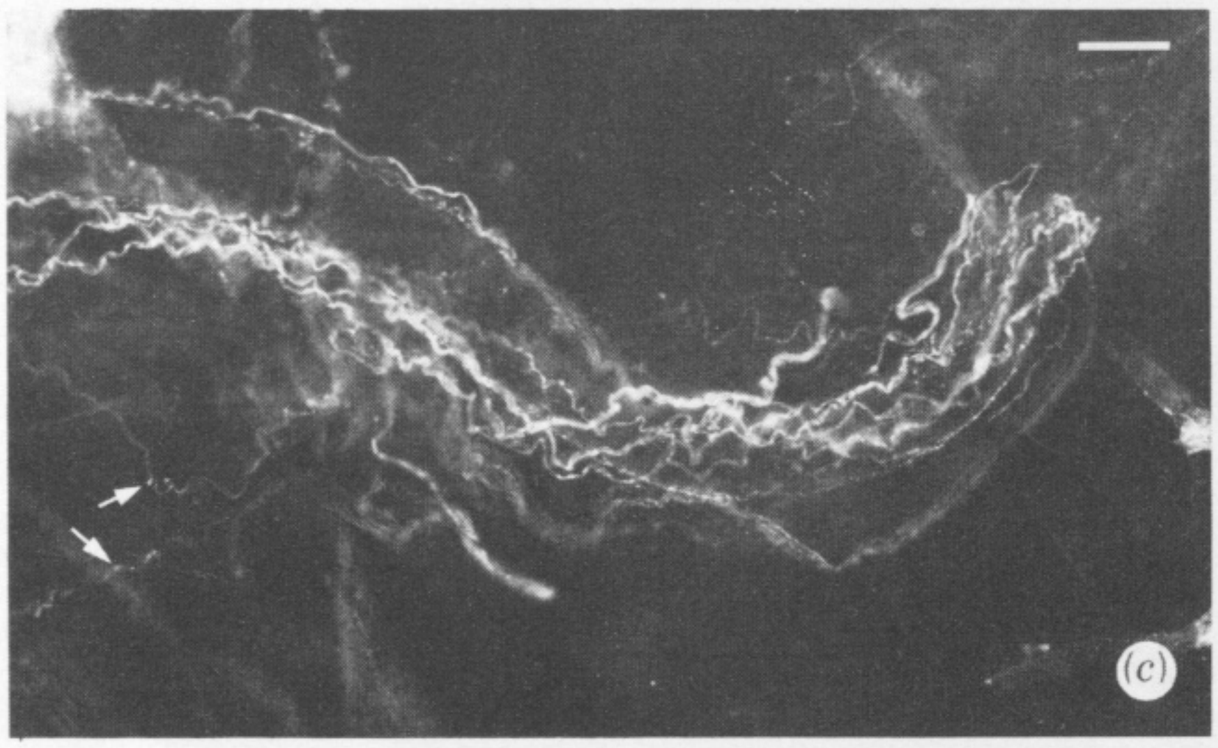
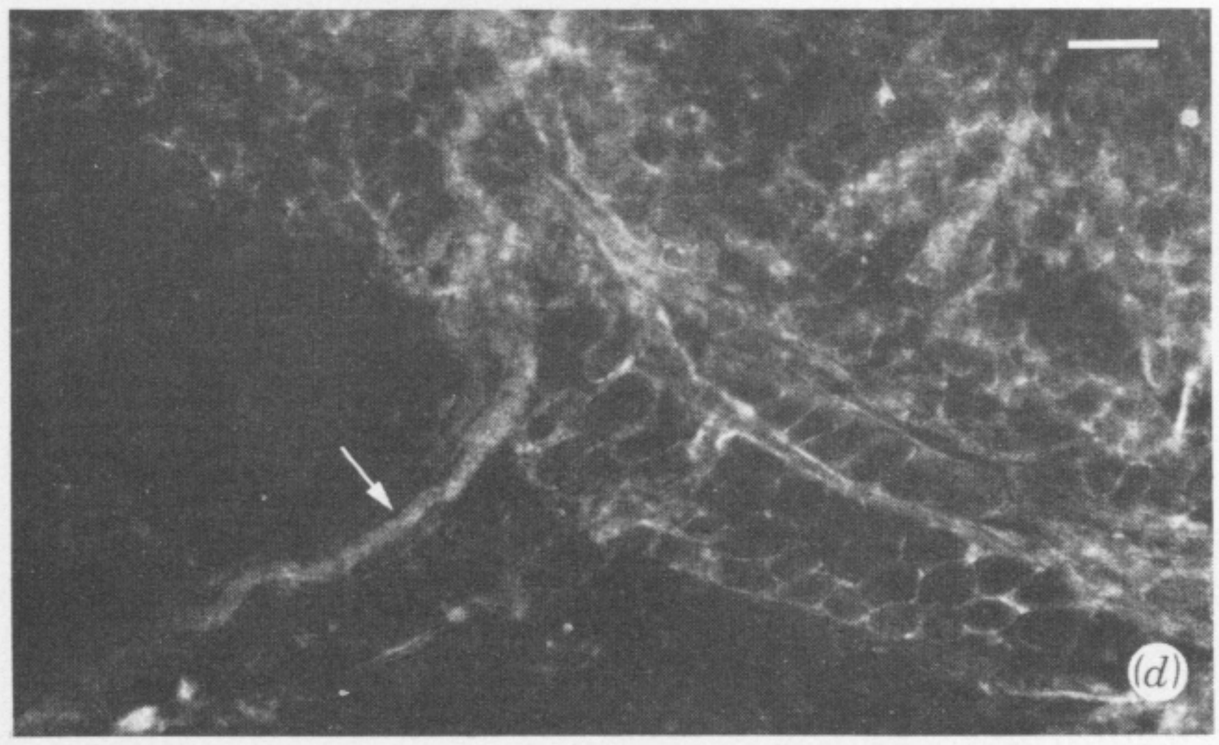


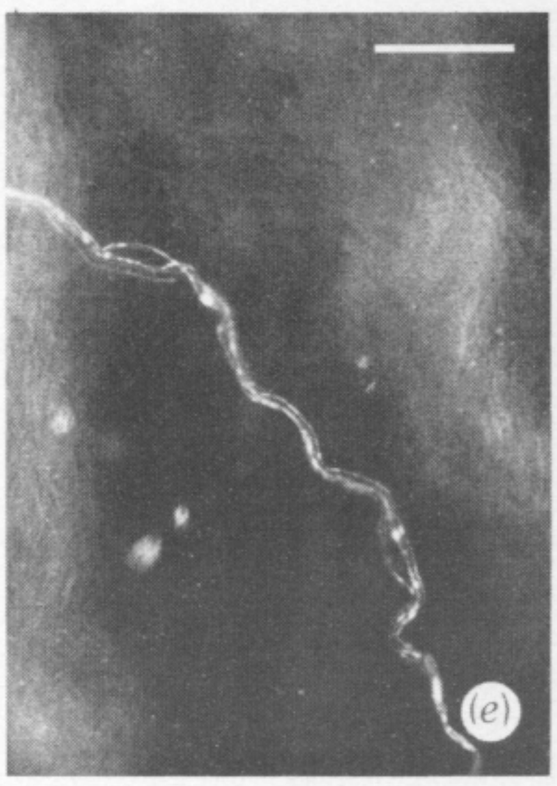
FIGURE 15. For description see p. 263.

