Zoology. — *A simple technique for the electron-microscopy of cell and tissue sections.* By L. H. BRETSCHNEIDER. (From the Zoological Laboratory, Utrecht, and the Department for Electron Microscopy, Delft.) (Communicated by Prof. J. BOEKE.)

(Communicated at the meeting of May 28, 1949.)

1. *Introductory:*

The relatively low penetration capacity of electronic rays limits electron-optical investigation to objects thinner than 10^{-3} mm. Isolated, or readily isolatable structures of vegetable or animal tissues or protozoa of such small dimensions, however, are not very numerous, so that the need to be able to make sections thinner than 10^{-3} mm was felt already when electronmicroscopy was in its first stages. The utilization of a high voltage electron microscope (made by Philips', Eindhoven, Holland), with an acceleration voltage of 100—400 kV, by the Institute for Electron Microscopy at Delft brought us nearer to the possibility to examine, when necessary, also thicker sections of, say 0,6 micron, since the penetration capacity rises with an increase in the voltage. When seeking after a suitable technique for this purpose, we found that it was possible to make plan-parallel sections through animal and vegetable tissues, of 0,6 micron, and successfully to examine these sections electronoptically. Although our aim, at present, is entirely directed towards the perfection of the technique employed, the provisional results obtained so far are already fit for publication, although we fully realize the numerous defects yet to be removed [1]).

2. *The old type of rocking microtome.*

All modern microtomes for light-microscopic investigation are adjusted to a thickness of the sections of 1 micron and multiples of this. About 60 years ago, the micron scale did not play such a dominating role, some microtomes not even having a scale division at all. This applies, for instance, to the earliest model of the "rocking microtome" made by the Cambridge Instrument Co. (London and Cambridge). In the calculation of the thickness of a section obtained with such a microtome on shifting one cog of the wheel, this thickness proved to be significantly less than 1 micron, namely, 0.59 micron. If we take care that certain conditions are fulfilled during embedding and sectioning of the object, we are able to make, with this microtome, serial sections of $\pm 0.6\ \mu$, which, already at an

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[1]) During printing of this manuscript we found a publication of D. C. PEASE and R. F. BAKER about "Sectioning techniques for Electron Microscopy using a conventional microtome", in Proc. of the Soc. f. exper. Biology and Medicine, Vol. 67, 1948.

acceleration voltage of 110 kV, are thin enough for electron-microscopic examination.

The rocking microtome was originally designed by Horace Darwin, and constructed for the first time in 1885. It is probable that our above-mentioned instrument is one of this earliest type. The microtome was developed from the older "Coldwell Automatic Microtome", and is characterized by the simplicity of its construction and manipulation. It owes the possibility of so minimal a shifting of the object towards the cutting blade to its typical transference of the screw movement to a lever, on which a hinge is fitted that turns a second lever carrying the object-holder. (See Fig. 1 and 2.) The nut *b* is placed in a bore in the longer extremity of the lever *a*; the spindle *c*, in this nut, moves the lever in an upward direction. This movement is communicated to the axle *d* of the lever *e* holding the object, which axle rests on the short extremity of the lever *a*. This axle describes a small sector of a circle, and thereby displaces the turning point of the object-holder *e*, and so moves it bodily towards the cutting blade *f*. The two axles *d* and *g* rest on the beam *h*, while each lever separately is held in place by a powerful steel spring *i* and *k*. The up-and-down movement of the object-holder, by which the object is drawn through the cutting blade, is effected by a cord running over a pulley towards the manipulation handle *l*. When the handle is pulled forward, the object-holder, against the tension of the spring *i*, is moved upwards, while the cogwheel is, simultaneously, and automatically, turned to the left. Only when the tension of the spring is eased, causing the manipulation handle to move backwards, does the object move through the blade.

Owing to the peculiar turning point of the lever of the object-holder, the section describes a line which is part on the surface of a cylinder. In view of the smallness of the object, e.g. 2 mm, and the length of the lever, the curvature is extremely slight. Since the cogwheel of our microtome has 260 cogs, while 6.5 revolutions — i.e. 1690 cogs — are necessary to shift the object 1 mm towards the blade, it follows that each section has a thickness of 0.59 micron. In our ancient instrument the automatic shifting of the cogwheel does not yet possess any scale-division at all; evidently it was adjusted by guesswork. In further developing the rocking-microtome into the modern model (List No. 184 of the Cambridge Instrument Co. Ltd.) the number of cogs and revolutions has been arranged in such a way that the smallest shift produces sections of 2 micron, so that this type is no longer suitable for our purpose. We have accordingly approached the firm in question, and very much hope that our suggestions and proposed improvements will lead to the construction of a microtome suitable for this purpose [2]).

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[2]) During printing of this manuscript the Cambridge Instrument Company communicated to us that building of this instrument is progressing and probably will be ready in a short time.

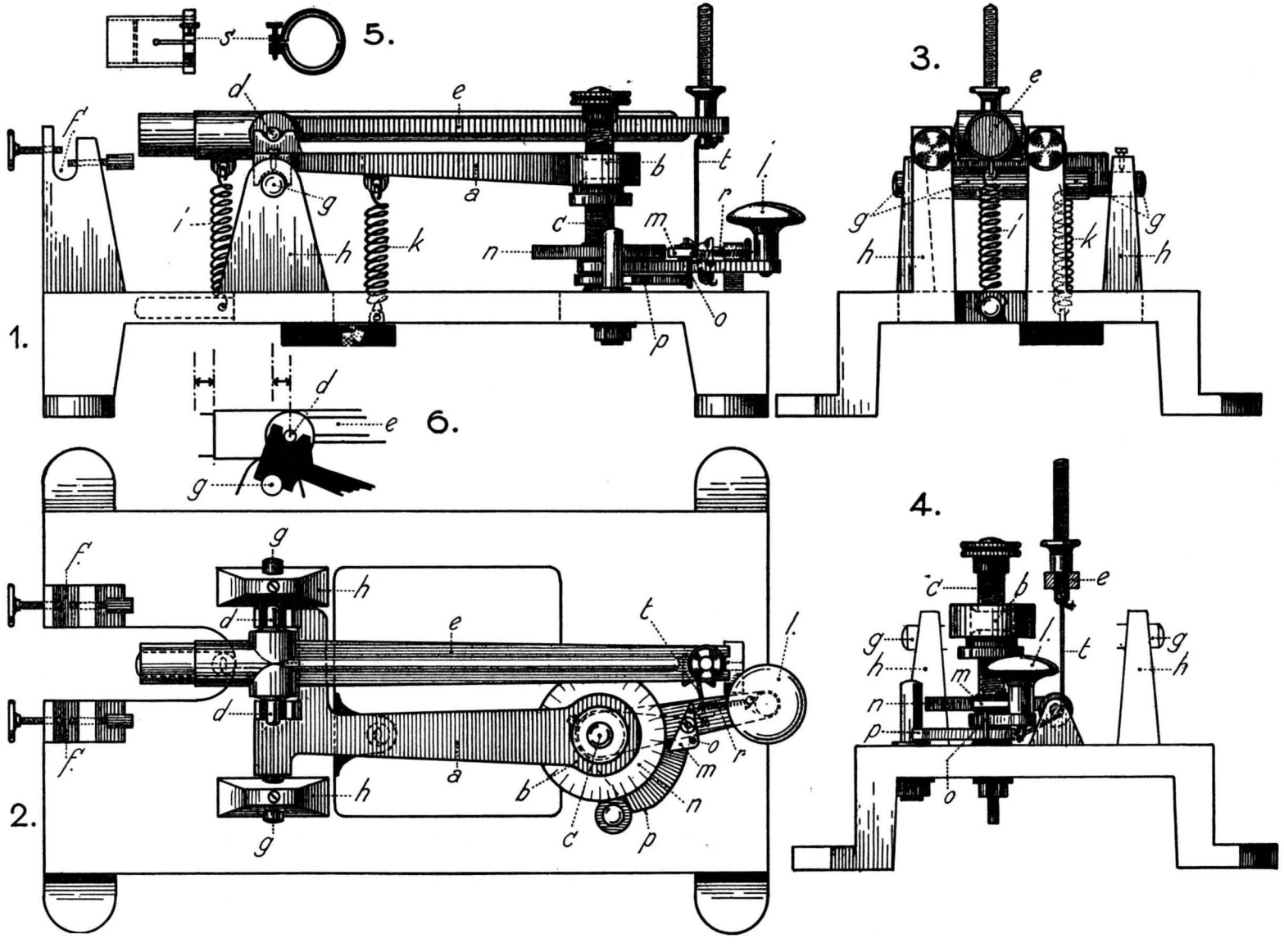
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Fig. 1.

