THE PATH OF THE SLOW CONTRACTILE WAVE IN ARTHROPOD MUSCLE FIBRE

By E. MATTHAEI and O. W. TIEGS, F.R.S.

Optical Laboratory and Department of Zoology, University of Melbourne

(*Received* 24 *February* 1954)

[Plates 33 to 37]

The passage of the slow contractile wave of arthropod muscle fibre has been recorded photographically in a type of fibre in which the pattern of cross-striation is helicoidal. The records are consistent with the hypothesis that the slow waves move along the helicoid.

INTRODUCTION

The work that is described below is an attempt to examine, by direct photographic recording, the path of the contractile wave within a striated muscle fibre. The final aim of such an investigation must be the recording of the normal twitch wave; but the technical difficulties arising out of the high speed of propagation of such a wave are formidable, and preclude any attempt at optical recording by methods that are generally available.

In contrast to the rapid twitch wave, the slow contractile waves of arthropod muscle readily admit of direct inspection. In the completely fresh fibre, examined intact through the transparent chitin of the living animal, the only observed movements are the rapid twitches; the slow waves come into being later, either when the muscle is isolated in saline, or when the animal is entering a moribund condition. They can readily be induced, even in the freshly removed transparent limb of the animal, by slight trauma, as, for example, by pressing on the chitin. Often many fibres are simultaneously affected—this is the 'Wellenspiel' of German authors; at other times only an occasional fibre is involved. The duration of the wave action varies greatly, from a few minutes up to half an hour or even more. There are also large differences in their rate of propagation. Initially some pass at considerable speed across the field of the microscope, and are then barely noticed. But soon slower waves appear and these can then readily be followed with the eye. As the wave action subsides, the fibres mostly revert to the relaxed condition; but sometimes a wave becomes retarded till it ceases to advance, so that within the wave the fibre remains permanently contracted, giving the familiar 'stationary' or 'fixed' contractile waves of authors.

We may suspect that the slow waves arise by retardation of the normal rapid twitch wave; and certainly this conviction has given point to the present work. We base this belief on the readily observed large differences in speed of the slow waves, and on the improbability that such a co-ordinated process, having no relation to the normal contractile wave, will come into being at a time when the tissue is no longer fresh. But experimental evidence to support this belief we cannot yet produce.

The only photographic recordings of the slow waves, hitherto obtained, seem to be those of Hürthle (1909). They were made on *Hydrophilus* muscle in saline under a cover-glass, not for the purpose of following the path of the contractile wave, but to investigate possible volume changes in the fibrils during contraction. Apart from the astonishing detail, exceeding that which we have ourselves obtained, the photographs show at once that the contractile wave is a highly co-ordinated process, the cross-bands of adjacent fibrils passing simultaneously into contraction, so that the wave advances along with the fibre in the plane of the cross-striation. The impression is given, in fact, that adjacent fibrils are contracting 'in step'. It is surprising that Hürthle does not even comment on the remarkable evidence contained in his photographs, since for the first time it gives a possible clue to the meaning of alined cross-striation in muscle fibre.

The evidence, if general, must have important consequences for theories of conduction within the fibre:

(i) *Is the path of excitation along the fibrils?* This seems least in accord with the observed facts, for unco-ordinated conduction along the fibrils must almost certainly bring with it some scatter as the wave advances. Our own photographs confirm Hürthle's to the extent that we do find a co-ordinated action between adjacent fibrils. There is, moreover, the basic difficulty of the actual form of the wave: it would seem that a wave advancing from striation to striation along the fibre, must initially have spread from the nerve-endings across the fibre before it began to move along it. This does not seem compatible with simple conduction along the fibrils nor, indeed, with any approved hypothesis of excitation.

(ii) *Is the sarcoplasm the conducting medium?* This hypothesis in itself seems inadequate; but if, as an ancillary hypothesis, we envisage the cross-membranes as barriers along which some interfacial charge is built up before propagation to the next membrane, then at least the main fact brought out by Hürthle's photographs might be accounted for.

(iii) *Is the sarcolemma the conducting medium?* In itself this hypothesis also seems inadequate; but if we envisage the cross-membranes as acting in the capacity of conducting membranes, carrying the excitation into the interior of the fibre, then co-ordination between adjacent fibrils could be fully accounted for.

(iv) *Do the helicoidal cross-membranes provide the path of conduction?* Conduction along these membranes would fully account for the co-ordination, and would, in addition, give meaning to the helicoidal pattern. The principal objection against this hypothesis is the existence of types of fibre without helicoidal pattern (see previous paper, Introduction, §E, p. 242); that it is a crucial objection is not assured.

Method

Our observations have been made principally on the leg muscles of a spider, *Pholcus littoralis*. The legs in this species are long and very slender; the chitin is thin and not excessively coloured, and in most individuals is sufficiently transparent to give a clear image of the underlying muscle fibres, which can thus be examined in their own natural medium.

The legs, removed from the living spider by cutting through the basal joint, were placed for examination on a glass slide, under a thin cover-glass, immersed in saline to which a small amount of wetting agent was added. The chitin usually wetted well, adhering air rarely causing trouble; indeed, bristles on the chitin have been a far more important source of trouble, since they tend to throw shadows across the field (the disturbing effect of the bristles is seen, for example, in the photographic series *A*, plate 33, but in this instance does not affect the fibre under observation). The preparation was then placed under the microscope, and examined for wave action, which commonly appears spontaneously after some time; if it failed to appear, it could usually be induced by pressing on the cover-glass at some small distance from the point of observation.

In recording the contractile wave, consideration had to be given to the following points: (i) the tissue is virtually transparent; (ii) the leg is of appreciable thickness; (iii) an adequate depth of focus is necessary for obtaining intelligible records, but this must not conflict unduly with resolution; (iv) exposure must be sufficiently brief to produce a stationary wave; (v) frequency of exposure must be high; (vi) the photographic emulsion must have a resolution able to record that achieved by the microscope, and must be capable of tenfold enlargement, without showing grain.

Consideration was given to the use of phase-contrast objectives to overcome lack of contrast in the material; but the thickness of the object, and the need to obtain maximal focal depth, forbade this. Although a 4 mm dry objective would have given the necessary magnification and resolution, a Zeiss 3 mm oil-immersion objective (N.A. 0·85) was eventually used; it produces better contrast than do dry lenses, and glare is eliminated; moreover, oil-immersion objectives have an increased focal depth over dry objectives, a depth of 3μ having been achieved in our work. The condenser (aplanatic 1·4) was immersed to eliminate air interfaces, and its diaphragm reduced to give, for reasons of depth of focus and contrast, a resultant N.A. for condenser and objective, of 0·5. A low-power projection eyepiece projected the image at 10 cm on to the film.