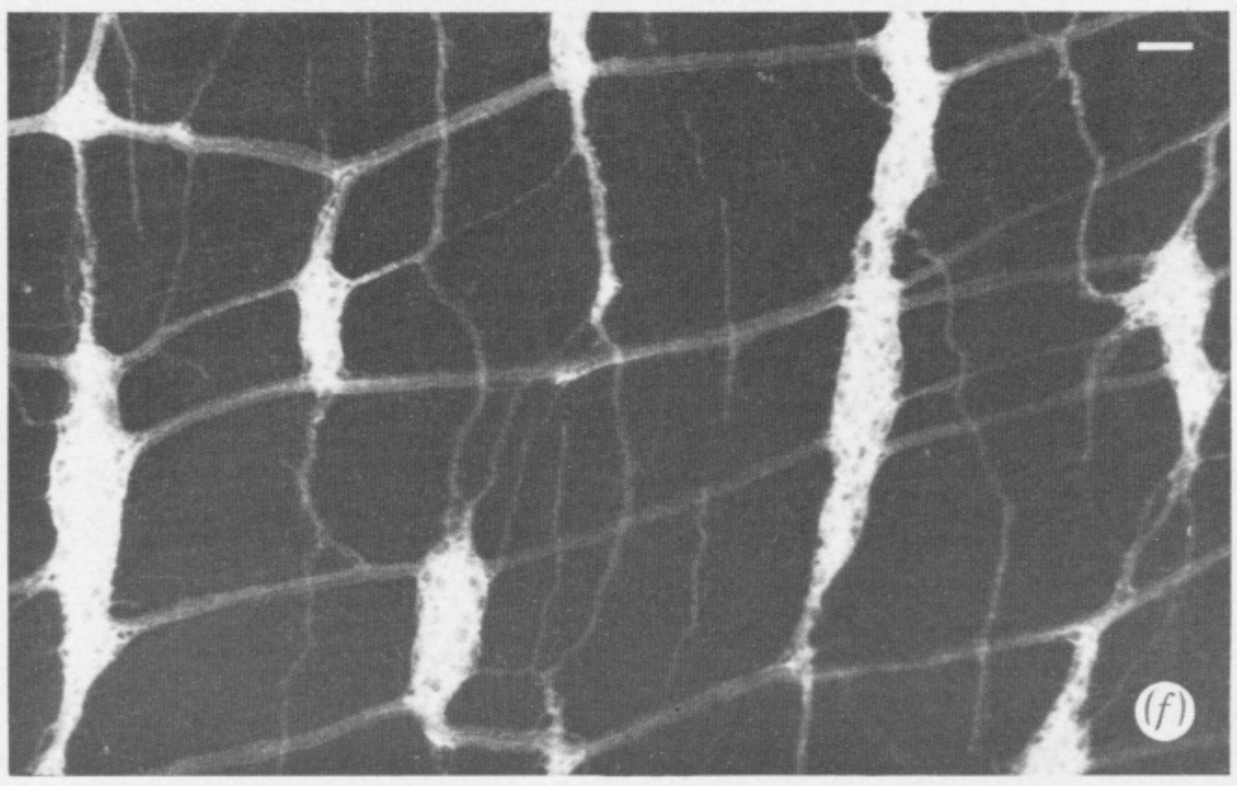


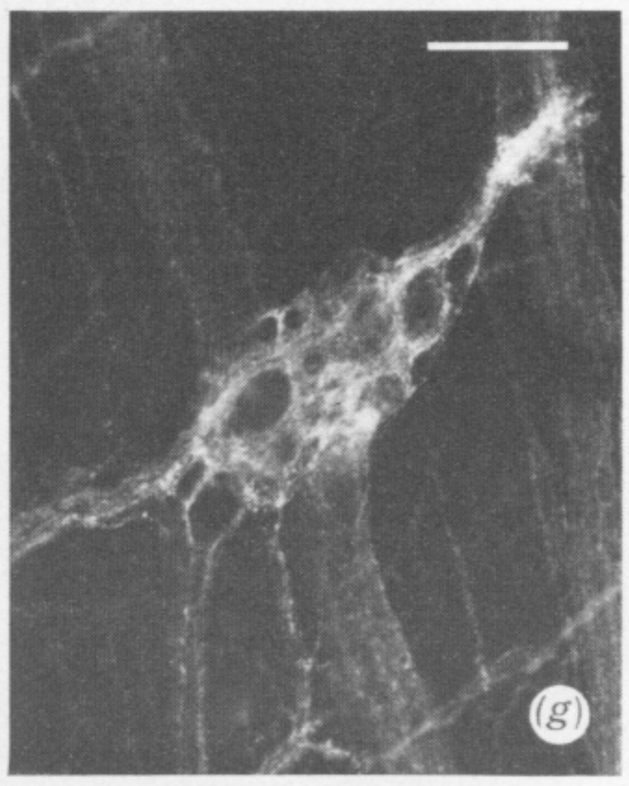


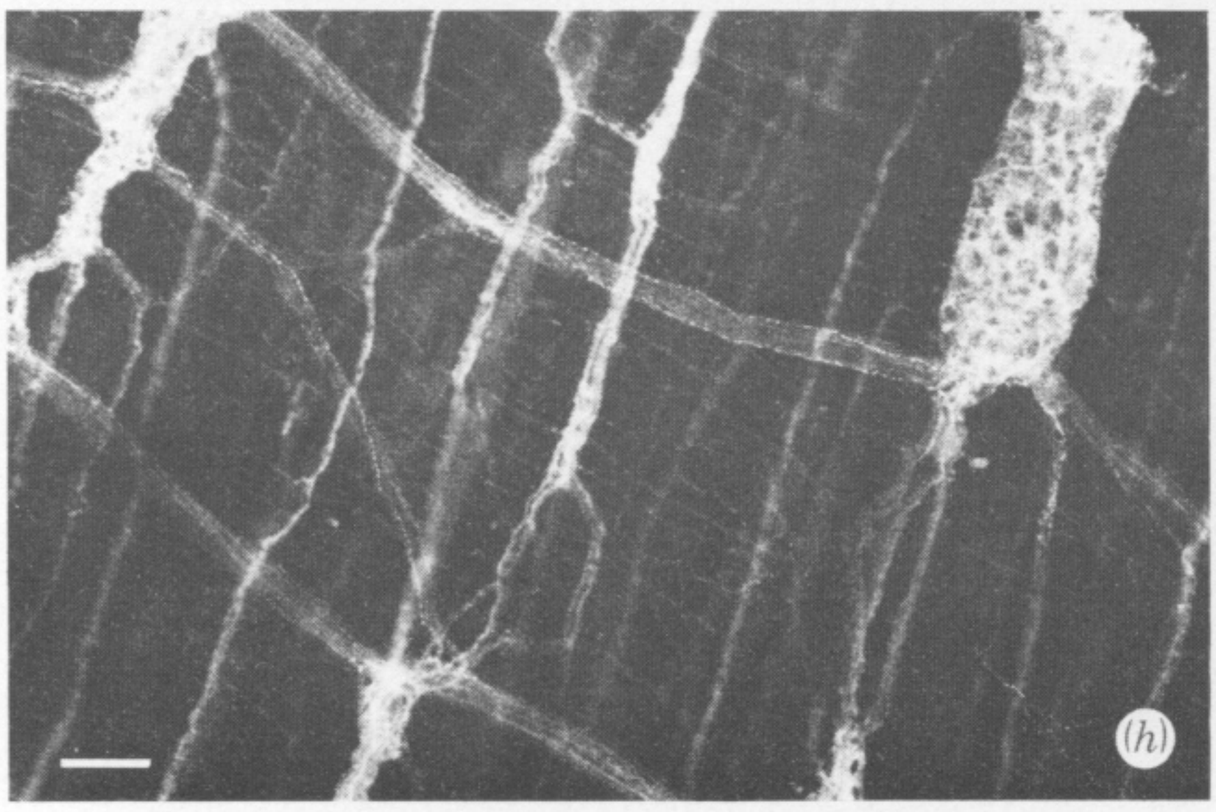












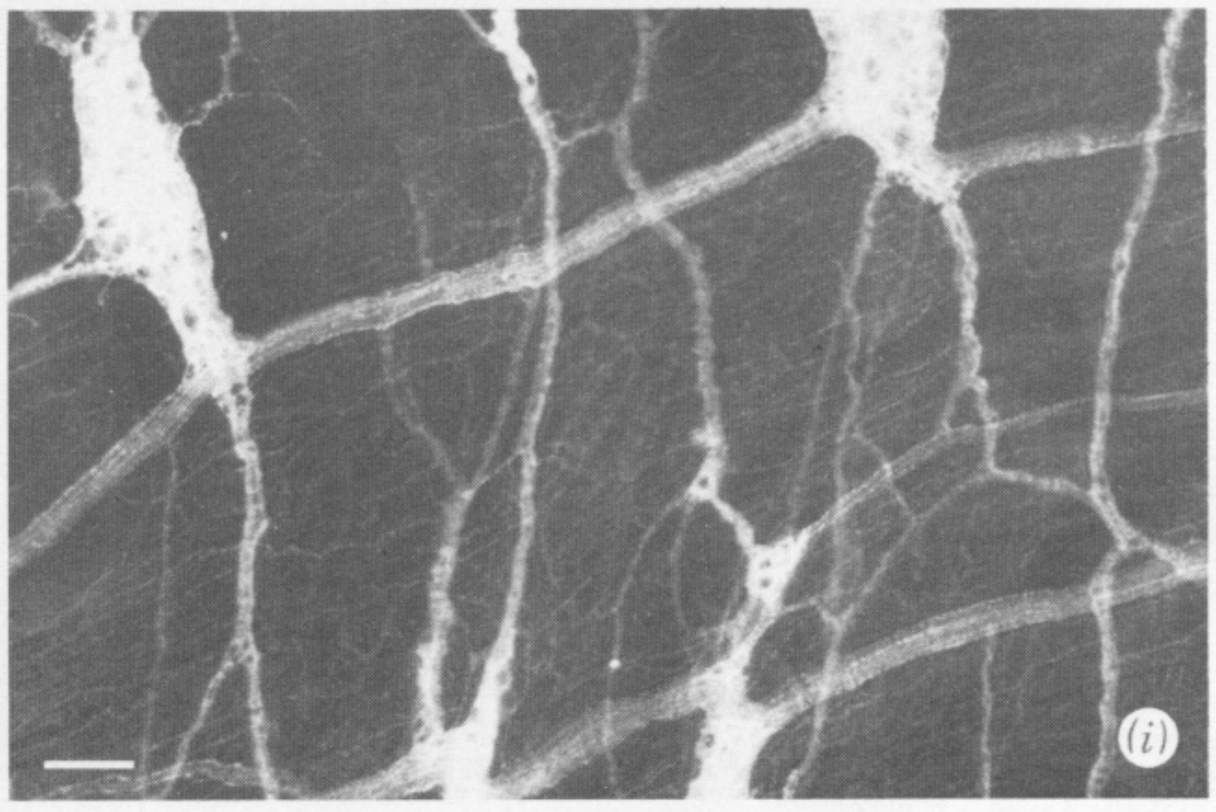
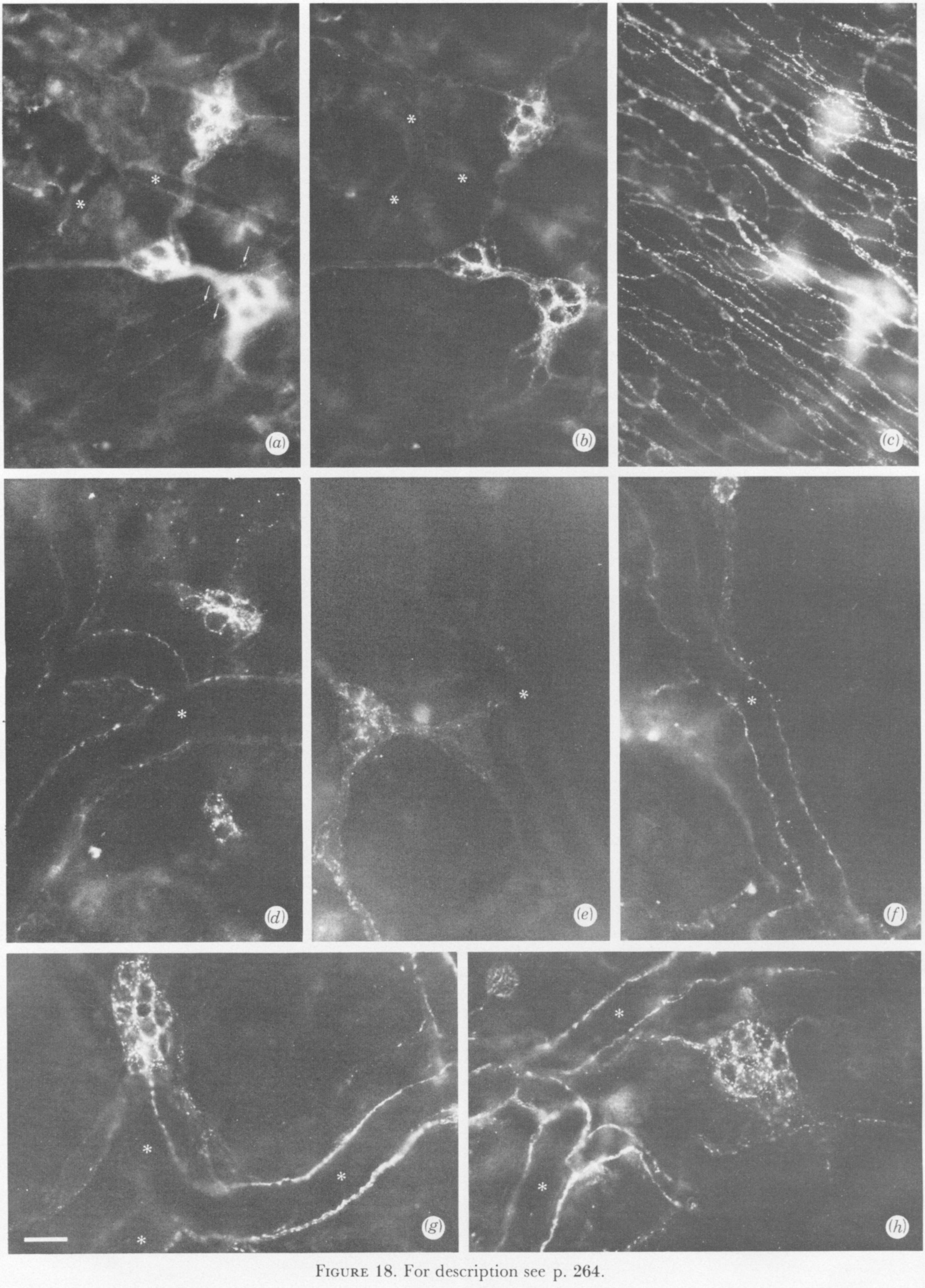


FIGURE 16. For description see opposite.

FIGURE 17. For description see opposite.



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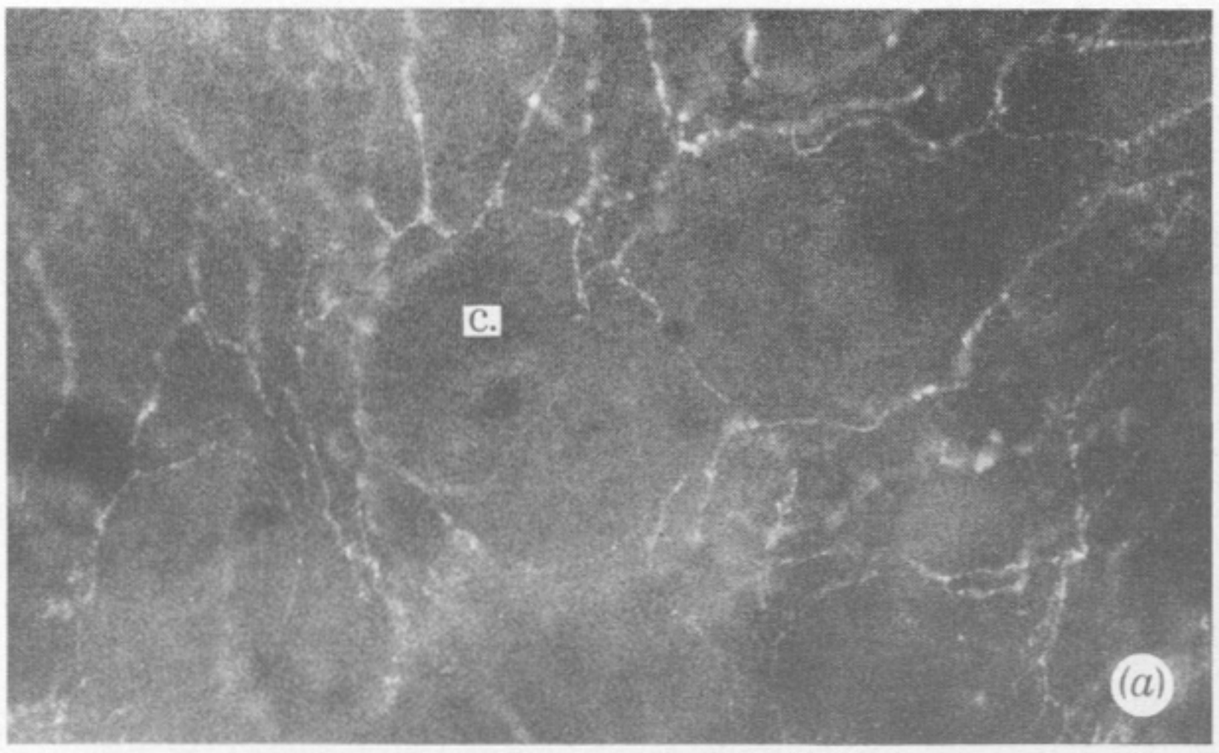