3. *The cutting blade.*

In making sections of such extreme thinness, special demands are made in regard to the properties of the cutting blade. Both the radius of curvature of the blade-point and the cutting angle should be as small as possible. The cutting edge of the blade must be polished sufficiently well to reduce to a minimum any flaws in the grinding. Although the objects (smaller than 2 mm), and the electron-microscopic field of view (less than 70 micron) are small and require only narrow blade-parts, we have, up to the present, made our sections with the ordinary, current type of microtome blades. We use the 6 cm long, concave Jung knives, which are first ground on a hone, and afterward polished on leather. We are at present seeking after a means to use Gilette blades, which more closely approach the ideal i.e. that the cutting edge passes gradually into the flat surface of the blade.

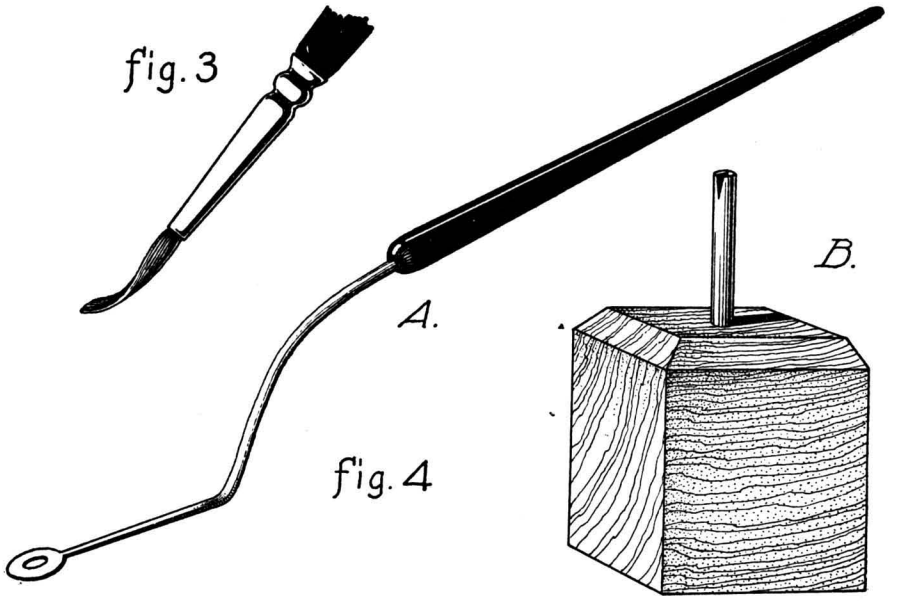
4. *Embedding, cutting and mounting of the sections.*

At ordinary room temperature, sections of such thinness, made from objects embedded in ordinary paraffin are driven up into narrow, useless strips. To prevent this, we sought after an embedding medium with a higher melting-point and a firmer consistency. For this purpose we found most useful a mixture of 2 parts yellow beeswax and one part paraffin (melting-point 72° C). The mixture is filtered a few times through a hardened filter, and kept liquid in the thermostat. Notwithstanding this raising of the melting-point, it is necessary to lower the room temperature to $\pm$ 10° C in order to prevent crushing of the sections. We have sectioned at 8° C at lowest, and up to 14° C as highest limit. Outside this temperature range the sections are no longer usable. We treat protozoa or small particles of tissue, from fixing to embedding, in centrifugal tubes. For embedding in wax-paraffin, which requires a small block not larger than 2 mm along the edge, we fold a container of thick tin-foil over a conical metal mould. On the outside, the folds are covered with a thick solution of celluloid in acetone and so closed. Objects and embedding medium are poured into this container, in the thermostat, and after sedimentation solidified in cold water. After this the container can be removed by just folding it open.

The rise in temperature of the object, placed in the usual way on to the object-holder with the aid of a heated spatula, is eliminated again by cooling in running water.

The blocks are, at most, 2 mm long and wide, and also flat in order to obviate their "giving way" and prevent any elasticity when the object is drawn through the blade. We cut small ribbons of 2—3 successive sections, which we then remove from the blade with the left hand, with the aid of a flat "water-colour" (painter's) brush. The handling of sections of such thinness requires some care. The hairs of the brush are cut level at the top. The brush is then dipped into 2 % solution of celloidin in ether-

ethanol; while drying, it is given a flat, somewhat curved form by pressing it between two fingers (fig. 3). The celloidin keeps the hairs together without making them less supple. With this brush, the sections are removed from the blade, and placed in rows into PETRI's dishes, in which they can



be kept dustfree and cool for a long time. While cutting, we from time to time make a section about 4—5 μ thick which, after staining, serves as light-microscopic control-object. During the cutting process and subsequent mounting of the sections, we successfully avail ourselves of the wellknown magnifying spectacles, which facilitate both the examination of the sections and their accurate mounting on the pellicle. In order to be able to section at a higher room temperature, we had a cooling device built for the microtome (see fig. 2, no. 2) consisting of a sectionally *L*-shaped case filled with ice chopped up fine, on which the microtome is placed. A tap serves to drain off the superfluous melted water which automatically flows away through an overflow. The front part, which is touched by the hands, is insulated by ebonite. At a room-temperature of 18° C there is a fall in temperature of the entire microtome including the object and the blade, to 12° C within 4 hours, and to 10° C after 6 hours- i.e. the optimal temperature for cutting purposes. Owing to the vertical back part of the refrigerator, the instrument, and particularly the blade, are also cooled from aside.

5. *Mounting the sections on to the object-carrier.*

The mounting of the sections on to the bore of the object-carrier of the electron-microscope has lately been considerably facilitated by the 3 mm electron microscope grids produced by the firm of Kodak (Rochester).

These small pieces of copper gauze, produced by electrolytic means and gilt, have a grille consisting of 55 μ thick wires intersecting each other at right angles, and enclosing $\pm$ 300 square openings of 70 μ. First, a parlodion pellicle is stretched over the surface of this gauze, obtained in the usual way by allowing one drop 2 % parlodion, dissolved in pure amyl acetate, to spread over the surface of aq, dest. The gauze is placed on this; it is then caught from below with the aid of a ring, at the same time one pierces the surrounding part of the pellicle with a needle (fig. 4*A*), and lays it upside down over a small anvil (fig. 4*B*), so that the pellicle now lies on top of the gauze. After drying away the superfluous water with the aid of blotting paper, and drying on a heated plate, the gauze, with its covering pellicle, is ready for the mounting of the section. The narrow ribbons referred to above are taken from the PETRI's dish by means of a brush as described earlier, and dropped on to the surface of a warm water bath. This consists of a water-tank, electrically heated to 40—45° C (fig. 2, no. 6), in which is placed a dish containing aq. dest. The heating of the bees-wax-paraffin mixture causes the surface tension to stretch the sections. When this stage is reached, the gauze, with its pellice covering, is placed into the aq. dest. with the aid of a fine pair of tweezers, exactly underneath the section, which is then lifted out of the water bath. The sections are then dried in a dustless, electrically heated box (fig. 2, no. 3) at $\pm$ 50° C, and, after drying, heated for a short time in the thermostat at the temperature of the meltingpoint of the embedding medium, which causes the object to remain quite flat on the pellicle. If the sections are not to be examined at once, the gauzes may be kept, in tubes of 4 mm diameter, with a rounded bottom and closed with a plug made of cigarette paper, in an exsiccator. Before the gauze is introduced into the electron-microscope, the embedding medium is dissolved by dipping it into xylene.

Although there is a chance that some parts of the objects are covered by the wires of the gauze, the latter's relatively large area nevertheless affords so many advantages in mounting the sections that this drawback may be regarded as being compensated to some extent. Moreover, the diameter of the openings in the gauze is greater than the diameter of the field of view of the Delft electron microscope, even at the smallest enlargement of 1500 $\times$.

6. *The double sections method.*

Different tissues are too compact and too hard, even at such a small size as 2 mm^2, to allow of their being sectioned to 0.6 μ. In such cases the blade may first slide a few times across the block, and then cuts far too thick a section. This applies, for example, to dense connective tissue, cartilage, bone, or horny layers. We managed, however, to obtain, also from such objects, sections of 0.6 μ thickness, by first cutting 20 μ thick sections on the freezing microtome, and afterwards embedding them, via

the alcoholic series, in the bees-wax paraffin mixture, when they can be cut to 0.6 μ. The same result was obtained with softer objects embedded in paraffin (50° C), which were first cut thick and afterwards embedded in the wax-paraffin mixture.