Koehler's system of illumination was used to reduce glare and heat. The light source was a 10 A a.c. arc lamp with light collector system to produce an image of the carbon arc just large enough to fill the condenser aperture of the microscope. By this means an effective exposure of $\frac{1}{50}$ s was achieved, and this sufficed for all purposes.

The camera was a 35 mm Zeiss Ikon ciné instrument, motor driven to give 24 frames per second. The shutter was used with an aperture of 180°. Vibration was eliminated from the microscope by mounting camera and microscope on separate concrete foundations.

The object was focused, and during the recording kept in view, by a beam-splitter mounted above the eyepiece.

After trials with a number of emulsions, Ilford Pan F was finally used, the normal contrast of 0·7 gamma being raised to 0·9 gamma by suitable development. Although this gave an increase in the silver grain, this was still sufficiently small ($0{\cdot}5\mu$) to permit a tenfold enlargement.

RESULTS

In interpreting the photographs it is necessary to emphasize that they present us with optical sections only of the contractile wave, the sections having under the conditions of the experiment a thickness of not more than 3μ. In particular, it will be necessary to examine the appearance that the wave will present, if it does, in fact, move along the helicoid.

The fibres of this spider have been described in the foregoing paper; they are of the 'tubular' type, with lamellar sarcostyles that enclose an axial core of nucleated sarcoplasm. In such fibres the helicoid is often wound about the axial sarcoplasm, but this need not necessarily be so; for example, in the photographic series *B*, plate 34, the fibre there under discussion is focused along the axis of the helicoid, but the axial sarcoplasm is not quite in focus; the latter, still not properly focused, is better seen in series *C*, plate 35.

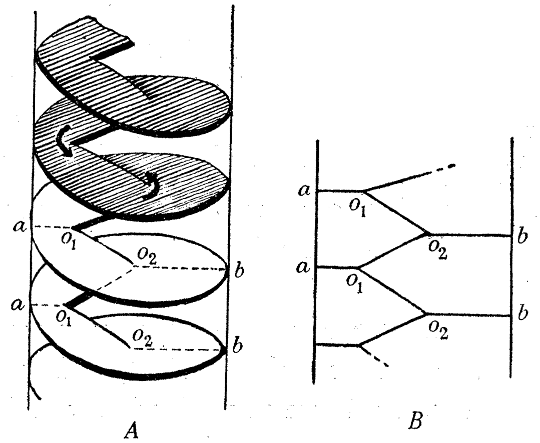


FIGURE 1

The commonest type of helicoid is one in which the axis is an imaginary surface within the fibre (figure 1). If such a helicoid is placed with its axis in the plane of focus, then a zigzag line with two abrupt turns results. Figure 1 *B* is a representation of such a section; figure 1 *A* shows how the section is derived. If the axis of the helicoid is tilted to intersect the focal plane in such a way as to bring O_1 of one striation, and O_2 of a more distant striation simultaneously into focus, then an 'oblique vernier' will result (for discussion of these points, see Tiegs (1934)). Both are, of course, different expressions of the same geometrical arrangement. An example is shown in the accompanying photographic series *A*: in no. 1 of the series the relaxed part of the fibre shows the zigzag line; a later member of the series (no. 10) shows, in the same part of the fibre, the 'oblique vernier', the contractile wave having evidently slightly tilted the fibre.

We may suppose that the path of the contractile wave, if it moves along the helicoid, will be roughly as indicated by the arrows in figure 1. The actual appearance of such a wave when viewed in optical section will depend on the focus of the microscope with respect to the axis of the helicoid.

(i) *The focal plane of the microscope intersects the axis of the helicoid* (series *A*, below). The optical expression of this is a 'vernier effect', oblique or otherwise. As the contractile wave advances along the fibre, the tip of the wave must, in this instance, develop a change in direction along the vernier; and this will be due to the fact that its movement from the 'near' to the 'far' turn of the helicoid has come under observation (for convenience of discussion we shall, in this paper, use the terms 'near' and 'far'; their choice is quite arbitrary, for their interchange will not affect the argument; we cannot, from the photographs, distinguish between 'near' and 'far').

(ii) *Axis in focus* (series *B*, below). Here the zigzag striation picture is obtained, and the most advanced tip of the wave must alternate from one side of the fibre to the other. In an ideal demonstration of this, the axis of the helicoid and focus of the microscope must completely coincide, depth of focus must suffice to give some recording just 'above' and just 'below' the axis, and finally the frequency of exposure must be so timed as to capture the wave as it alternates from one turn of the helicoid to the other. In practice we cannot hope to achieve this; but much can be learnt if, during passage of the wave, focus relative to the axis changes to bring 'near' and 'far' turns of the helicoid separately into focus.

(iii) *Axis not in focus* (series *C*, below). If the frequency of recording is low compared with the speed of the wave, then we may get a picture similar to that already given by Hürthle, i.e. an entire striation may have fallen into at least partial contraction before there is any perceptible contraction in any part of the striation next in advance (an example is shown in series *C*, numbers 23, 24). Such a picture brings out forcibly the basic point that adjacent fibrils do not contract independently, and it even suggests that they contract 'in step'. For purposes of analysis higher frequency recording relative to the speed of the wave is needed. If the contractile wave moves along the helicoid in the manner indicated in figure 1, then we should expect the contraction to start at the axis of the helicoid, and spread outwards across the striation, and we might even get some axial contraction in advance of the main front of the wave. If, on the other hand, the contraction spreads inward from the sarcolemma, then we should expect the contractile wave, even at axial focus, to develop from the margin of the fibre inwards. But in either case the 'in-step' analogy would acquire a modified meaning.

(iv) *Start of the contractile wave* (series *D* below). We should expect that a wave that advances along the fibre with a wave front in the plane of cross-striation, should initially have spread from the nerve ending across the fibre, before there was any pronounced movement along it. The only hint of such spread across the fibre, known to us, is in a paper by Rollet (1891) who figures an abortive (fixed) wave emanating from the motor ending in *Cassida equestris*.

Of the many recordings that we have obtained we give here four only, as follows: series *A*, comprising twelve separate exposures; series *B*, comprising fourteen exposures; series *C*, comprising twenty-four exposures; series *D*, comprising forty-four exposures. The reader will probably have little difficulty with series *A* to *C*, but series *D*, which is the only record that we have so far obtained of the start of a contractile wave at the motor-nerve ending, suffers from a blemish due to a bristle on the overlying chitin, which we could not remove, and which has thrown a shadow across the field; even despite this, the essential information that we require from this series has not been obscured.