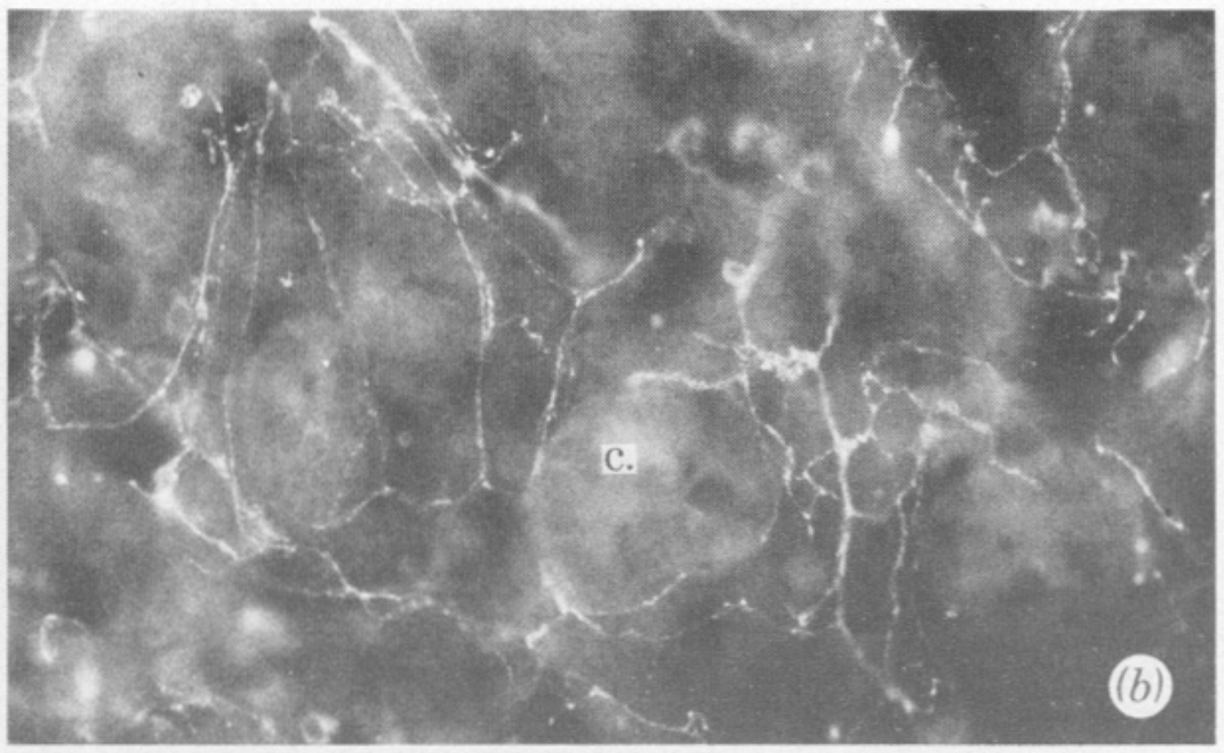

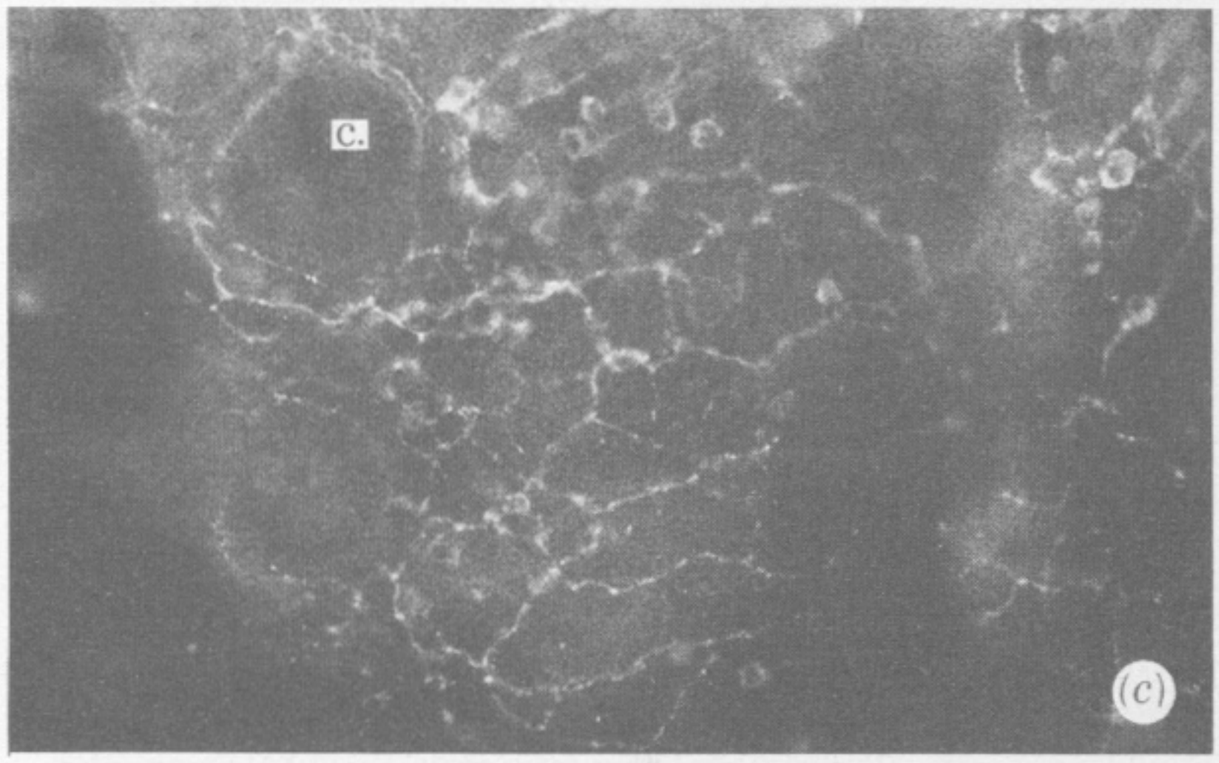

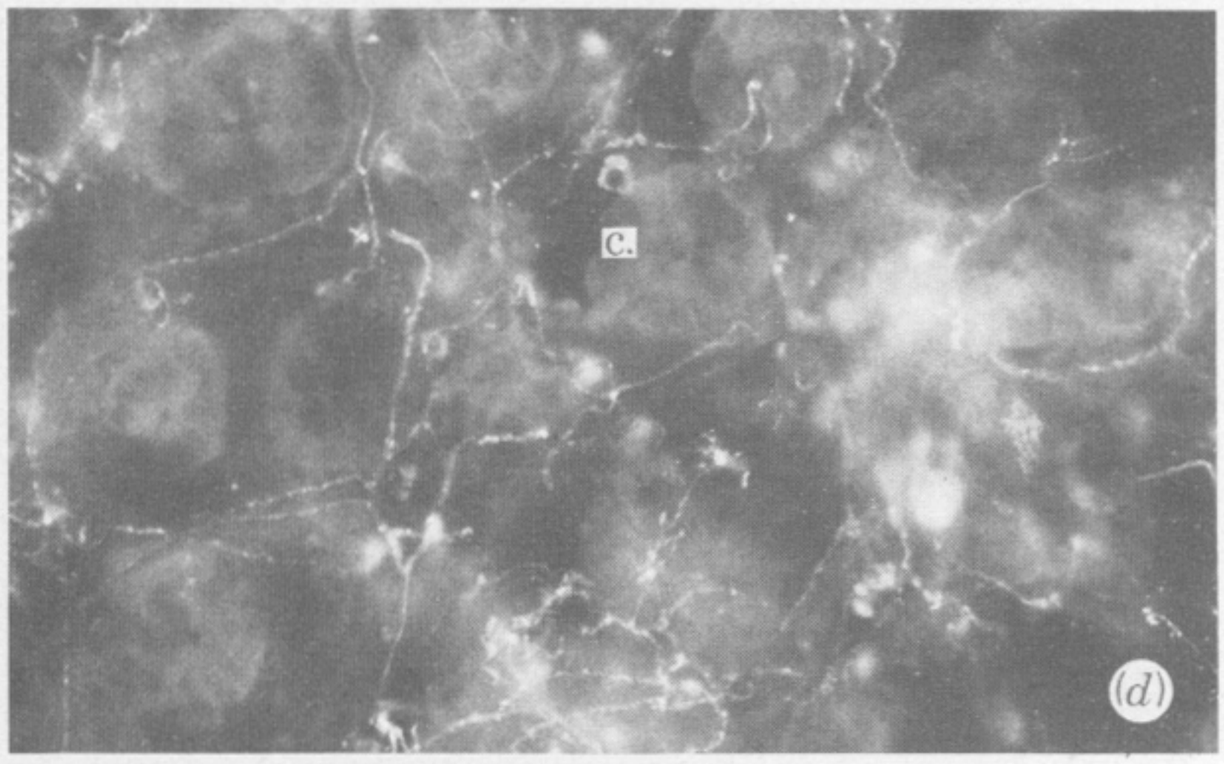

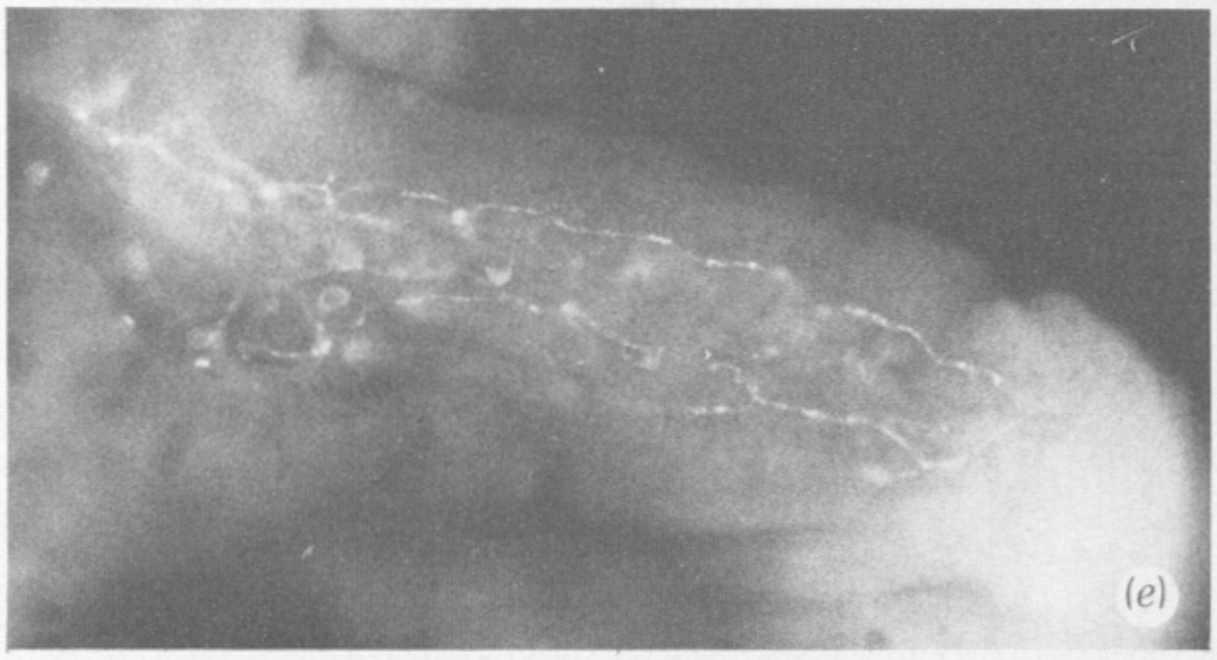

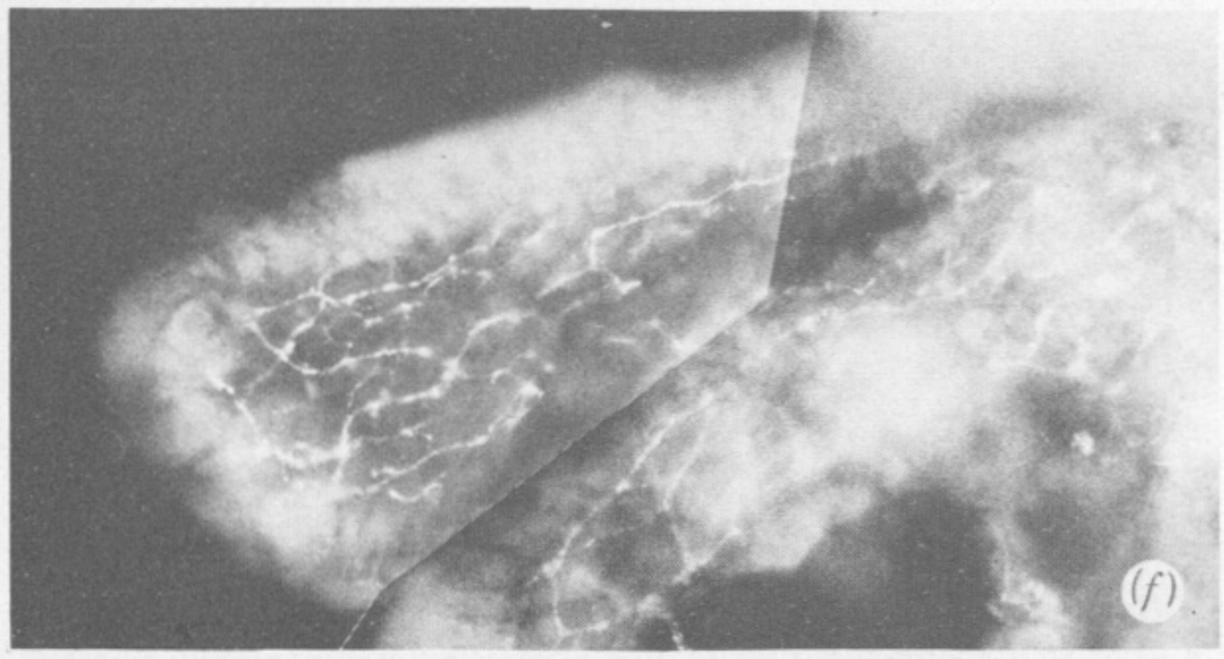

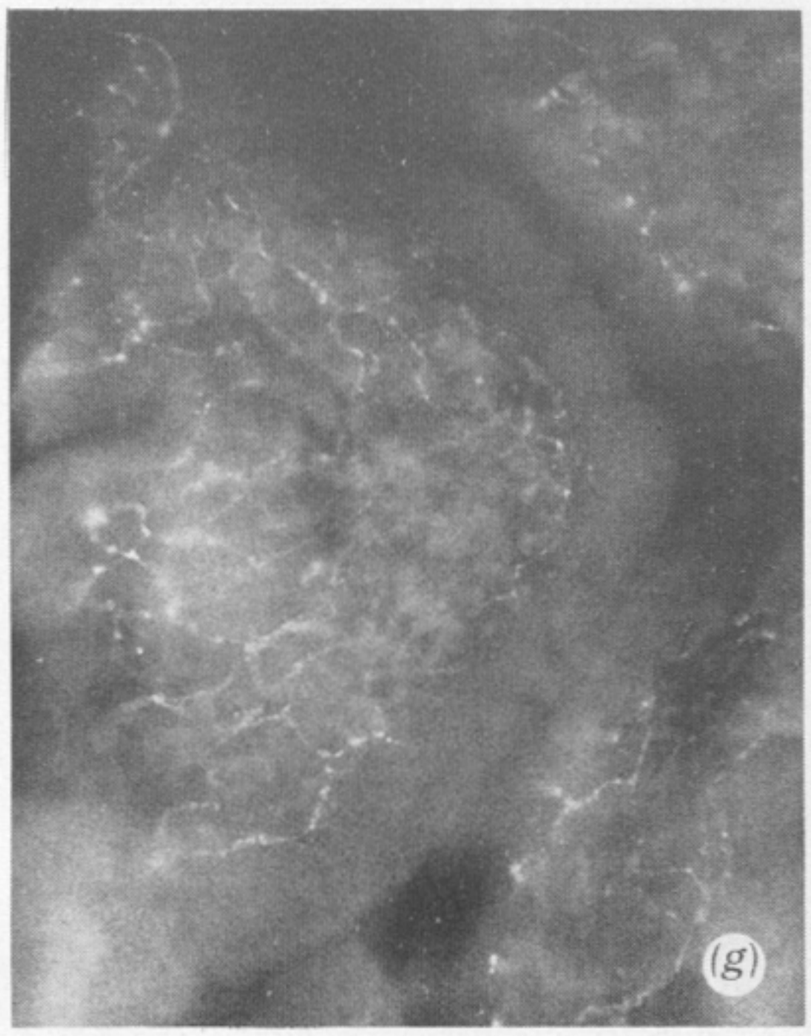