7. *Electron-microscopic documentation and analysis of the photographs.*

It is obvious that the Delft electron microscope, with its photographic documentation on a film band, by means of which 26 pictures can be taken without changing the film-holder, allows of more extensive documentation than the technique of other electron microscopes, which have to work with separate plates. The instrument is not always adjusted sharply enough for every picture to be eligible for reproduction, although they may still be usable in the actual investigation. For this reason the number of photographs taken is on the large side, with the consequence that the question of an easily surveyable registration has arisen in our laboratory.

We make contact prints of all pictures taken, from the films. Each print is pasted separately on a special card, which also contains the necessary data. These cards are arranged in a filing system according to the subjects to which they refer. The actual enlargements are made from the best prints, in sizes of 9 × 9 cm or 18 × 18 cm; intended for both research and publication purposes. They are pasted on drawing cardboard, and placed into boxes, numbered in the order of sequence in which they appear in the recording diary. With the aid of a magnifying glass (10 ×), moreover, the contact prints can be examined quite well for research purposes, and, if desired, be reproduced in drawing, by using a drawing-prism. What we lack in electron-microscopy, as compared to light-microscopy, is direct, subjective observation of the object itself, a thing which greatly facilitates analysis especially when the object is drawn. We compensate this drawback by drawing the electron-microscopic pictures, either free-hand or with the aid of a drawing-prism. This mnemotechnical analysis has many advantages, because one realizes more consciously the significance, topography, etc., while drawing the object. In cases where covering or mutually intercrossing structures are not, at first, clearly distinguished, graphic reconstructions or plastic models come to our assistance. We derive the dimensions of the structure with the aid of a weakly magnifying lens (preparation lens) with a calibrated ocular micrometer, from the enlargements. Optically, this is arranged in such a way that the distance between the ocular lines is 0.2 mm, whereby any errors in reading are reduced.

8. *Experiments with sections of different thicknesses.*

We have not so far observed any essential differences in the irradiatability of tissues at the voltages used by us (110 and 240 kV). Sections thicker than 0.6 micron are definitely useless. This is clear from fig. 6 of a smooth muscle of *Mytilus edulis* (mussel), of which we recorded sections

of different thicknesses, but with the same voltage and enlargement. In the case of sections of 1.2 μ we see — electron-microscopically — the muscle fibres as 3 μ-thick, dark and seemingly compact fibres, i.e. in the same form as shown by the light-microscope. Only the enveloping, thinner reticular fibrils are plainly seen. In the case of a thickness of 1 μ, we see that the muscular fibres are built up of a number (6—10) of smaller units of $\pm$ 200 mμ thickness. Only sections of 0.6 μ, however, are thin enough to render visible the composition of these secondary fibres from the 60 mμ-thick primary smooth muscle fibrils. From this picture it is also clear that, between adjoining secondary fibres, primary fibrils invariably pass from one to the other, thus ensuring their firm interconnection. The secondary fibres are embedded in a fine reticular syncytium, of which the fibrils are about 20 mμ thick.

Owing to some slight defects, due to wear and tear, in the cogwheel of our microtome, the object was shifted, in the places in question, by the distance of half instead of one cog; with the result, however that we still obtained usable sections of only 0.3 μ (see fig. 5*b*). The two sections through the ciliate *Isotricha ruminantium,* from the bovine stomach, were made from the same object. Whereas, in the case of the section of 0.6 μ thickness (fig. 5*a*), the cilium roots in the ectoplasm lie in several layers one above the other, we see, in the 0.3 μ-thick section, only 1—2 layers. The actual ectoplasm, after fixation with mercuric chloride-alcohol-acetic acid, mainly consists of microsomes of 50—70 mμ, which fill in the space between the cilium roots, and are quite plainly visible in the 0.3 μ-thick section.

In this ciliate, the ectoplasm is separated from the endoplasm by a membrane of a thickness of about 200 mμ, in which the cilia have their roots. In the 0.3 μ-thick section a few cilia are even visible in a pure cross-section.

This — quite accidental — observation of still thinner sections than those generally used by us, has led us to point out to the Cambridge Instrument Company that, in constructing a modern microtome, the possibility of using thinner sections than 0.6 μ might be borne in mind.

9. *Some of the results:*

To illustrate the possibilities of the technique described, we will cite a few of the objects examined by us, without, for the moment, going into precise details.

Electron-microscopy of tissues aims, on the one hand, at the direct clarification of already — more or less — known light-microscopic observations on the borderline of the distinguishing capacity of the light-microscope. For this reason, electron microscopy, in these cases, consciously follows in the footsteps of the results of light-microscopy, in order to find, beyond the light-microscopic borderline, fresh details, and to check up on old ones. From this it follows at once that the enlargements to be chosen

should be increased gradually as from the light-microscopic limit, so that the relation between the total picture and the details shall not be lost. In this connection, too, the thickness of the structures to be examined should be such that this interrelation shall be maintained. The drawback — adduced as argument — that the analysis of electron-microscopic photographs of thicker structures is hindered on account of the presence of several layers, one covering the other, is less significant than the advantage derived from maintenance of the interrelation between the structural details. Radiology, too, was faced with the same difficulties and overcame them.

On the other hand, electron-microscopy consciously seeks after factual material which hitherto has been completely unknown light-microscopically, with a view to establishing more universal fundamental principles in regard to the finer structure of the organic material, and the question of the ultimate size and arrangement of its component parts. Our own investigations have shown that these two directions of research go hand in hand and are mutually complementary.

A. The cilia.

Fig. 7, of *Opalina dimidiata*, a ciliate from the intestine of *Rana esculenta*, shows, among other things, the structure and implantation of the cilia. As we see from the pictures, the cilia, which measure ± 300 mμ in diameter, consist of an axial fibril of 70 mμ thickness and a cilium sheath, or outer layer, of ± 100 mμ thickness, through which runs a spiral fibril of ± 30 mμ (fig. 7*c*). Each cilium separately is inserted in the ectoplasm by a tube-shaped cilium root, the direct continuation of the cilium sheath. This is plainly shown by the rings which we find under the pellicles in the ectoplasm, in each series of cilia caught tangentially. We also found the same structure and implantation in *Parameciu*m. In *Opalina*, each cilium is surrounded at the base by a ring, while between this ring and the cilium base, 8—9 ectoplasm filaments are stretched. May be the ring and the filaments serve to keep the cilia in place.

B. The nematocysts.

Fig. 8 shows longitudinal sections through the small nematocysts of the tentacles of a sea-anemone, *Corynactis viridis*. The coils of the spirally rolled nematocyst filament in the capsule are sectioned laterally, and their structure appears complicated. As its chief characteristic, the filament which has a diameter of 500 mμ, shows a torsion which gives it the shape of a deeply cut infinite screw-thread. In the wall of the filament there are two spiral bands, each 100 mμ wide, which appear in the projection on the photographs as two bands intersecting each other. Around these coils lies the actual closed capsule membrane, which is extremely thin (see fig. 8*b*). The mechanism of a nematocyst is based on the action of a temporary, higher inside pressure in this capsule, which causes the filament to be expelled. For this reason it seemed to us of interest to ascertain whether

this membrane might perhaps possess a certain electron-microscopic structure, which would be adapted to this turgescence function. It appears, indeed, from greater enlargements, that the capsule wall consists of parallel fibrils of ± 90 Å thickness, running in the longitudinal direction of the nematocyst capsule. These fibrils show a very regular striation at right angles with their direction due to the fact that denser and less dense bands alternate at equal distances. The periodicity of this striation is ± 300 Å units, and very probably represents the macromolecular arrangement of the protein chains forming the structure of this membrane.

C. Bone structure.

Fig. 9 shows a section through a lamella spongiosa of the vertebral bone of the bluewhale *Balaenoptera musculus*. The bone was fixed in formalin immediately after the animal was caught; it was then decalcified, and further treated according to the double section method described on p. 659. The picture was made from the outer zone of a lamella, and shows a fine three-dimensional network of collagenic fibrils oriented longitudinally. The fibrils measure between 100 and 200 mμ, while the interspaces are between 100 and 300 mμ wide. In these spaces, and around the fibrils we see spherical bodies of about 100 mμ in diameter, lying like a cloak around the fibrils. This, quite probably, is the mucoid secreted by the bone-cell and masking the collagenic fibrils. Owing to the many anastomoses between the fibrils, the interspaces also appear in the guise of a three-dimensional network, in which the calcium salts were originally deposited.