There is one point to which special reference must be made, namely, the blurring of the cross-striation immediately in advance of, and in the wake of, the contractile wave. This is very apparent, for example, in series *B*. It is not a defect of technique, but arises from 'striation reversal' attending contraction, the *Q*-band of the relaxed zone disappearing as the contraction band along the *Z*-membrane develops. Events that initiate contraction are therefore in progress well in advance of the actual contractile wave. Blurred striation, far from confusing the picture, usually facilitates interpretation, for it helps to localize the beginning of the contractile wave.

SERIES *A* (plate 33)

Of this series we have twenty-six exposures, during which the wave advances over twenty-two cross-striations; only twelve of the series (numbers 13 to 24), which alone are relevant, are here reproduced, and are renumbered 1 to 12. The frequency of exposure is, of course, far too low to disclose the development of the contractile band within a single cross-striation, and the effect discussed above under heading (iii) cannot therefore be recorded. On the other hand, as numbers 1 and 2 of the series show particularly well, the wave is advancing into a length of fibre in which the axis of the helicoid is focused (note zigzag striation). In later members of the series the advance of the contractile wave into this part of the fibre has caused a slight tilt, so that an oblique vernier replaces the zigzag.

In the circumstances the effect discussed above under heading (i) appears. The lead in the contractile wave, which in numbers 1 and 2 is on the left side of the fibre, has swung, in numbers 5 to 12, to the right side. This is, of course, dependent on change in direction of the cross-bands; but this change in direction is itself conditioned by the oblique vernier, indicating a change in focus from the 'far' to the 'near' turn of the helicoid (in this instance the terms 'far' and 'near' are used with specific reference to the diagram, figure 1, reference to which will aid interpretation). If in any of the later members of the series the focus had been slightly lowered, then the wave-front would have reverted to its original direction; note that the detached fragment of striation to the right of the vernier, which is dart of the lower turn of the helicoid, has the required direction.

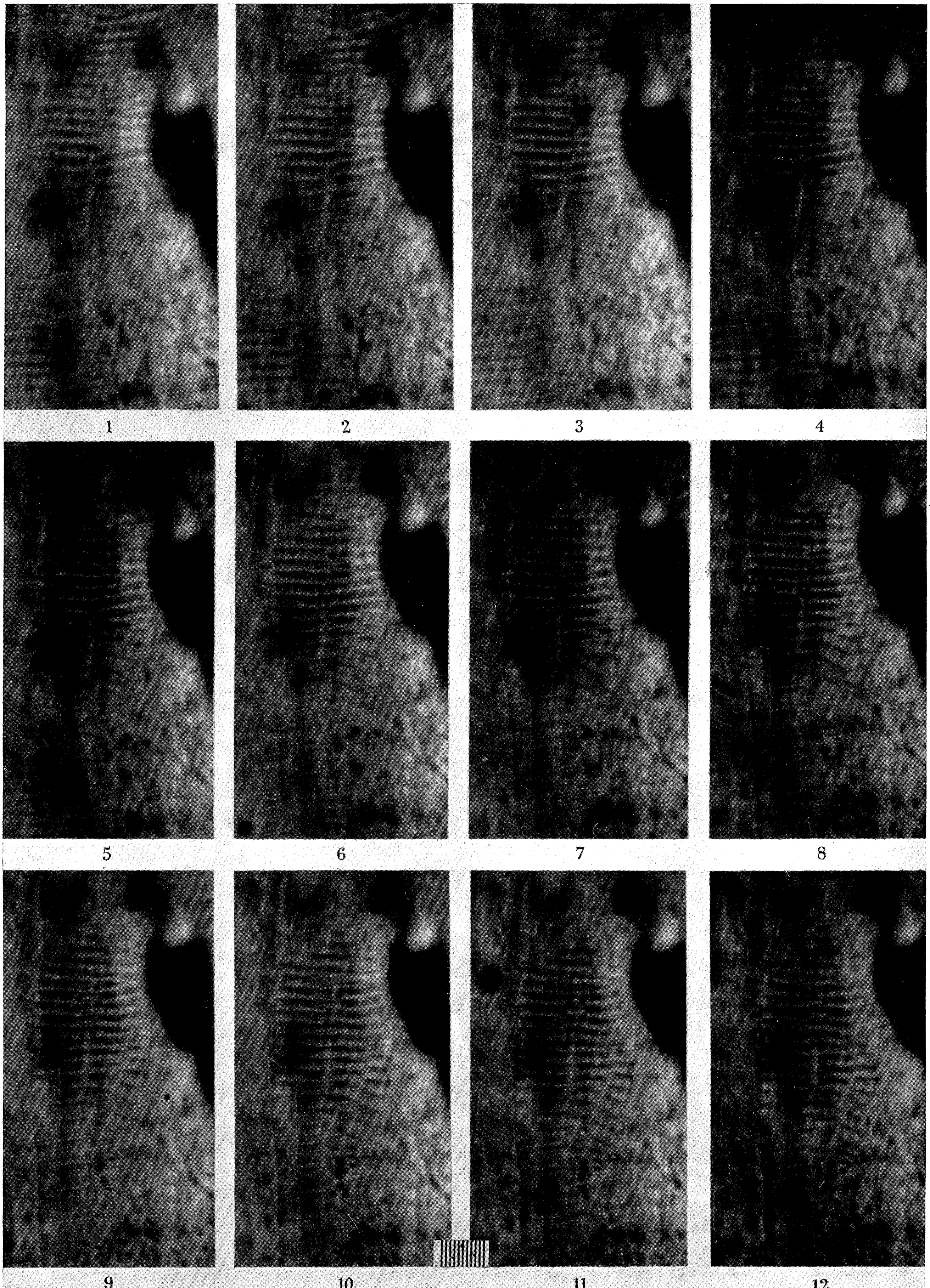
The evidence of this series is clearly consistent with the hypothesis that the contractile wave moves along the helicoid, and is probably not consistent with any other hypothesis. But the recording is far too infrequent to reveal the actual spread of contraction from one turn of the helicoid to the other, and in that respect the series falls short of demonstration.

Incidentally this fibre is of interest because the *Q*-band is dim, the cross-bands in the relaxed zone being actually the *Z*-bands; 'striation reversal' is therefore not recorded in this series, and consequently there is no 'blurring of striation' preceding the wave.

The large blemish to the right of the fibre is the unfocused base of a bristle in the overlying chitin.