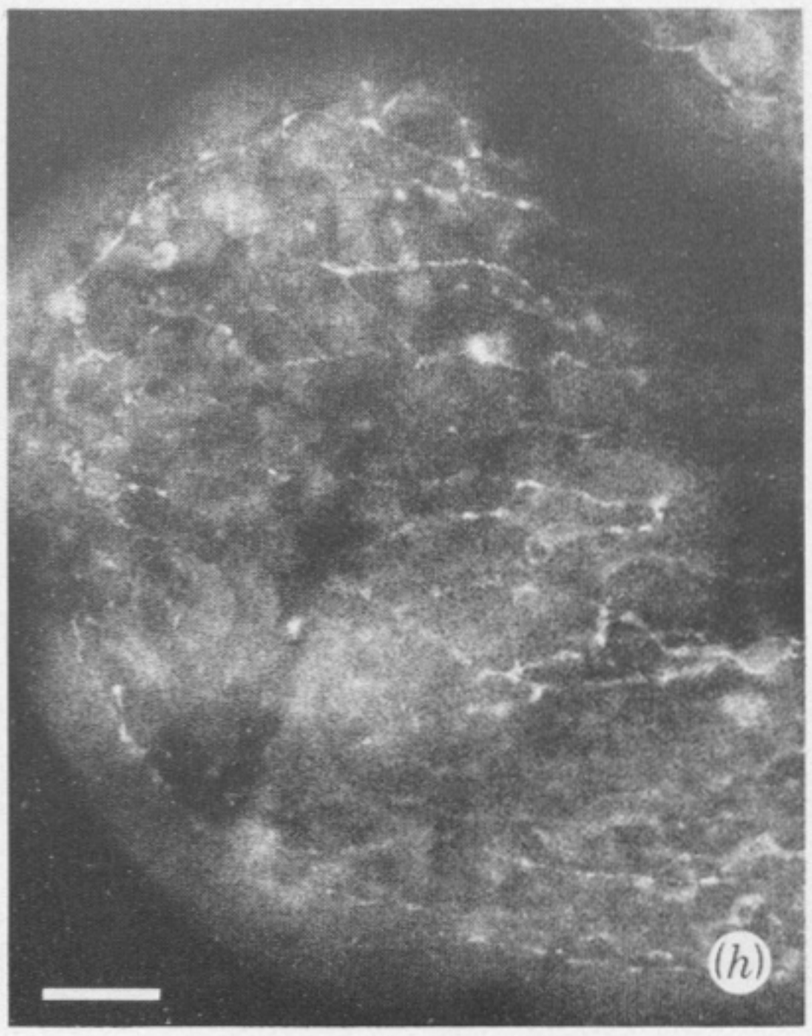

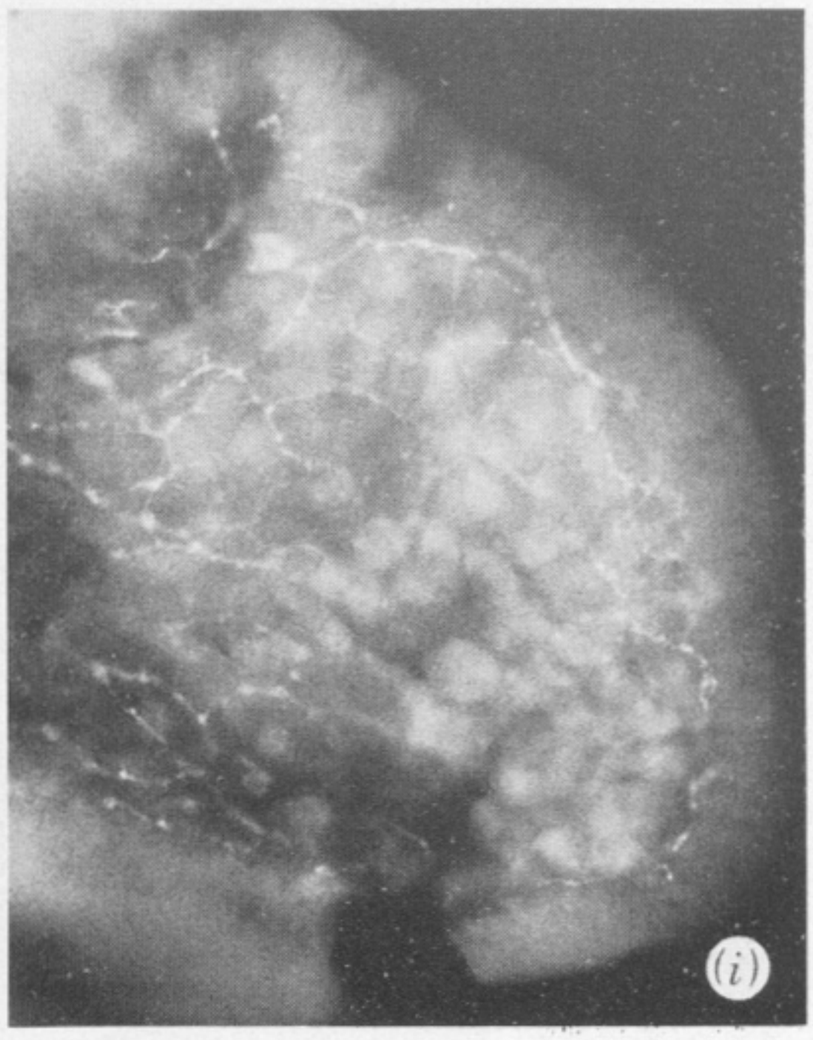

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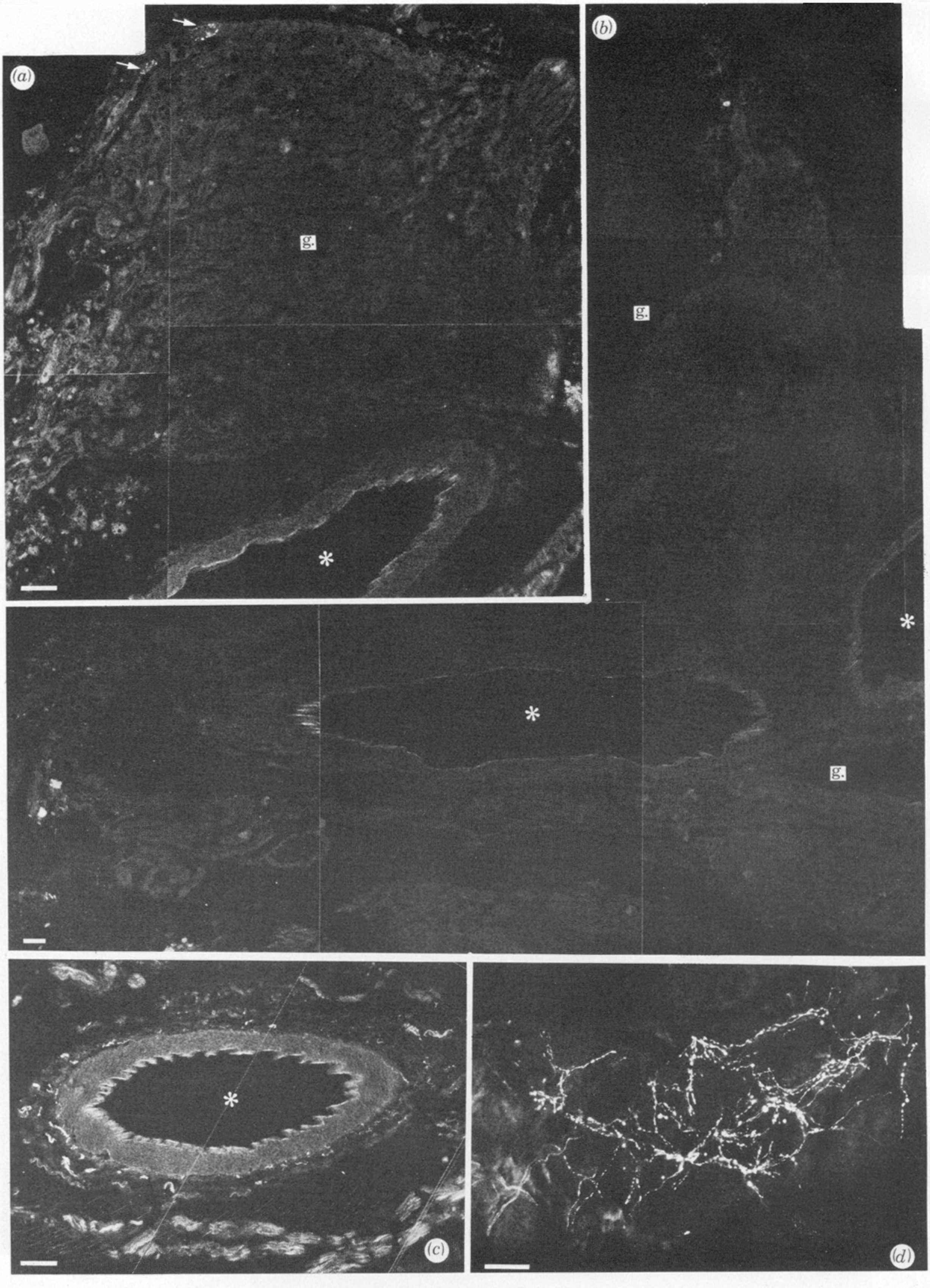


FIGURE 20. For description see p. 265.