D. Structure of cross-striated muscles.

The pictures in fig. 10 and 11 derive from sections through the wing-muscle of a dragon-fly *Aeschna cyanea*. Here, we invariably find only a single layer of the 300 mμ-thick myofibrils in evidence. The sarcomeres, about 2—2.5 μ in length, are distinctly bounded by ring-shaped *Z* bands (see fig. 11). On other photographs — not reproduced here — these *Z* bands consist of membranes transversely intersecting the muscle syncytium. As homologues of the *Q* band we see here ± 1.5 μ large bodies, the sarcosomes, which surround each myofibrilla like a sheath (see fig. 11), and, in fixation, are shorter than the sarcomeres. These sarcosomes have an alveolar structure. In greater enlargements we see that each myofibril consists of a larger number of protofibrillae (see especially near the *Z* bands, fig. 11), which measure ± 25 mμ in diameter. In fibrils without sarcosomes we notice a transverse striation, with a periodicity of 400—500 Å units, and probably due to the actomyosin.

We also investigated the myogenesis in very young larvae of *Triton taeniatus*, because there the myoblasts in the tail are younger and less far differentiated than in the trunk which enables us to observe the genesis of the cross striation in one and the same object (see fig. 12 and 13).

In the myoblasts of the tail we see the presence of primary fibrillae with a diameter of 120 mμ which are not yet transversely striated, and are joined together in bundles. After this, parallel septa are formed in the protoplasm eventually becoming the well-known *Z*- and *M* bands, on the fibrillae and forming the links between the myofibrillae mutually. We plainly see here that the *Q* band is thicker than the *I* band, giving the impression as if, also in vertebrates, the fibrilla in the *Q* band is surrounded by a sheath, at any rate during the period of its genesis. During the genesis of these myofibrils we find, in the plasm of the myoblasts, spherical, 1 μ-thick sarcosomes accompanying the still non-striated fibrillae at regular distances; they may possibly be related in some way to the *Q* zone and its genesis.

E. Plasm Structures.

Fig. 14 shows the local structural differences, fixation and object being the same — which may occur in the plasm of part of the fertilized oocyte of *Ascaris megalocephala*. In this worm, the egg shell is formed immediately after insemination by the secretion of ascarylic acid from the oocyte; a process still taking place here. It will be seen how the coarse vacuolar plasm structure in the remaining part of the oocyte (at the bottom of the picture), at the point where the secretion is still proceeding, takes on the character of an extremely fine vacuolar structure, while the cortex of the oocyte is being broken by a liquid current. The fact that the egg membrane is not yet fully formed in this place is evident from its thinness and from the hiatus. The egg-membrane itself possesses a large number of pores from 20—40 mμ in diameter, and is built up in layers.

F. Intestinal cell.

The structure of the striated border, and the plasm zone below it, in the intestinal cell of *Ascaris*, are shown in fig. 15. The striated border consists of 5 μ-long and 80 mμ-thick filaments kept together by means of thin transverse links. They pierce the thin limiting membrane of the intestinal cell and pass into the plasmatic structure of the cortex, which latter shows an extremely dense configuration. Thereunder begins the endoplasm, which contains numerous mitochondria (Osmium fixation). These consist of filaments with a thickness of 160 mμ, whose substance, on the greater enlargements, gives the impression of consisting of fibrillae in a parallel arrangement. At the surface of these seemingly homogeneous mitochondria we find, may be as a product of condensation, numerous denser granula. The larger granula of 1—1.5 μ, strongly impregnated by OsO4, consist of reserve fat.

It may be remarked even now, from the above concise commentary of the photographs here published, that in each of the objects investigated, there appear not only additions to what was already known light-microscopically, but also structures newly discovered by electron microscopy.

The further development of the technique described above must now be directed more especially towards an extensive investigation of the most efficient fixation and staining methods to be applied in the electron-microscopic analysis.

10. *Summary.*

(1) A description is given of a simple microtome (old model "Rocking Microtome, Cambridge"), and a fairly simple histological technique by which sections of 0.6 microns can be made through protozoa, animal and vegetable tissues. An ice-cooled installation is described, by means of which sectioning is possible also at room temperature.

(2) Electron-microscopic photographs are described, of sections of 1.2 μ, 1 μ, 0.6 μ and 0.3 μ, from which it appears that sections of 0.6 μ are already thin enough for examination with the Delft electromagnetic electron microscope (*vide* J. B. LE POOLE, *Philips Technical Review,* Vol. 9. pp. 35—46, 1947), at an emission voltage of 110 kV and with the electron microscope with high acceleration voltage for 400 kV (A. C. VAN DORSTEN, W. J. OOSTERKAMP, and J. B. LE POOLE, *Philips Technical Review,* Vol. 9, pp. 195—201, 1947), at emission voltages of 240 kV.

(3) Some results, elucidated by photographs, obtained with the following objects, are discussed to illustrate the technique described:

(*a*) Ciliary structure and implantation in *Isotricha, Opalina* and *Paramecium*;

(*b*) nematocysts in *Corynactis*;

(*c*) bone structure in *Balaenoptera*;

(*d*) wing muscle in *Aeschna*; cross-striated muscle in the *Triton* larva;

(*e*) egg membrane secretion and plasm structure of the oocyte in *Ascaris*, and

(*f*) striated border and mitochondria in the intestinal cell of *Ascaris*.

We wish to express our sincere thanks to Mr. J. B. LE POOLE, C.E. and the staff of the Department for Electron Microscopy, Delft, for their untiring and valuable cooperation. Our investigations would, moreover, not have been possible without the financial assistance of the "Netherlands Organization for Scientific Research" (Z.W.O.), to which body we hereby also tender our grateful thanks.

Fig. 2. General view of the materials.

Fig. 5. Sections of *Isotricha*; fixation with FLEMMING's fluid; thickness a) 0'6 μ and b) 0'3 μ; orig. magn. 25.000 $\times$; 110 KV.

Fig. 6. Sections of a muscle of *Mytilus;* fixation with BOUIN's fluid; thickness a) 1'2 μ, b) 0'9 μ and c) 0'6 μ; orig. magn. 10.000 $\times$; 110 KV.

Fig. 7. Sections of *Opalina* and *Paramecium*; fixation with FLEMMING's fluid; thickness 0'6 μ; a) orig. magn. 24.000 $\times$, b) and c) 20.000 $\times$; 110 KV.

Fig. 8. Sections of nematocysts of *Corynactis*; fixation with BOUIN's fluid; thickness 0'6 μ; orig. magn. a) and c) 15.000 $\times$, b) 30.000 $\times$; 110 KV.

Fig. 9. Section of bone of *Balaenoptera*; fixation with formalin; thickness 0'6 μ; orig. magn. 20.000 $\times$; 110 KV.

Fig. 10. Section of a muscle of *Aeschna*; fixation with BOUIN's fluid; thickness 0'6 μ; orig. magn. 16.000 $\times$; 240 KV.