SERIES *B* (plate 34)

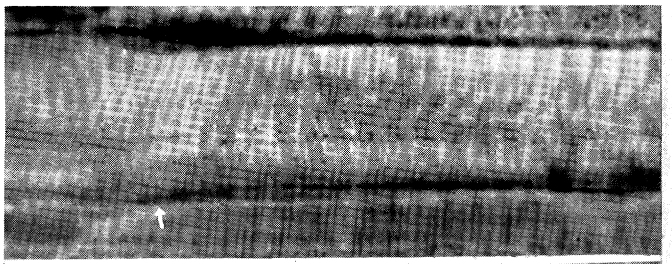
This is an unbroken series of fourteen photographs, recording an advance of the contractile wave to the right over sixteen cross-striations. The wave is moving into a length of fibre in which the helicoid has been focused along its axis, the zigzag striation being readily seen. Since the frequency of recording is almost in phase with the frequency with which the wave traverses successive cross-striations, we should not expect to register any alternation in advance of the wave from one side of the fibre to the other. Actually this consideration does not wholly cover the case, the position being also affected by the fact that the passage of the wave along the fibre has, in this case, perceptibly altered its level of focus. For example, in numbers 10 and 11 of the series the passage of the wave has lifted into focus an axial nucleus (incidated by *N* in number 10); it is progressively less distinctly focused in 9, 8, 7 and 6, while in 5 and preceding photographs it is unfocused; similarly, beyond 11 it gradually fades from focus. It is as though the fine focusing adjustment of the microscope were very slightly altered during passage of the wave; in this

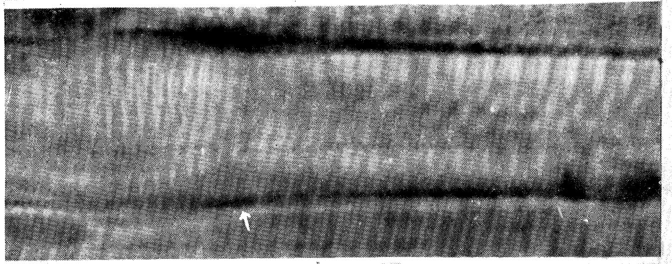

1 2 3 4

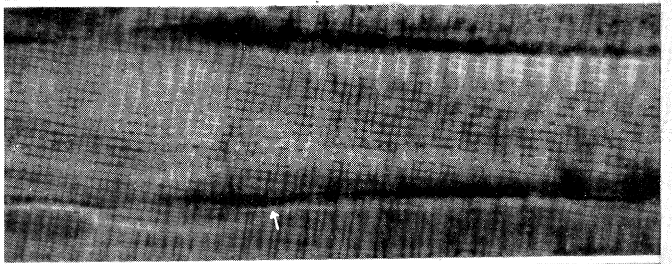
5 6 7 8

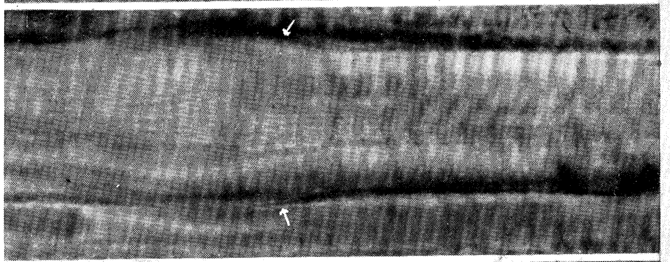
9 10 11 12

Series A. Scale: 1 division = 1μ.