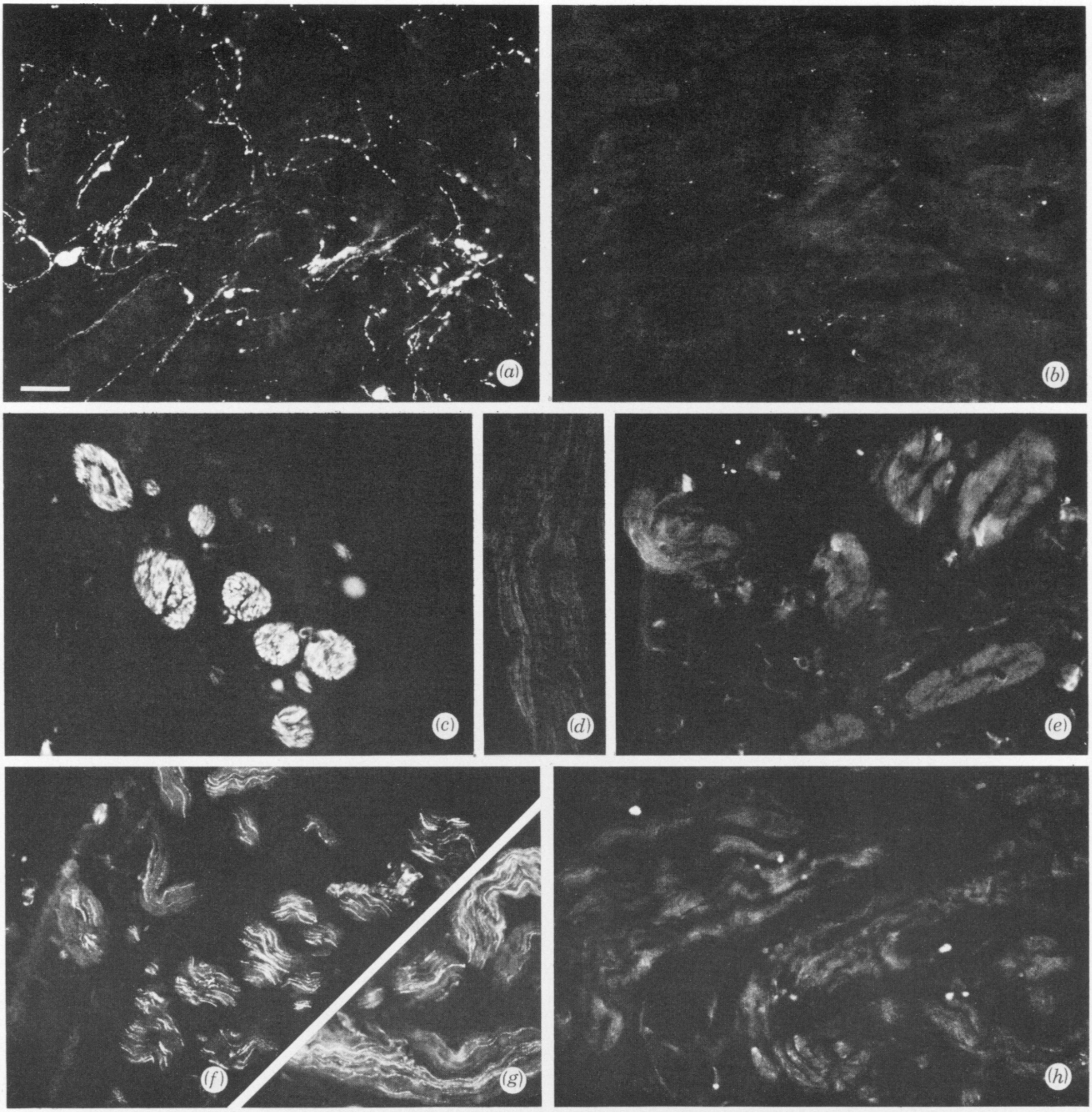


FIGURE 21. For description see p. 265.

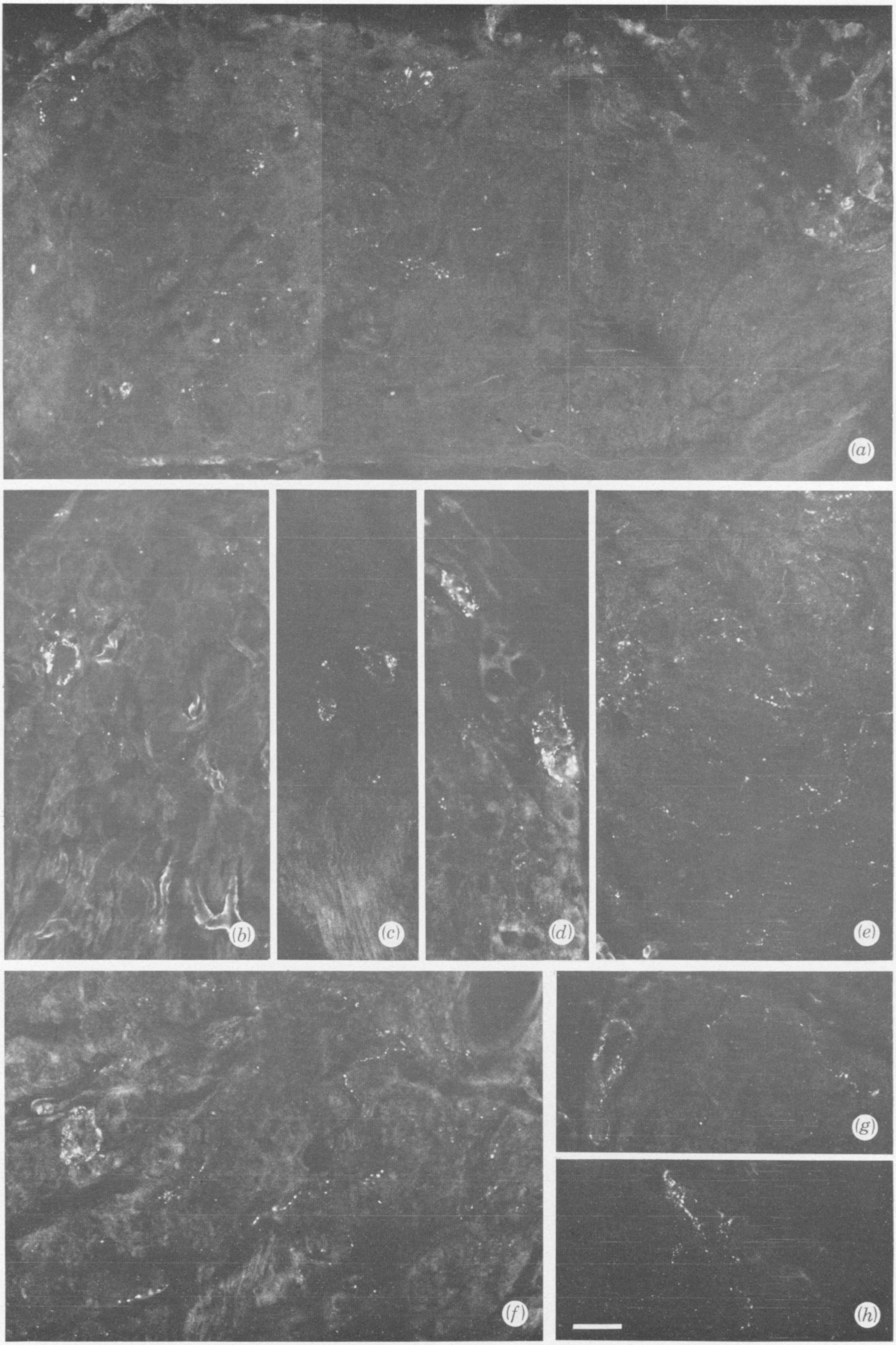


FIGURE 22. For description see opposite.