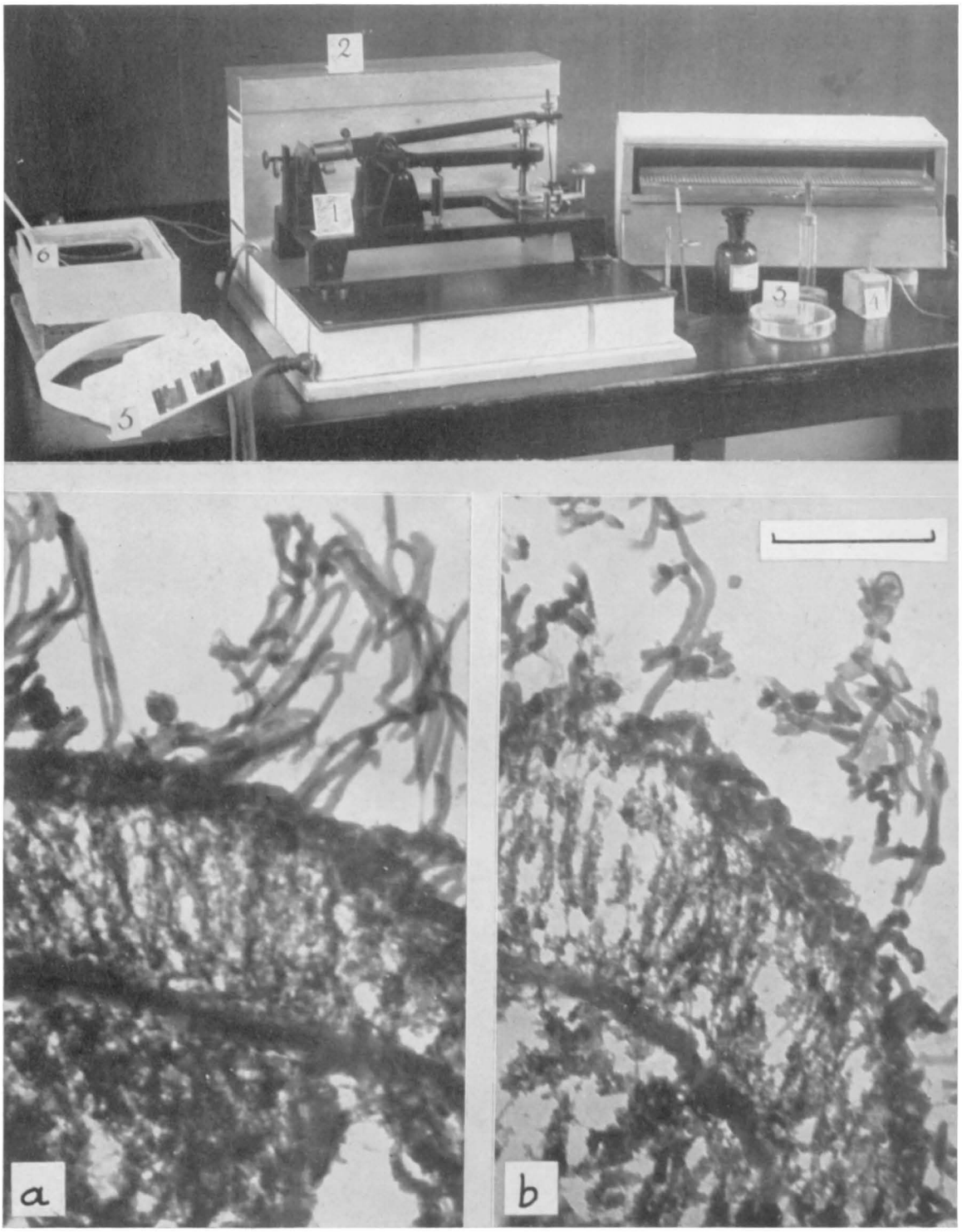
Fig. 11. Section of a muscle of *Aeschna*; fixation with BOUIN's fluid; thickness 0'6 μ; orig. magn. 16.000 $\times$; 240 KV.

Fig. 12. Section of a myoblast of *Triton*; fixation with LENHOSSEK's fluid; thickness 0'6 μ; orig. magn. 8.000 $\times$; 110 KV.

Fig. 13. Section of a myoblast of *Triton*; fixation with LENHOSSEK's fluid; thickness 0'6 μ; orig. magn. 12.000 $\times$; 110 KV.

Fig. 14. Section of an oocyte of *Ascaris*; fixation with CARNOY's fluid; thickness 0'6 μ; orig. magn. 12.000 $\times$; 110 KV.

Fig. 15. Section of an intestinal epithelial cell of *Ascaris*; fixation with CHAMPY's fluid; thickness 0'6 μ; orig. magn. 8.000 $\times$. 110 KV.



|————————| Indicates 1 μ in all pictures.

Figs. 2 and 5.

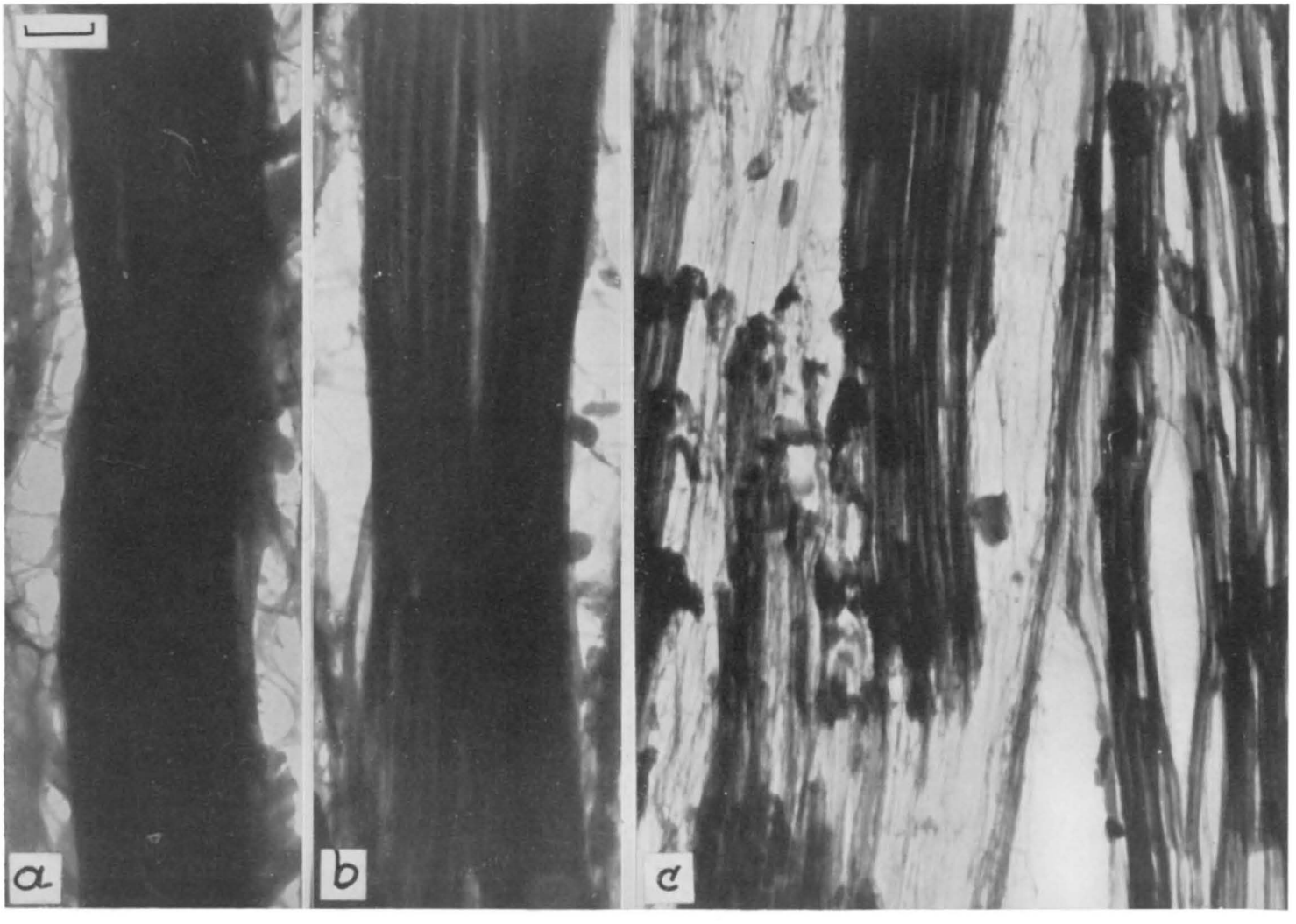


Fig. 6.