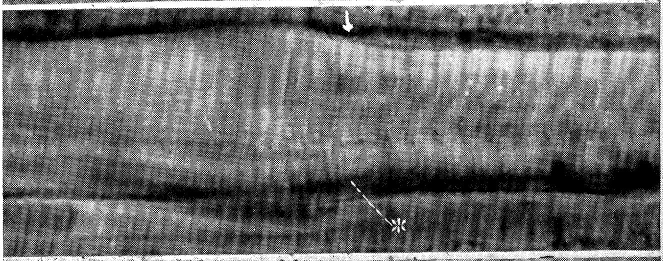

1

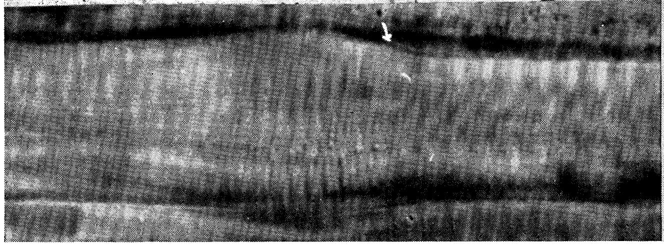

2

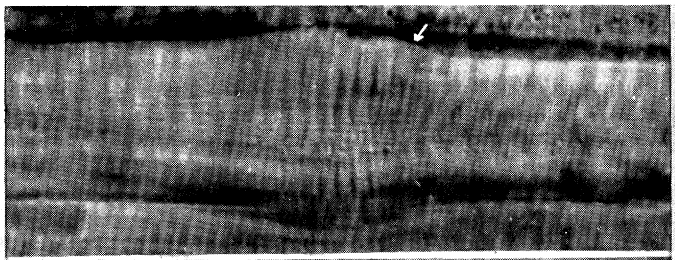

3

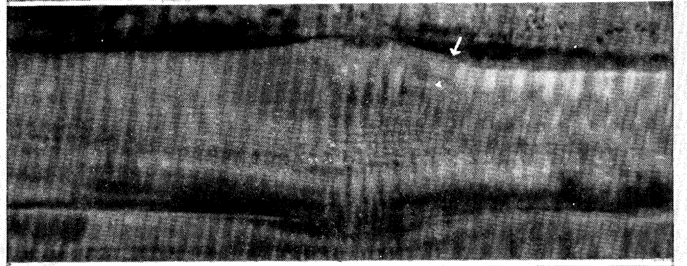

4

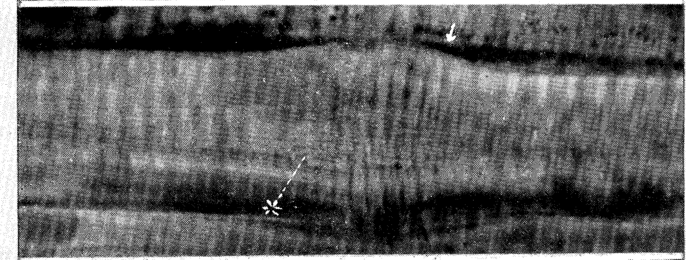

5

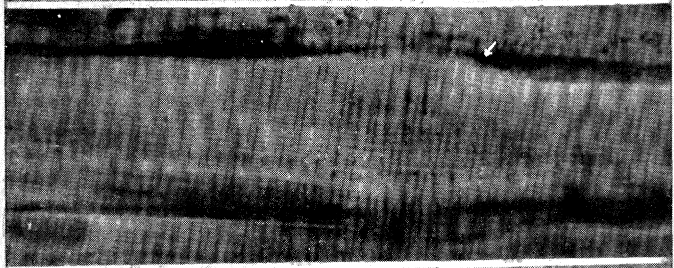

6

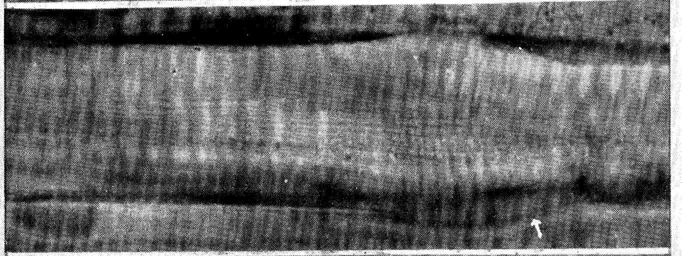

7

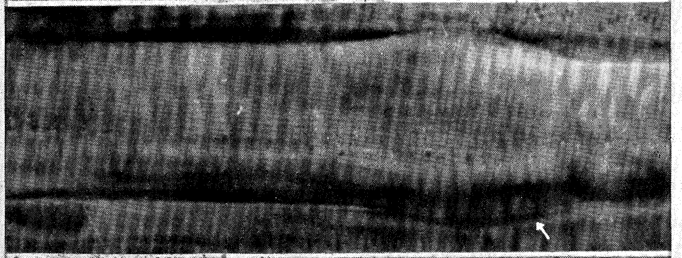

8

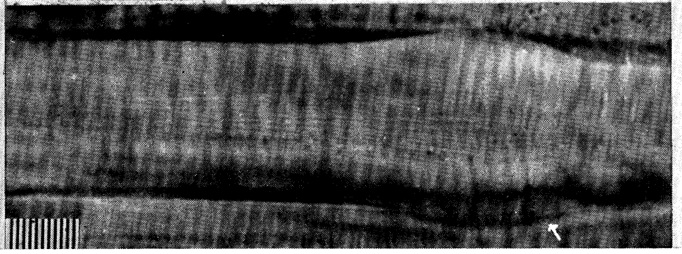

9


10

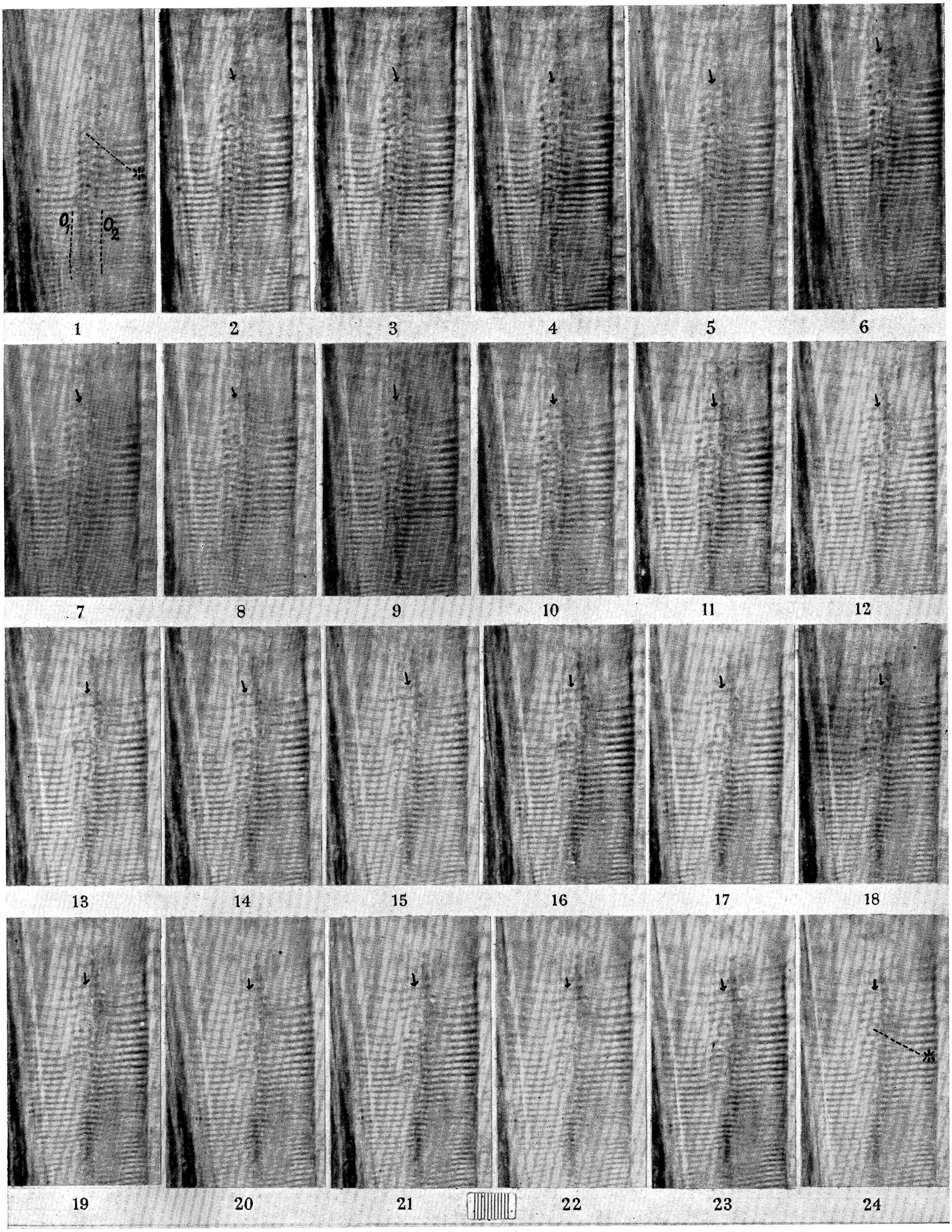

11


12

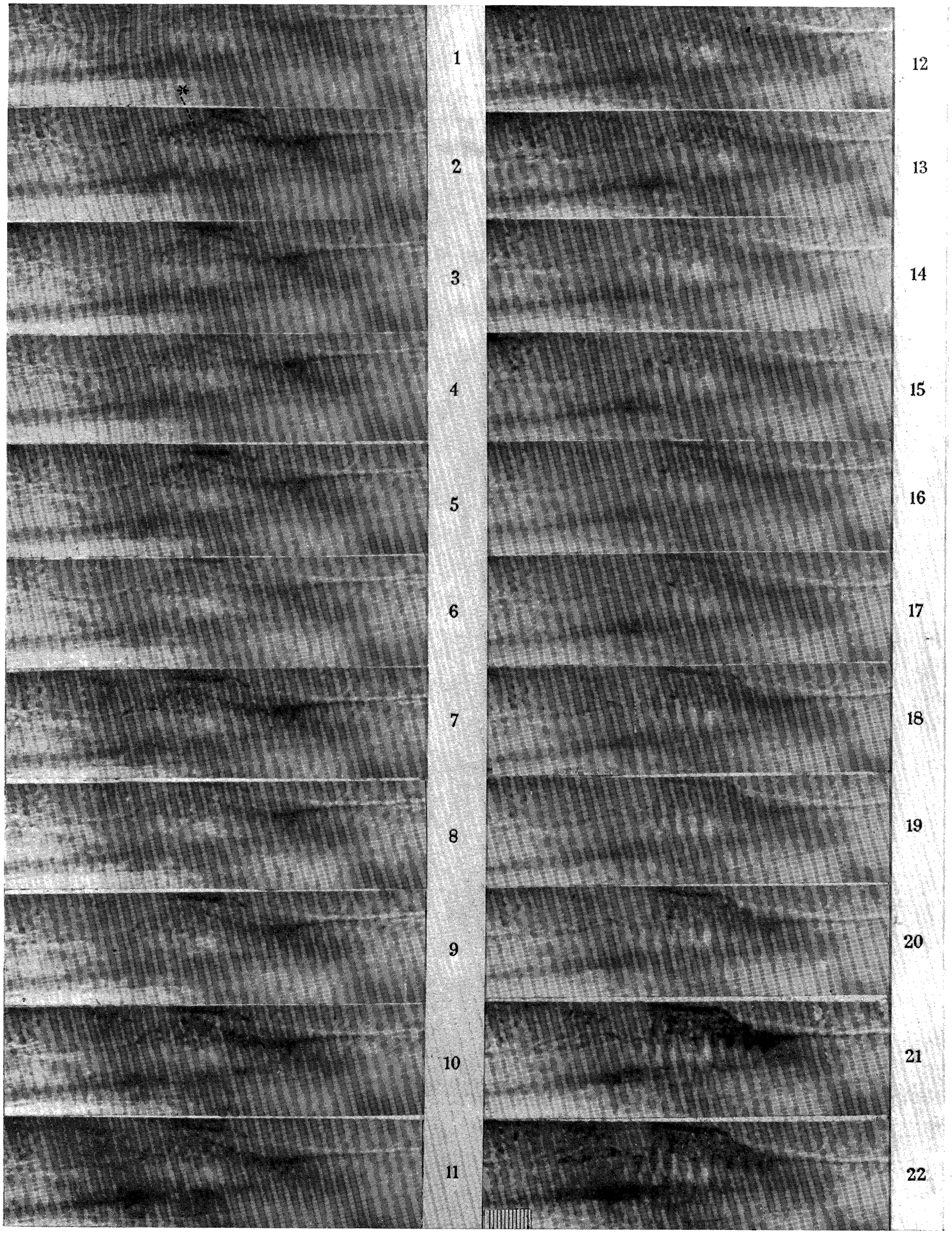

13


14

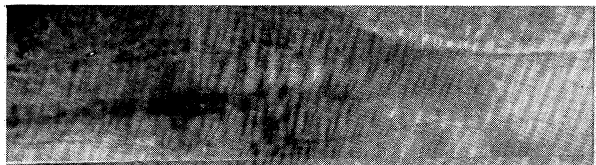
SERIES B. Scale: 1 division = 1μ.

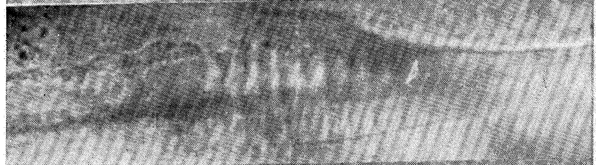


Series C. Scale: 1 division = 1μ.



SERIES D. Origin of contractile wave from nerve ending. 1 division = 1μ.


23

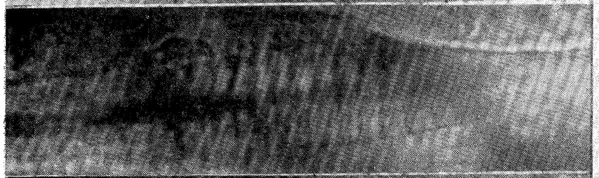

24


25


26


27


28

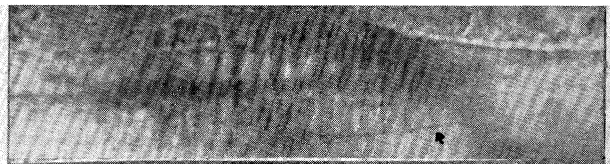

29