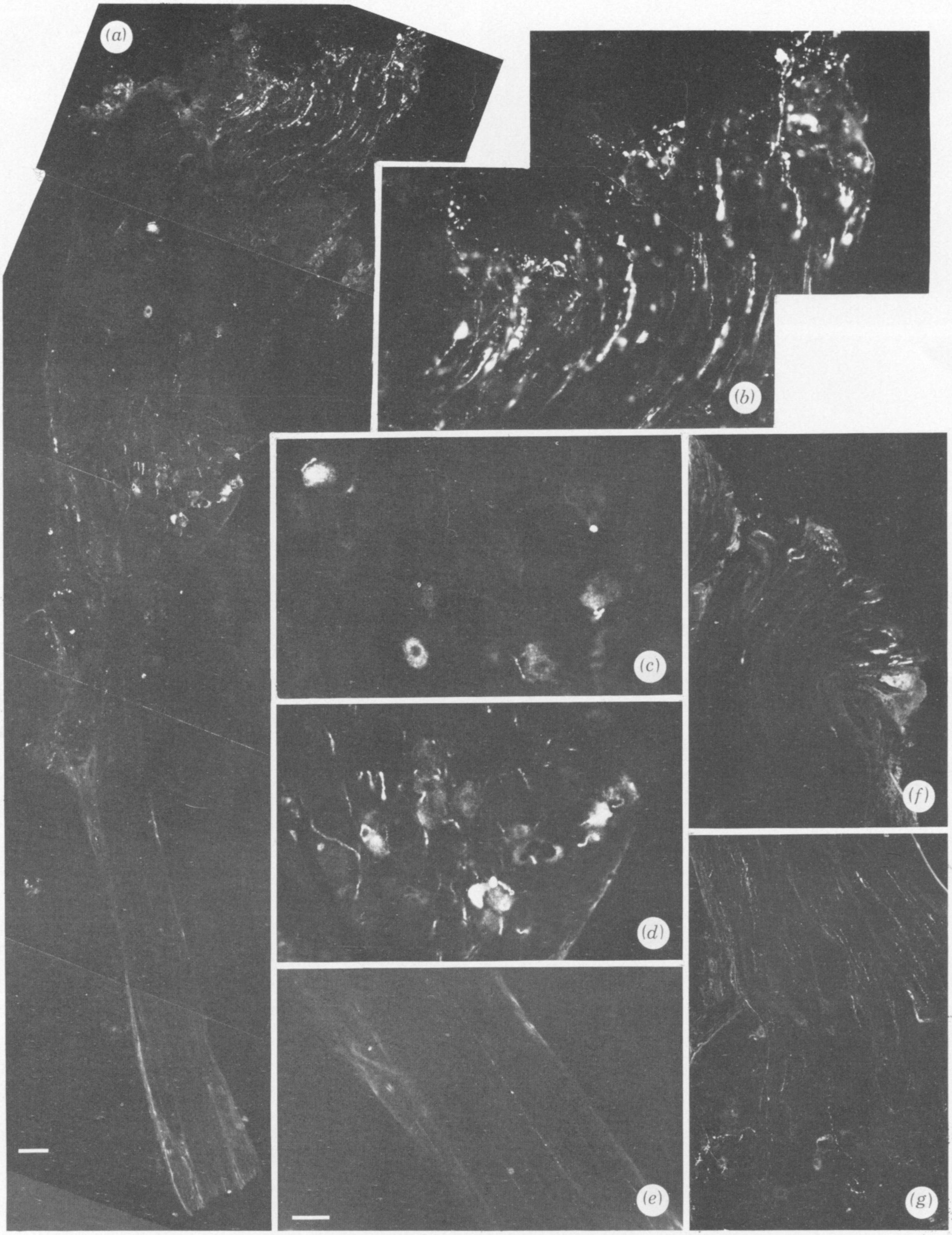
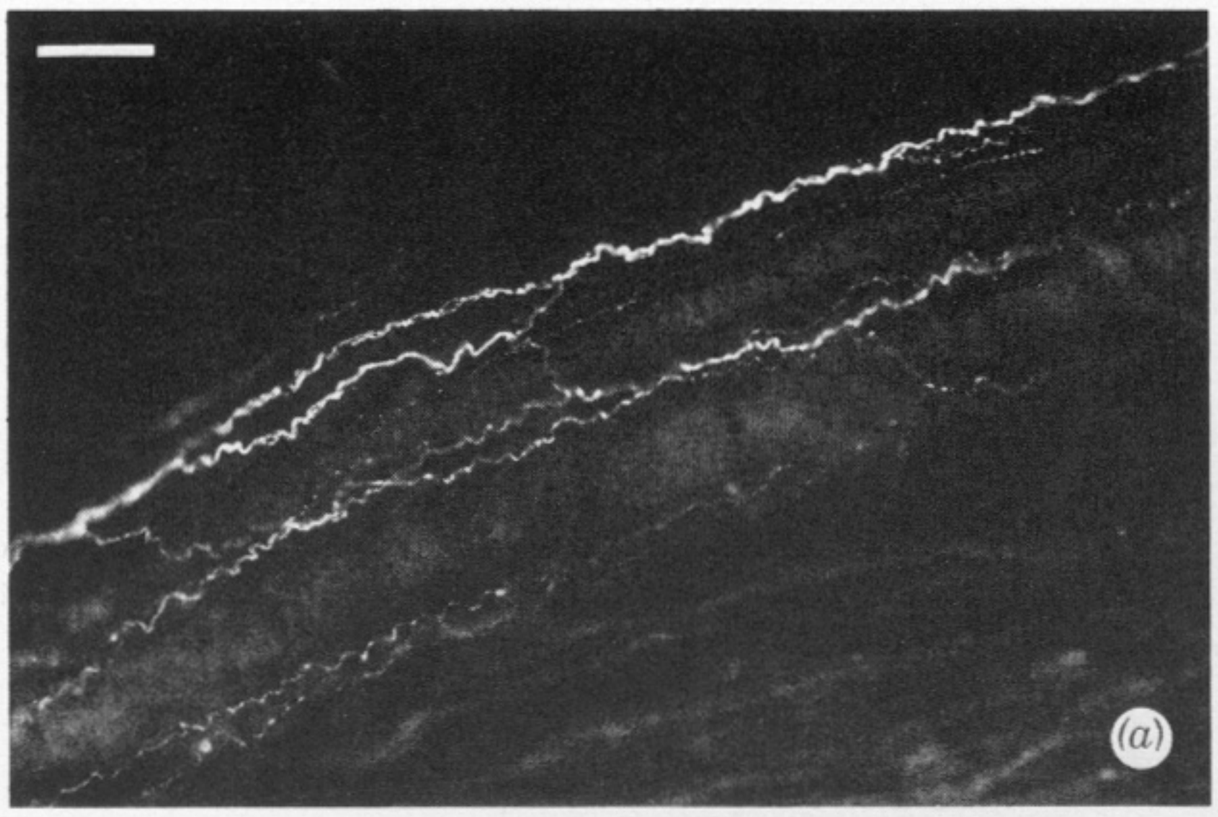
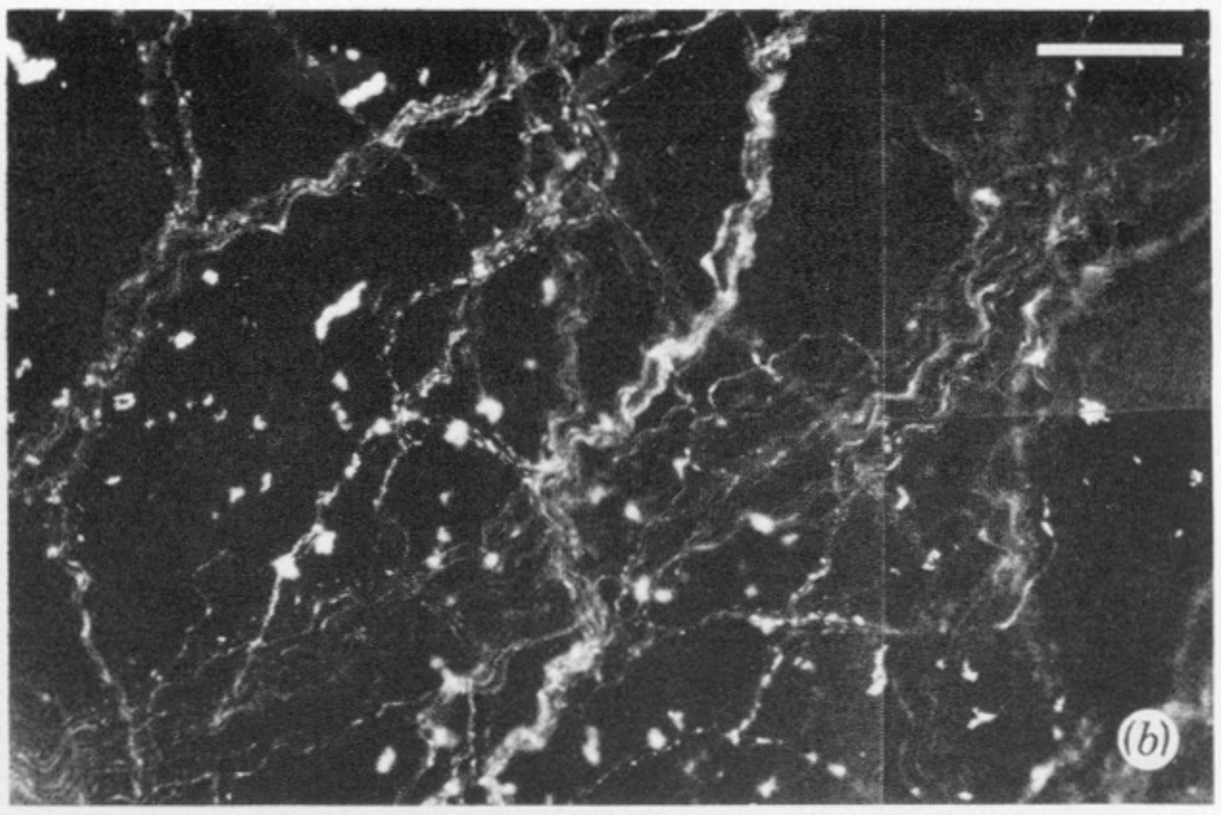
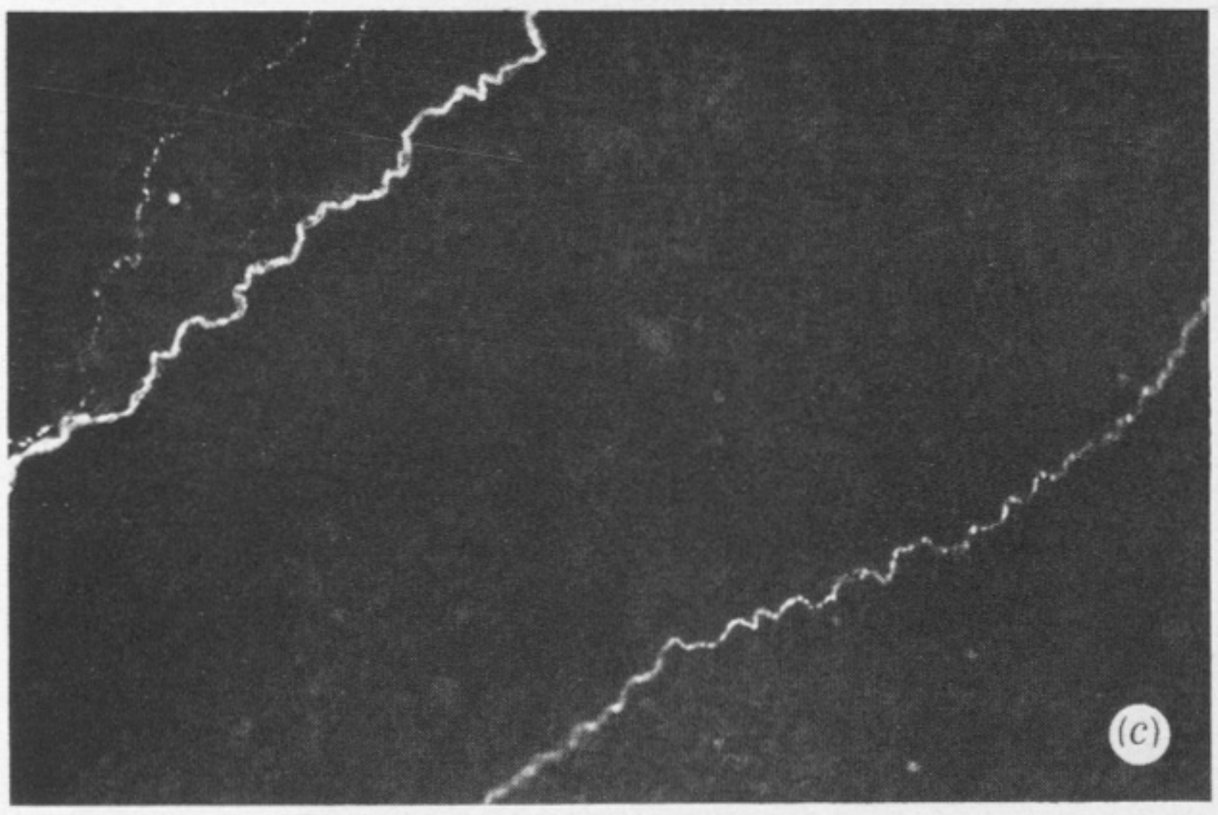
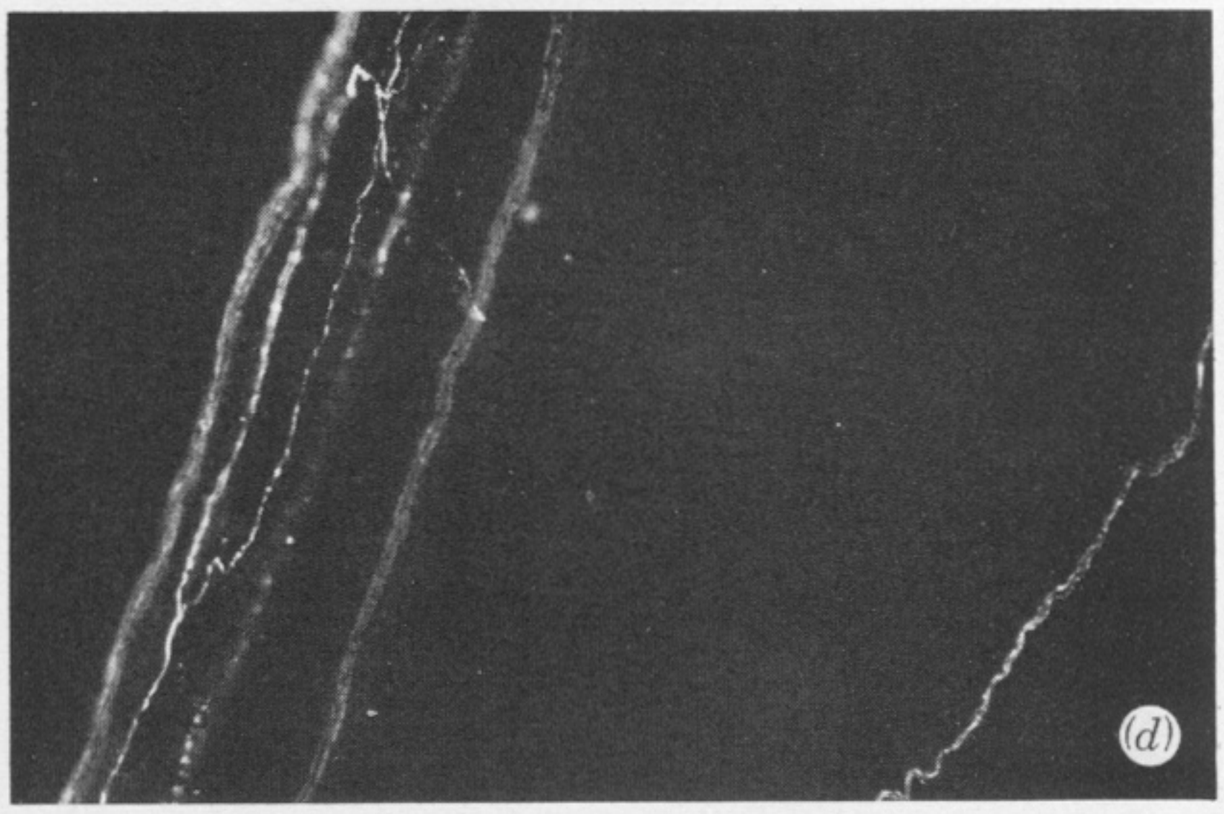