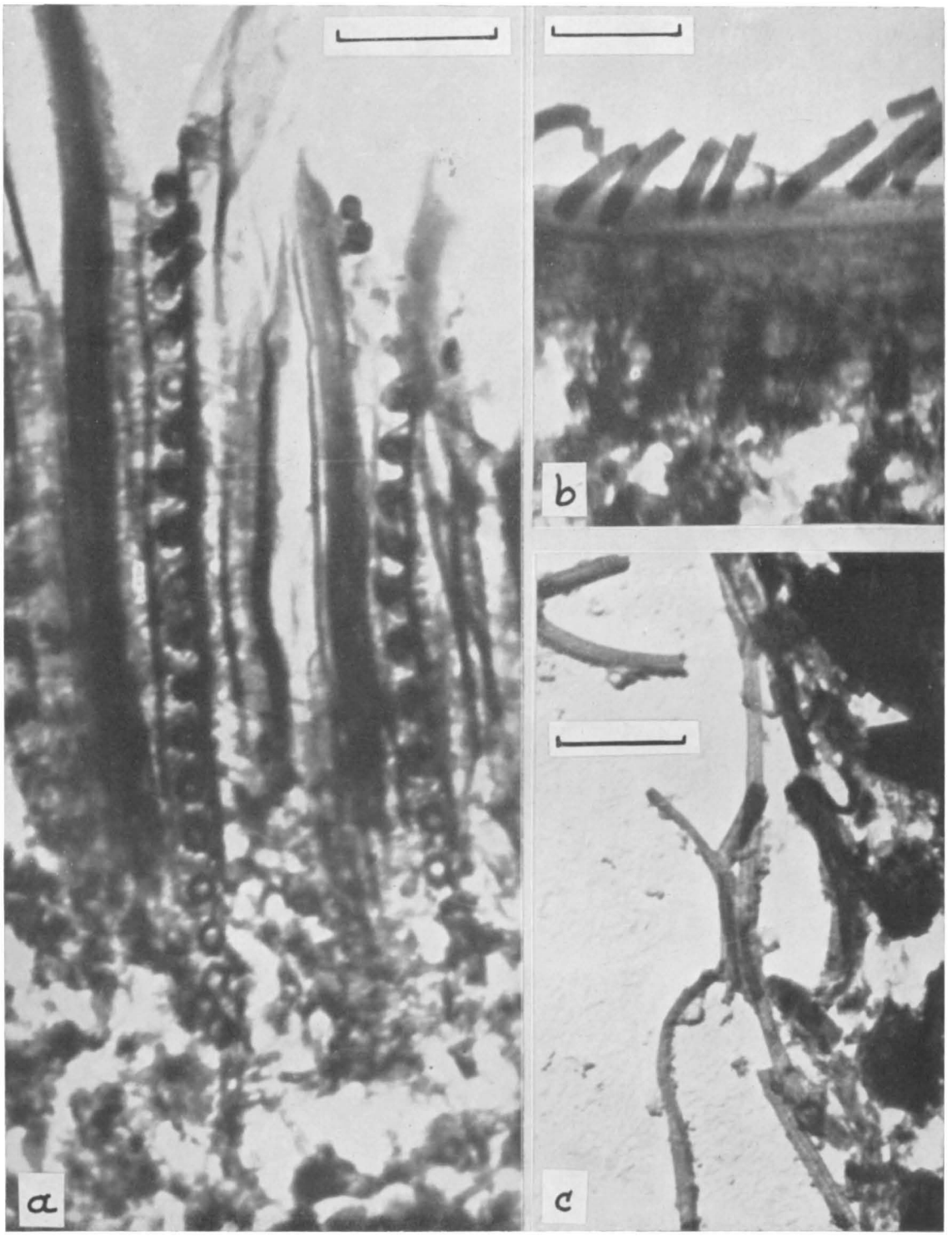


Fig. 7.

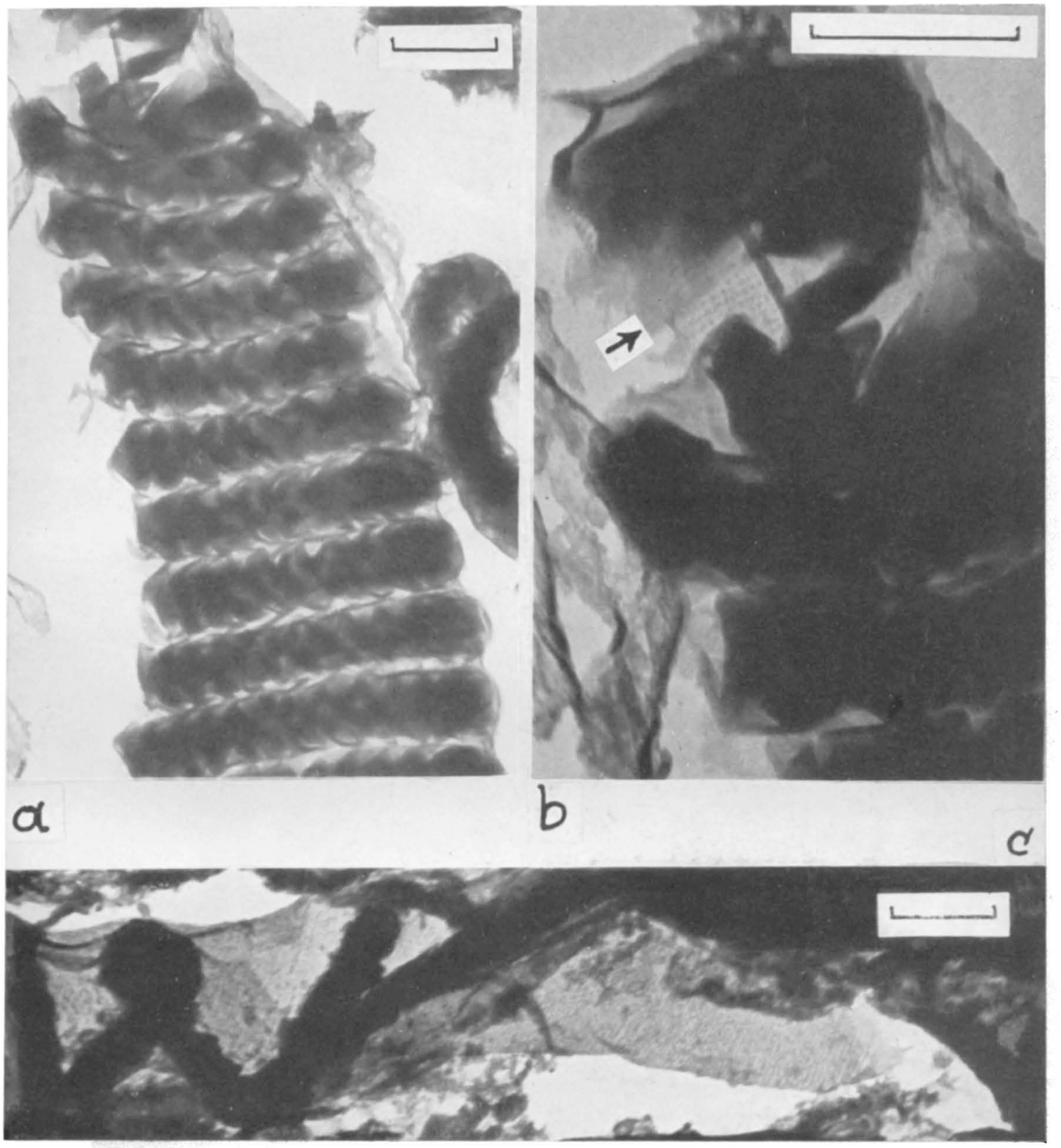


Fig. 8.