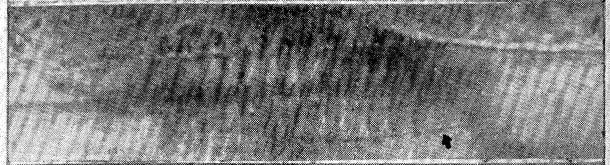

30


31


32

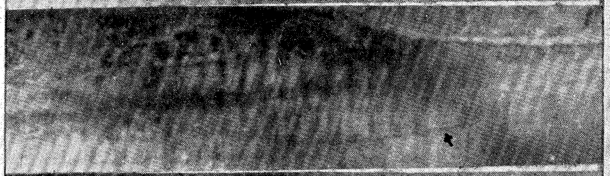

33


34


35


36


37

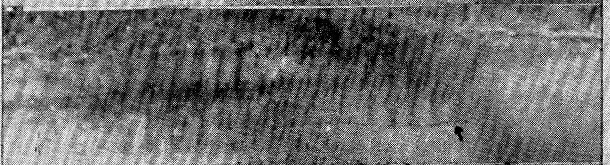

38


39


40


41


42


43

44

Series D (continued)

particular instance, as will be evident shortly, it has had the effect of changing the focus from the 'near' to the 'far' turn of the helicoid (choice of 'near' and 'far' are again quite arbitrary).

In numbers 1, 2 and 3 of the series the most advanced part of the wave, indicated by the arrow, is on the arrow side of the fibre. The criterion for judging the beginning of contraction is reappearance of cross-striation in the wake of the zone of 'striation blurring'. There is necessarily some doubt, for example in 2, as to the exact beginning of the wave; it will, however, be agreed that contraction is more advanced on the arrow side than on the opposite side of the fibre.