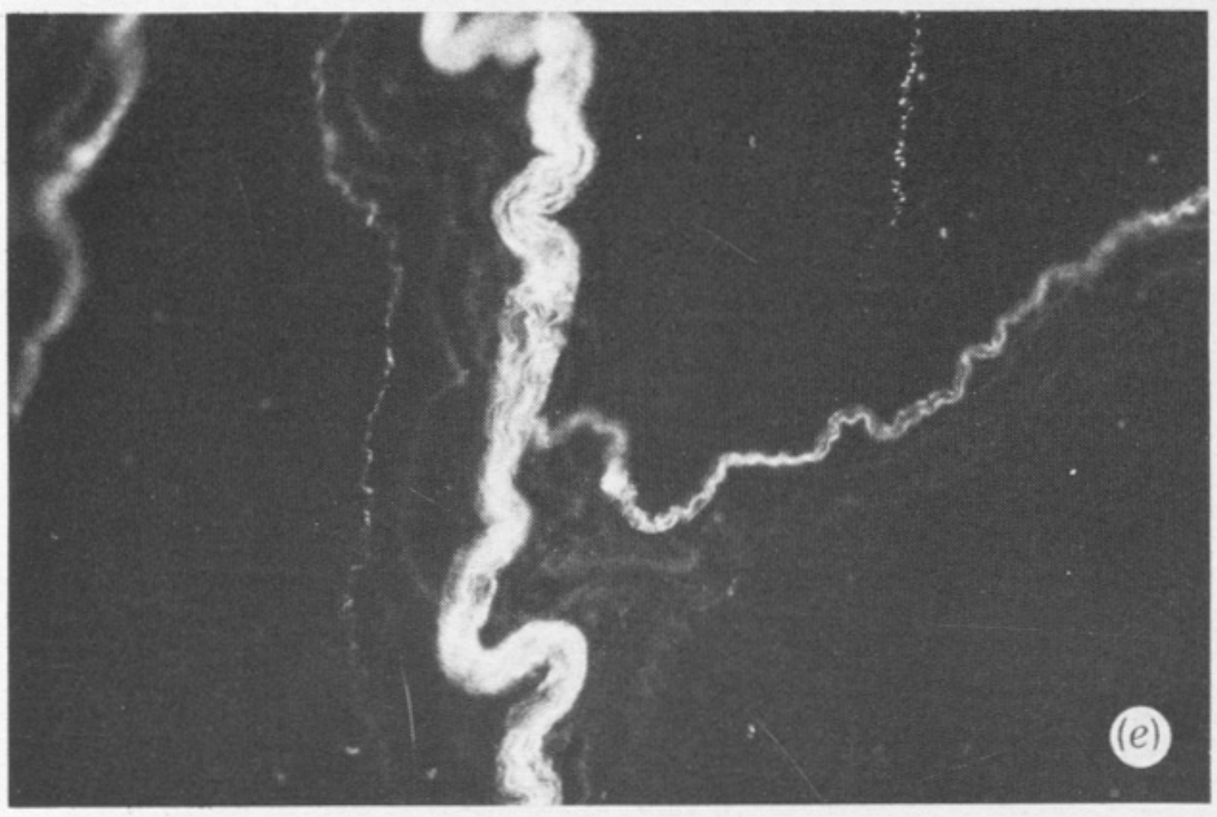
FIGURE 23. For description see opposite.