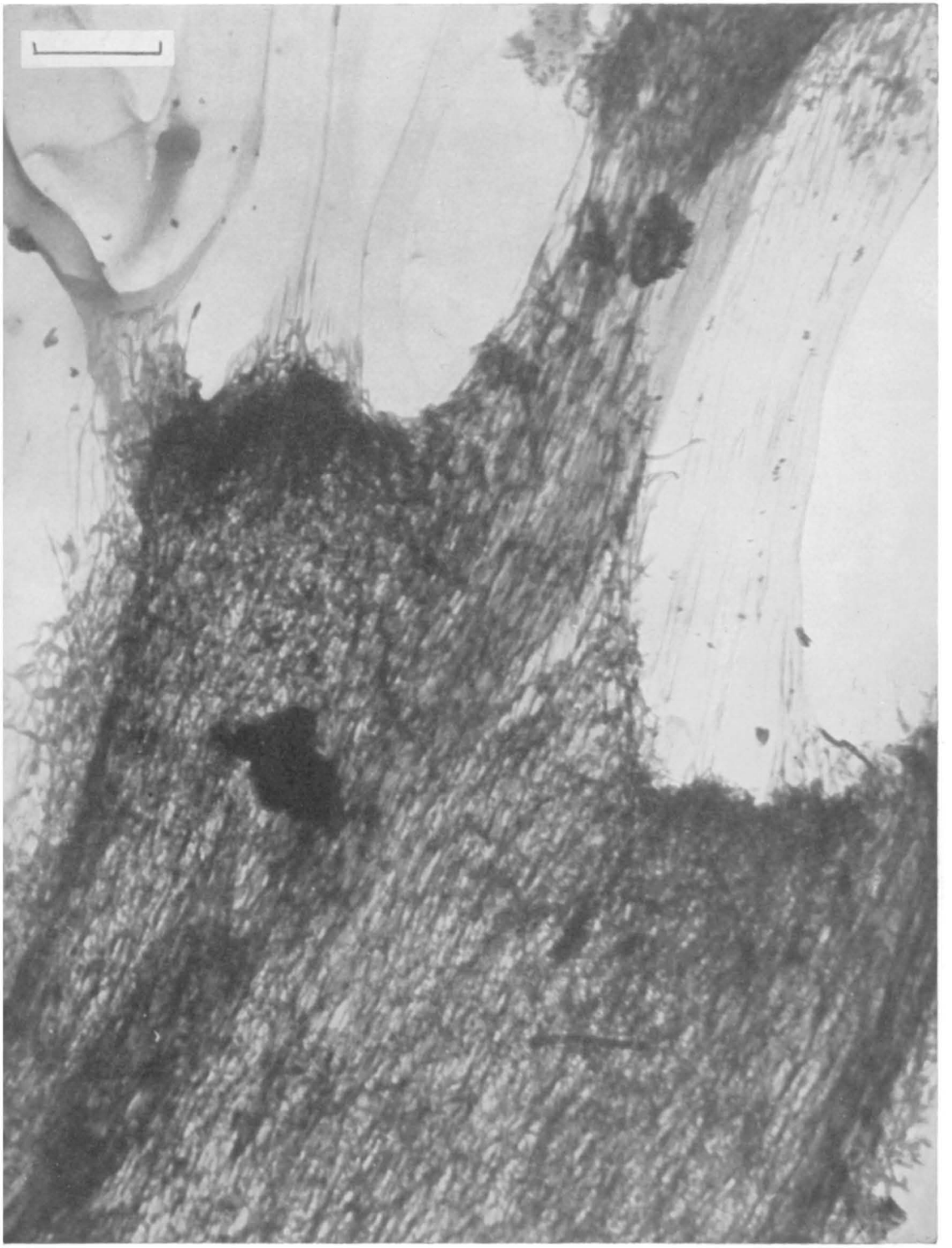


Fig. 9.

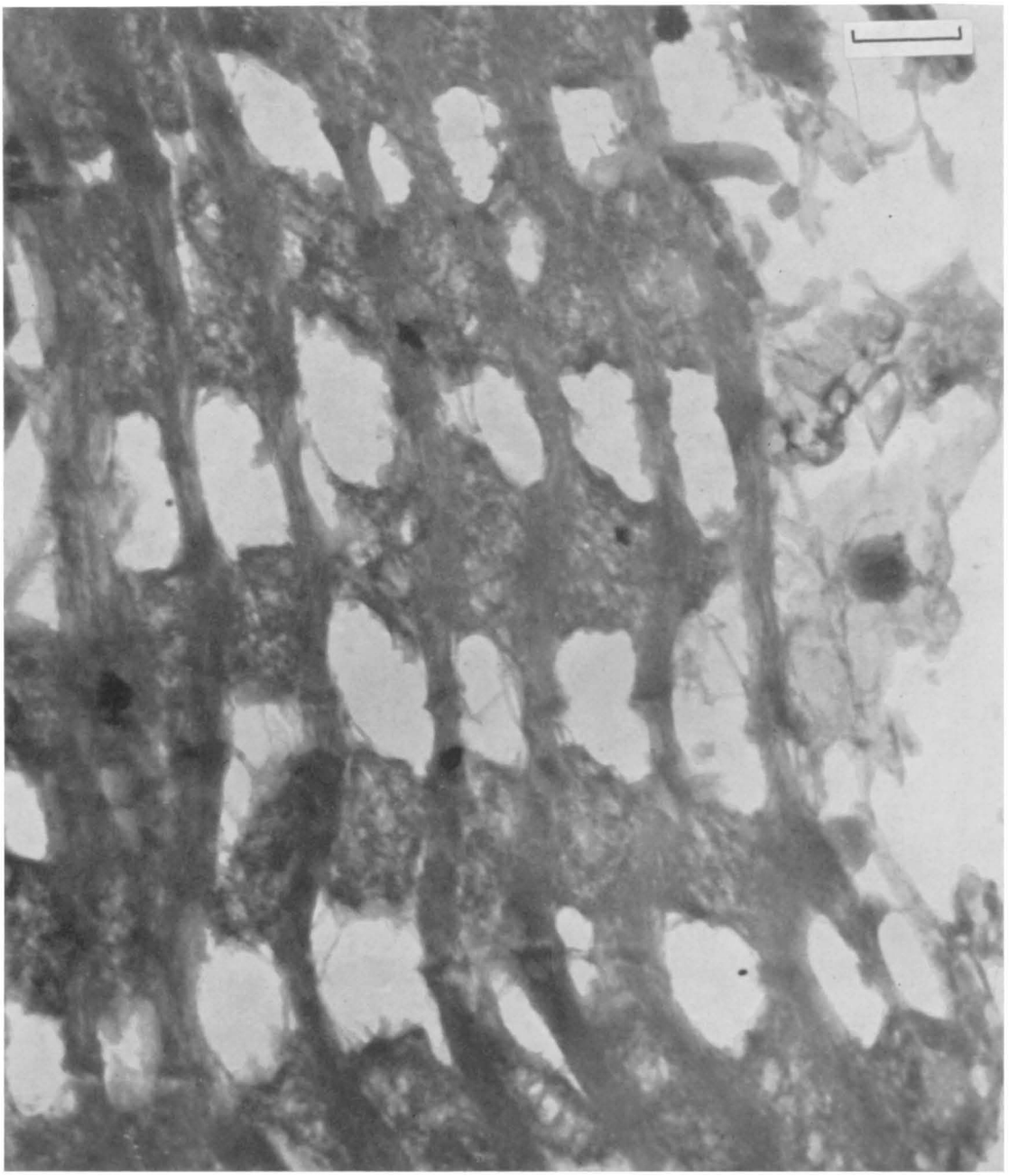


Fig. 10.

Fig. 11.

Fig. 12.

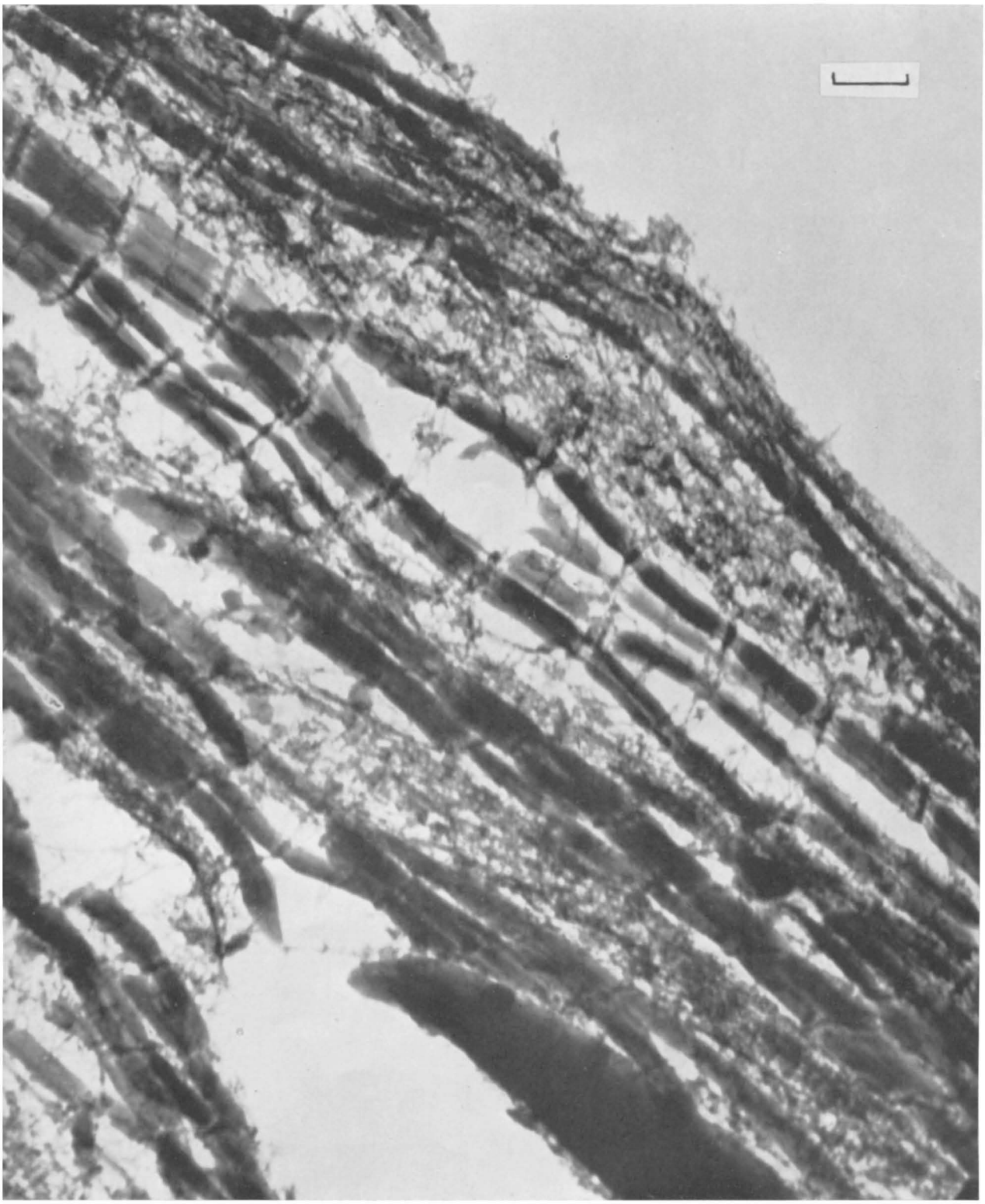


Fig. 13.