In 4 the wave has a uniform front across the fibre; in 5 it is rather more advanced on the original side, while in 6 a swing to the opposite side of the fibre has developed. The reader should note that in 5 and 6 the striations indicated by the asterisk are not contraction bands, but represent incipient striation reversal. In 7 the lead on to the far side of the fibre has become better defined, and is maintained in 8, 9, 10 and 11, but reverts to the original side in 12, 13 and 14.

The reader who attentively examines the photographs will have no difficulty in seeing how advance from one side of the fibre to the other has been brought about. It is plain that even within the contractile wave the axis of the helicoid has been focused—hence, of course, the discontinuity in the cross-bands in the middle of the wave. The slight movement of the fibre, arising from passage of the wave, has had the effect of altering the focal plane with respect to the axis of the helicoid, sufficing, however, to change the focus from its 'near' to its 'far' turn. This is at once shown by the abrupt change in direction of the cross-striation, and therefore of the contractile wave, in numbers 6 and 7. In 12 the focus reverts to the 'near' turn of the helicoid, and so the lead in the wave is again on the initial side.

It must be observed that the alternation of direction shown by the photographs does not, in itself, suffice to demonstrate movement of the contractile wave along the helicoid; it will be necessary to show, in addition, that the contraction band actually spreads across the fibre from the wave tip. The frequency of recording is, of course, too low to disclose this. Yet the need for inferring such spread is evident even in this series. Thus in 10, 11, 12 and 14 the particular striation in which, on one side of the fibre, the lead has been established, is on the other side of the fibre barely emerging from the phase of 'blurred striation'. This is particularly well seen in 11; in 12 contraction of this part of the striation (two to left of arrow) is completed.

The inference from this series is that in the earlier members (up to 5) we have recorded the wave as it is entering the 'near' turn of the helicoid, and is spreading across the fibre; that in 6 to 11, with 'far' turn in focus, we are recording it as it moves into the 'far' turn, spreading therefore in the opposite direction across the fibre; and that in the final members (12 to 14), with 'near' turn refocused, it reverts to its original side and direction.

It may be objected that we are merely recording inward spread from an excitation advancing unevenly along the sarcolemma. This, of course, overlooks the fact that it plainly depends on minute focal adjustment from one turn of the helicoid to the other. The next series (series *C*) will show that the excitation does not seem to spread in from the sarcolemma at all.

SERIES *C* (plate 35)

This series records an extremely slow contractile wave, an advance of only nine cross-striations being covered by 175 exposures. The series is too long to reproduce here in full; we give, instead, number 1 of the series, and 55 to 175, and since the gradation in the series is too small to be visible, we give only every fifth exposure, renumbering them 2 to 24 (1 is, of course, the initial member of the series). In 1 and 24 an identifiable object of the series is indicated by asterisk, and this will enable the reader to gauge the advance of the wave (actually about nine striations). A little behind the tip of the wave the helicoid axis is focused; it is approximately indicated by dotted lines in number 1, the notation having reference to the diagram (figure 1). Within the recorded advance of the wave, the axis of the helicoid is not properly focused, so that we are observing events within a single turn of the helicoid, just alongside the axis. Definition is good, and there is complete 'striation blurring' immediately in advance of the wave, so that the tip of the latter can be identified with fair precision. This series must therefore disclose the much needed information about events along the axis of the helicoid.

We begin with number 9 of the series, the arrow indicating the most advanced tip of the wave, and throughout the series this point will be kept under observation, indicating it by an arrow even when the wave has moved ahead of it (it is actually the fifth striation beyond the object indicated by asterisk in numbers 1 and 24, but of course approaches nearer to the object as contraction develops).

If we retrace it towards 2 of the series, we find that it arises by fusion of two separate elements, and is therefore a true contraction band arising by 'striation reversal'. If we follow it into the later members of the series, we find it spreading progressively across the striation to the left margin of the fibre (events to the right of the axis are partially obscured by the main column of sarcoplasm veering in that direction—see numbers 20 to 24).

The contraction bands that have meantime developed behind the one indicated show the same lateral spread from an initial fragment that develops in the middle of the fibre, and this is evident also in those that later develop in front of it; for instance, the contraction band that develops immediately in advance of the one indicated by arrow comes into being at about 14 of the series, and is completed at about 22. The next contraction band appears at about 17, but is not fully developed by the end of the recording.

Building up of the contraction band from the axial portion of the helicoid is what we should have expected on the hypothesis of spread of excitation along the helicoid, and this we do now actually observe. A surprising feature is that contraction at the axis may sometimes be a few striations in advance of the wave front; this is certainly so in the earlier members of the series, though not in the later ones.

It is pertinent to ask why the effect brought out by series *C* is not also evident in series *B*, since the focal levels are almost the same. Clearly the infrequent recording of that series could only by accident disclose a recognizable axial contraction in advance of the main wave. This accident has occurred in number 10 of that series; note a V-shaped axial contraction band developing in the striation immediately in front of that indicated by the arrow; note the still 'blurred striation' to the sides of it.

The final members of series *C* (25, 26), are comparable with those recorded by Hürthle, i.e. the most advanced contraction band occupies a complete cross-striation, and there is scarcely any evidence of contraction in front of this; but plainly the 'in-step' analogy which we have drawn above, is useful now only in so far as it emphasizes the co-ordinated contraction of adjacent fibrils, in contrast to irregular scatter along the fibre.

SERIES *D* (plates 36 and 37)

This series is the only recording that we have so far obtained of the start of the slow contractile wave at the motor-nerve ending. The fibre is an unusually narrow one; the nerve ending—or, more correctly the Doyère eminence with its granular sole protoplasm—is seen in profile (indicated by asterisk in number 2 of series). In this series a shadow, caused by a bristle on the chitin, interferes with visibility though not to the extent of obscuring the field; indeed, attentive examination can extract from the series all the information that we need.

The record is that of a wave arising spontaneously at the motor-nerve ending, not by electric stimulation. It has been our experience that the slow waves that pass at intervals along a fibre tend to arise at a definite locus on the fibre. Whether this is always at the site of a nerve ending is uncertain, for such endings are not readily seen except in profile. In this particular instance the nerve ending was at once evident, and as the waves appeared at intervals of about 10 s, the recording could be timed within a short interval of the beginning of the wave.

In 1 of the series we see the normal resting fibre, in which the Doyère ending spans six cross-striations. In 8 these six cross-striations have disappeared though the adjacent striations are still visible. This is the usual 'striation blurring' preceding the development of the contraction band; the blurring is in progress in 3 to 7, the confused picture at the nerve ending being occasioned, not by the shadow across the field, but by normal 'striation reversal'.