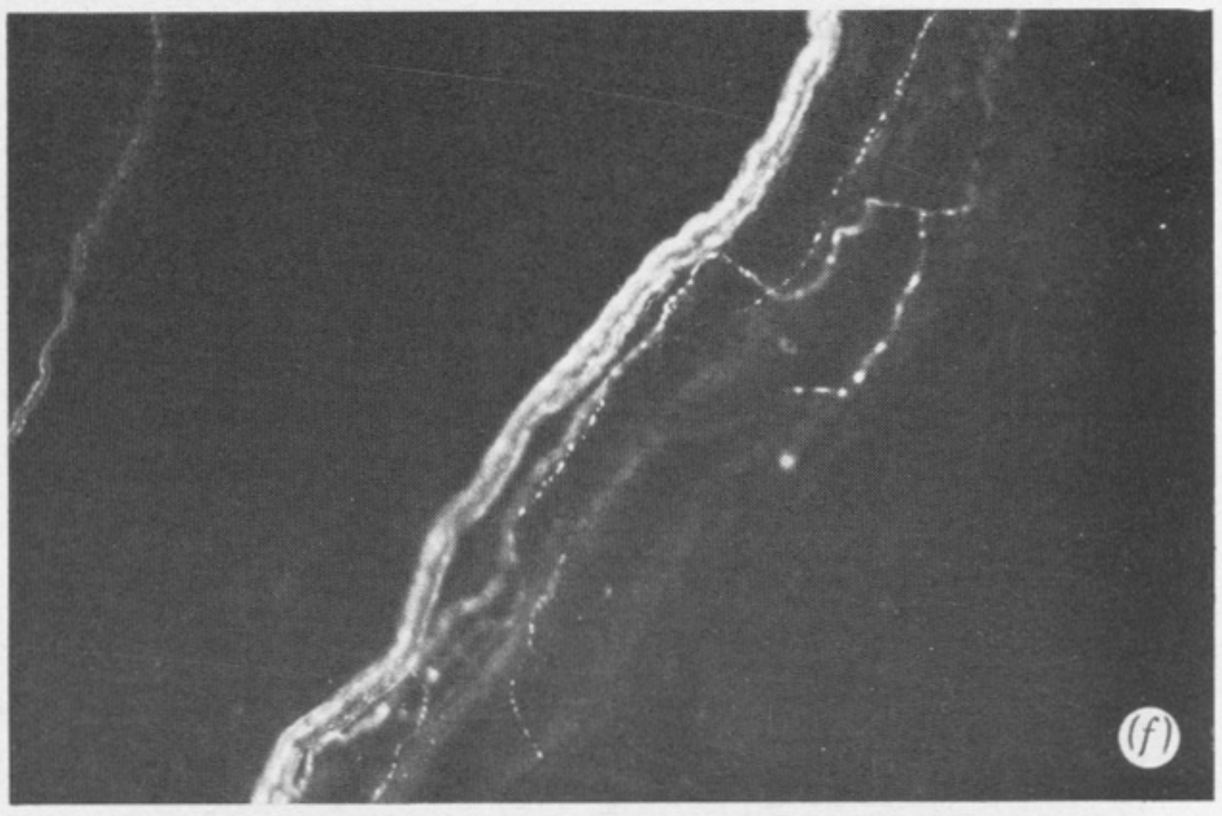


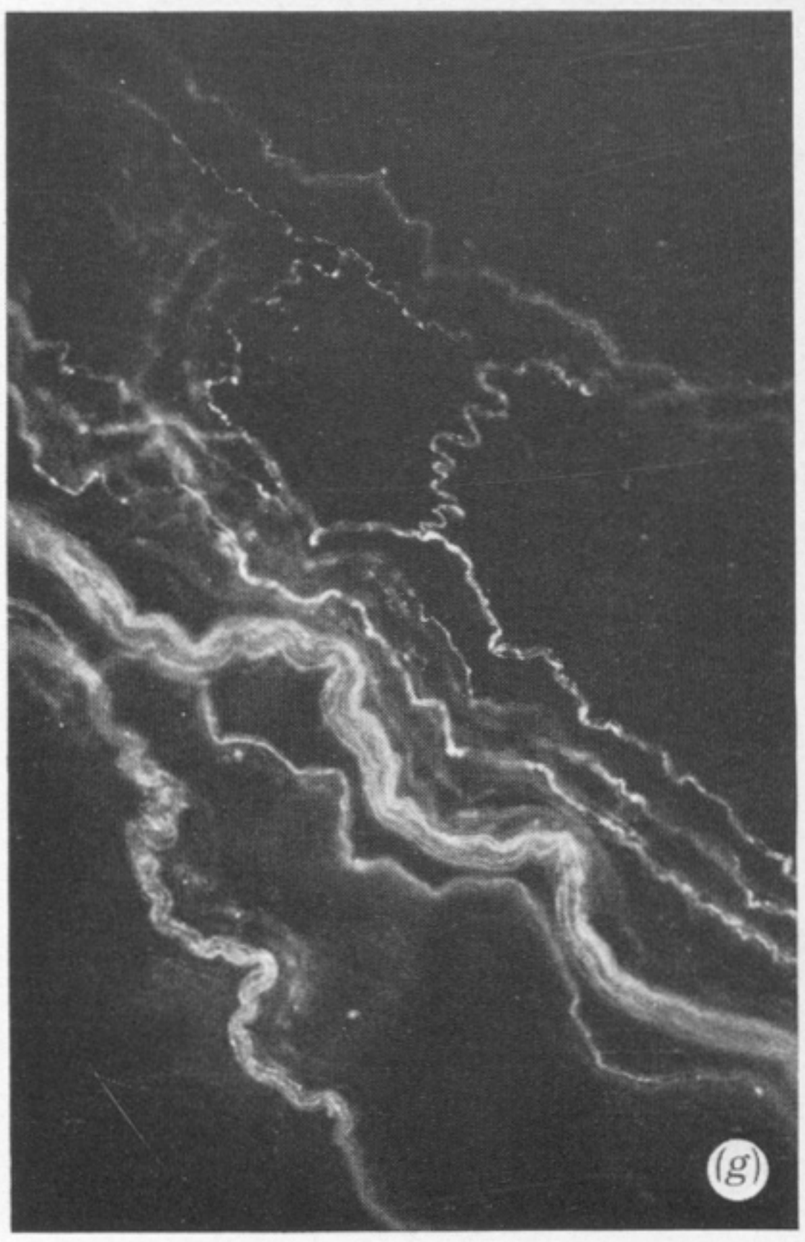












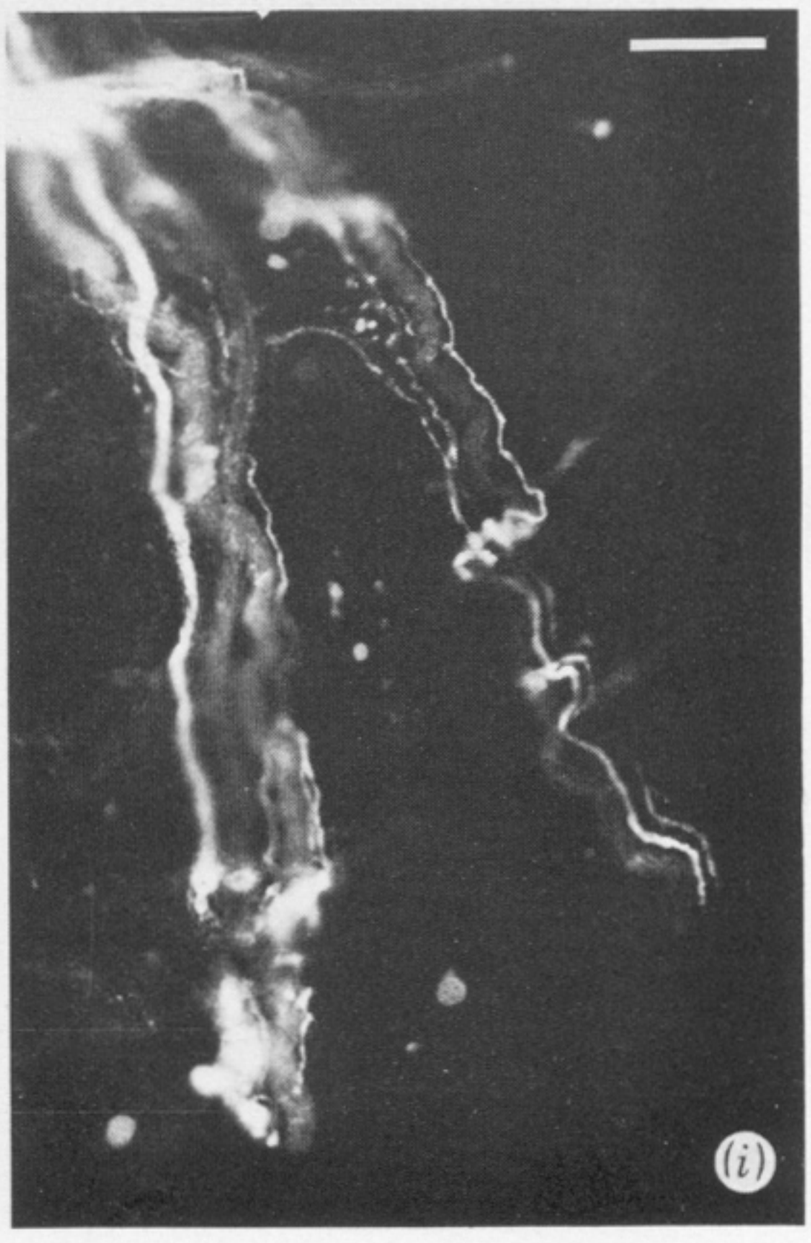


FIGURE 24. For description see opposite.

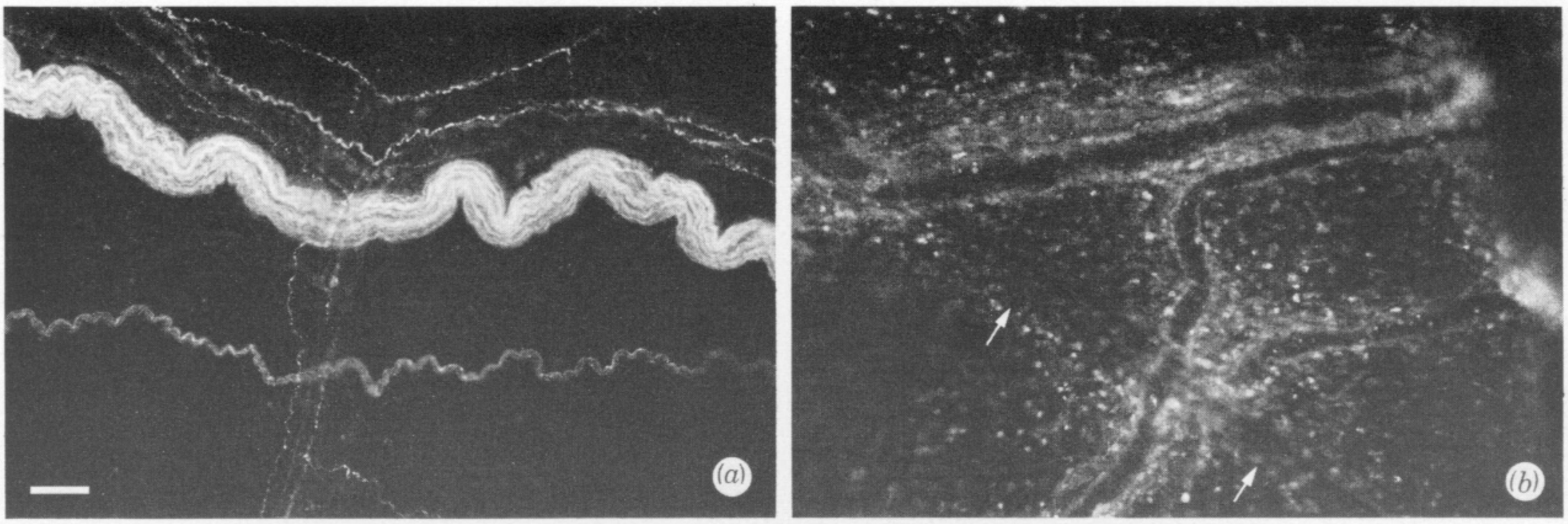


FIGURE 25. For description see opposite plate 22.

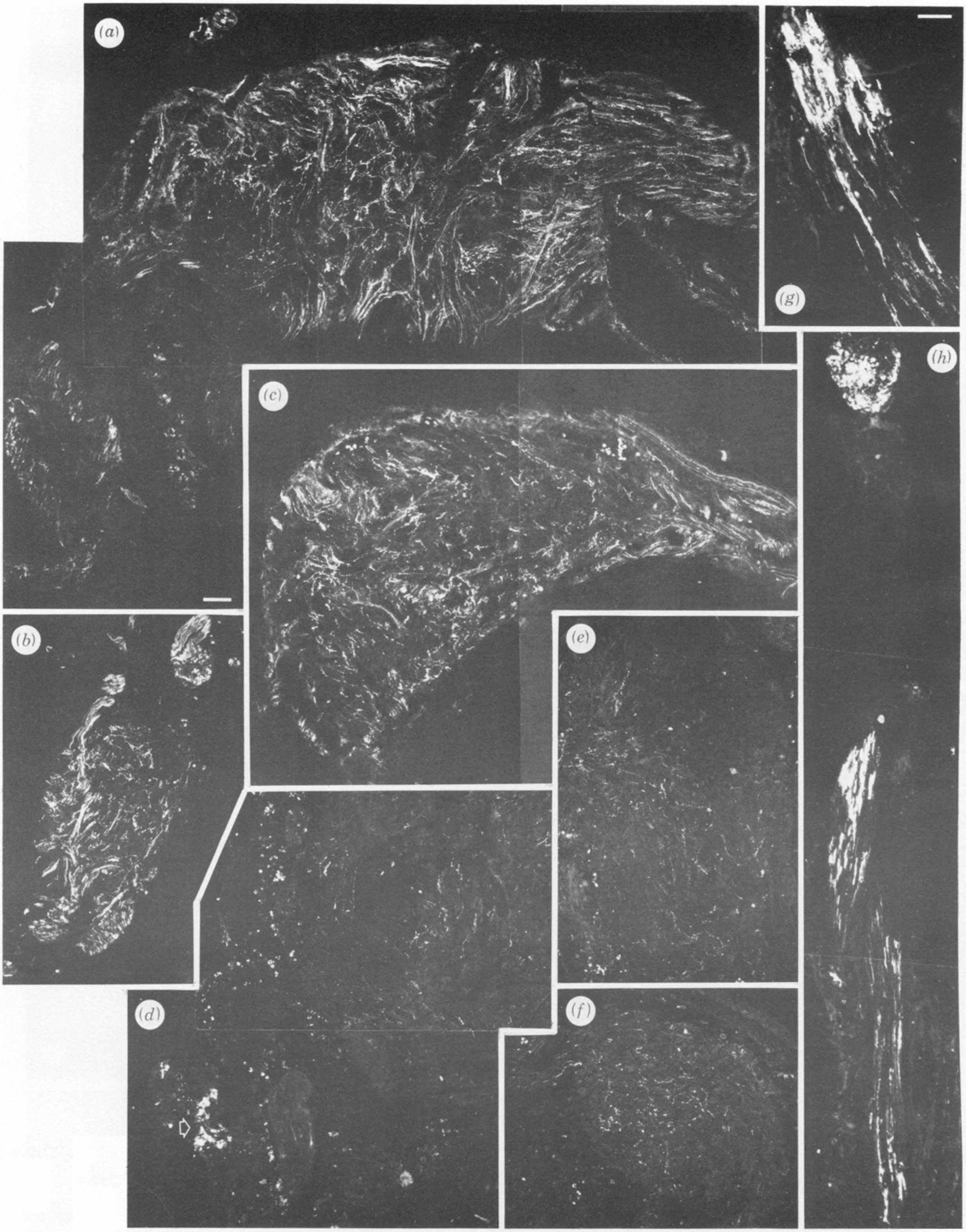


FIGURE 26. For description see opposite plate 22.

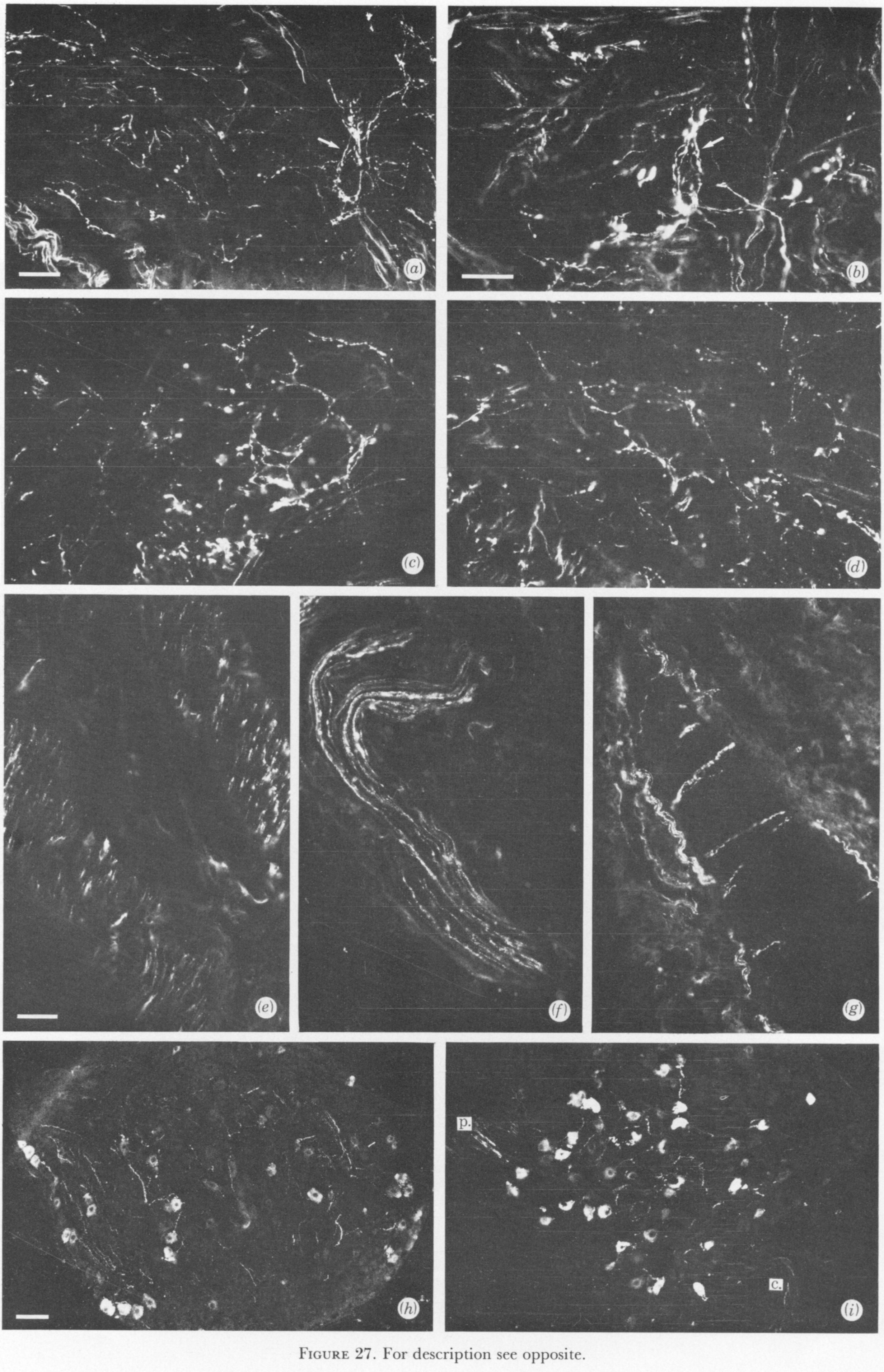


FIGURE 27. For description see opposite.