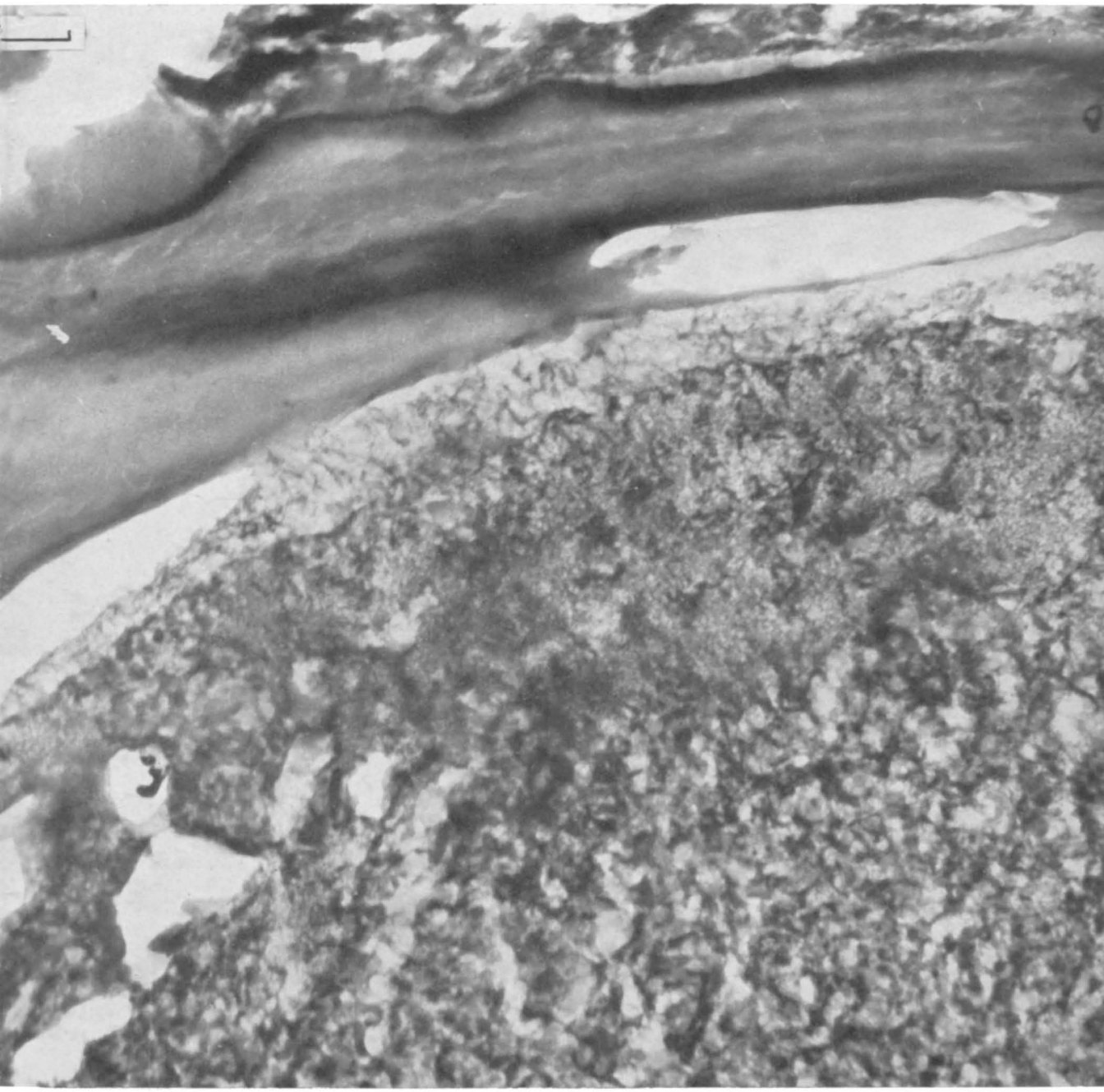


Fig. 14.

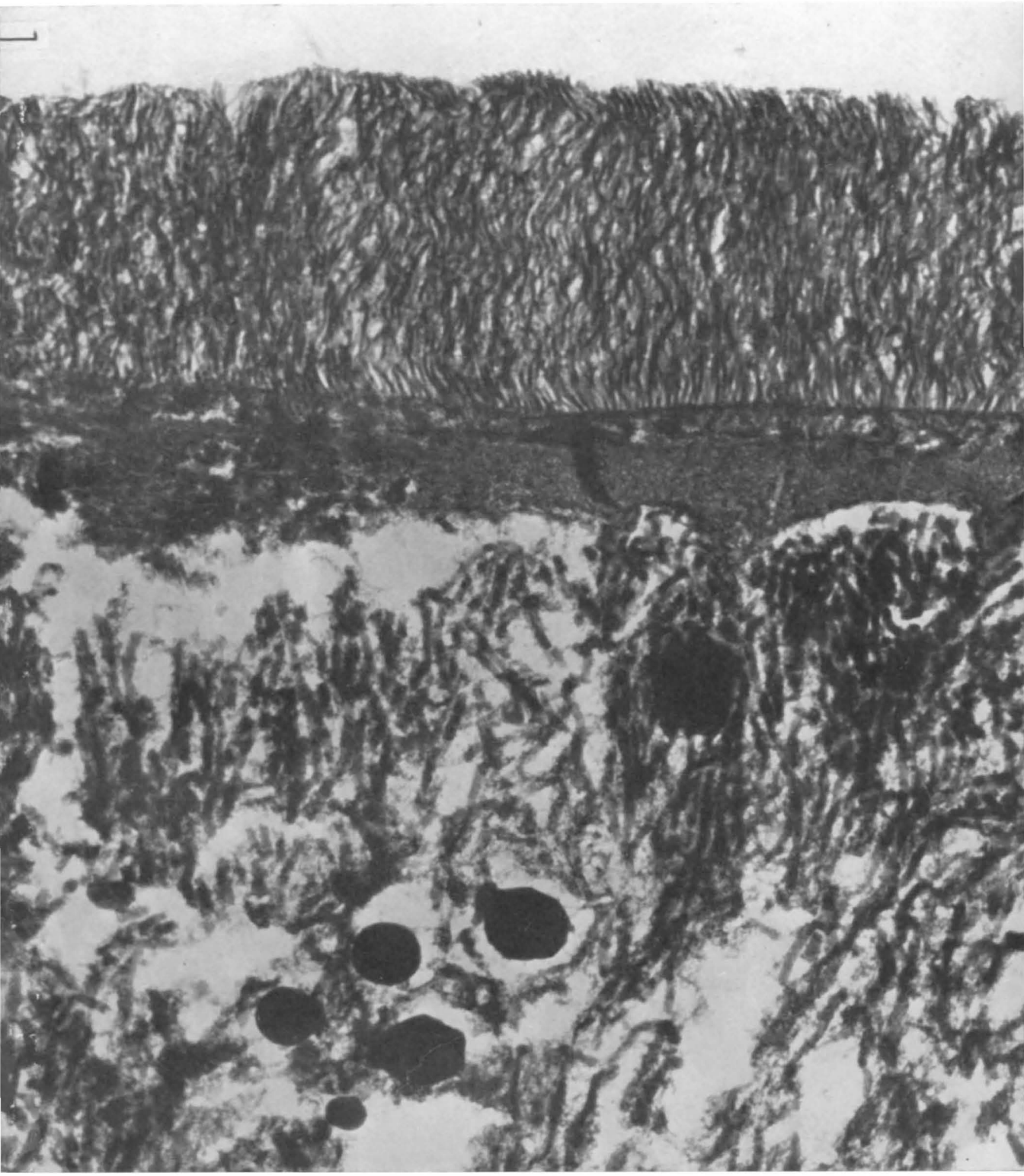


Fig. 15.