In 16 the cross-striation has reappeared, but in the form of contraction bands (incidentally the helicoid, vaguely suggested in 1, is here plainly evident); the first indication of the contraction band spreading across the fibre from the nerve ending is in 8 (or perhaps even 7). Meanwhile blurring of the hitherto intact striation to either side of the incipient contractile wave has begun, leading to the usual confused appearance immediately in advance of the wave tip, out of which a new contraction band then develops (18 to 21).

Owing to its importance for the problem at hand, we give this series at some length (up to number 44), covering the period from the inception of the wave to the time when it divides into two parts which then advance in opposite directions along the fibre. Several points are to be noted in this series: (i) everywhere we find the 'blurred striation' in advance of the wave tip, the striation later recovering as a contraction band; (ii) in 40 and 41, where the wave is splitting into two, there is a lag in the disappearance of the contractile band on the side of the fibre opposite the nerve ending, which was also the last part of the striation actually to pass into contraction; (iii) the thirty exposures, 16 to 44, cover an advance of the wave, to the right of the nerve ending, by only four or five cross-striations. The wave is therefore a slow one, and the record should therefore bring out features dependent on relatively fast recording. Consider, in 44, the contraction band indicated by the arrow, and located just behind the most advanced tip of the wave; this band, indicated also by arrows in preceding members of the series, can be retraced to a minute fragment that develops some distance from the margin of the fibre in 32; in 34 it has spread to the near margin of the fibre, but does not reach the opposite margin till near the end of the series. This is the effect that we have recorded in greater detail in series *C*.

The importance of series *D* for the present work lies in the fact that it discloses the manner in which the contractile wave is initially built up before it begins to move along the fibre. We should have expected, in order to account for Hürthle's photographs, that the contractile wave would first spread across the fibre before it moved appreciably along it, and this we do, in fact, find (14 to 16). Conduction along the fibrils or along the sarcolemma could not, we think, produce the result; on the hypothesis of conduction along the helicoid we should expect it. We should also expect, on this hypothesis, that the most advanced tip of the wave could at times be on the side of the fibre opposite the nerve ending; in the later members of the series (26 to 35) this is plainly the case.

The reader will note also, from about 23 onwards, an abrupt change in direction of the striation on the right side of the nerve-ending, this being attended, in places (38 to 44), by a clear vernier effect (indicated by numbers in 44). Plainly the focus is intersecting the axis of the helicoid. Initially the wave spread from the nerve ending across the fibre; it is now spreading, on the opposite turn of the helicoid, back to the initial side of the fibre. But is not this movement along the helicoid?

Discussion

The foregoing results are in essential agreement with the evidence contained in Hürthle's photographs, to which reference has repeatedly been made above: the slow contractile wave is propagated along the fibre as an all-or-none process, in the sense that the whole of the fibril bundle is involved, and in its propagation co-ordination is exerted on the contraction of its constituent fibrils, this being in some way determined by the pattern of the cross-striation. We cannot easily escape the conviction that transmission of excitation, which must be at the basis of this co-ordination, is the property of some definite component of the muscle fibre; and the hypothesis which we now advance is that the structure in question is the system of cross-membranes (see also Tiegs, 1934).

In an earlier paper (Tiegs 1932), photographic evidence was given that at the motor-nerve ending the hypolemmal nerve twigs are intimately associated with the *Z*-membranes of the fibre. This statement was made, not on the basis of general experience with motor endings, but on occasional examples in which the relation was evident. Certainly in most motor endings, prepared by conventional gold or silver methods, we do not see this connexion; but neither, in such preparations, do we usually see the cross-membranes. Further examination of this point, using methods that are capable of bringing out the cross-membranes consistently, is needed; meantime it may be noted that Beams & Evans (1953), using electron micrographs of sectioned motor endings, have made a similar claim.

If the point could be generally established, it would strengthen the hypothesis that it is the function of the cross-membranes to transmit the excitation within the interior of the fibre. The evidence given above is consistent with passage of excitation along helicoidally disposed cross-membranes, and seems to give meaning both to the existence of cross-striation, and to its helicoidal arrangement.

There are, however, objections which it would be futile to overlook. Barer (1948) has described observations on chironomid larvae, indicating that the slow contractile wave may not have the all-or-none character above referred to, but may pass 'down only one side of the fibre', involving therefore only part of the fibril bundle, and presumably implying conduction of excitation along those fibrils that are actually involved in contraction. The observation is based on a remarkable, and indeed unique, muscle fibre, arising from within each posterior proleg, within which it branches into two, the medial branches crossing to the opposite side, and uniting there with the uncrossed branch of that side; a slow wave, passing along the uncrossed component, is said to be restricted to that side of the complex fibre even beyond the point of junction with the opposite crossed branch. In *Chironomus duplex*, which is the only local species available to us, we can recognize the structure described by Barer, but we find that it is composed actually of four fibres, of which the middle two, described as crossing, are joined to one another, and then separate again. But we must concede that Barer's statement, if established for another species, could not be reconciled with the all-or-none interpretation that the helicoid theory requires.

A second difficulty is the existence of types of fibre in which the cross-striation lacks the helicoid pattern. Reference is made to these in the foregoing paper (Tiegs 1955). We cannot concede that the existence of such fibres must invalidate the hypothesis, for it is plain that they could not give slow contractile waves having the character of those that we have examined. Indeed, we do not know whether they give slow contractile waves at all. But the slow waves, we have supposed, though without adequate proof, arise by the retardation of the normal twitch wave; and we are left with the difficulty that the path of excitation in different types of fibre may be basically different. Even then we cannot agree that this inevitably commits us to conduction along the sarcolemma in all types of striated fibre, for there are some in which the known innervation directly contradicts this. A striking example is in the flight muscles of Diptera, in which, as described in the foregoing paper, the motor-nerve fibres penetrate deeply into the interior of the muscle fibres, and have no relation whatever to the sarcolemma. The scanty evidence suggests, rather, that in structurally different types of fibre, the conducting system may be quite different, and that the helicoidal system, which we find in vertebrate muscle, and in the coarser arthropod fibres, is only one of them. In what relation conduction along such a helicoidal membrane stands to the action potential propagated along the sarcolemma is obscure.

Finally, it is necessary to keep in mind that in vertebrate muscle, and at least in some, possibly all, arthropod striated fibres, there are actually two systems of cross-membranes—*Z* and *M*. We are at present even without a hint as to the meaning of this.

REFERENCES

Barer, R. 1948 *Biol. Rev.* **23**, 159.

Beams, H. W. & Evans, T. C. 1953 *Proc. Soc. Exp. Biol., N.Y.*, **82**, 344.

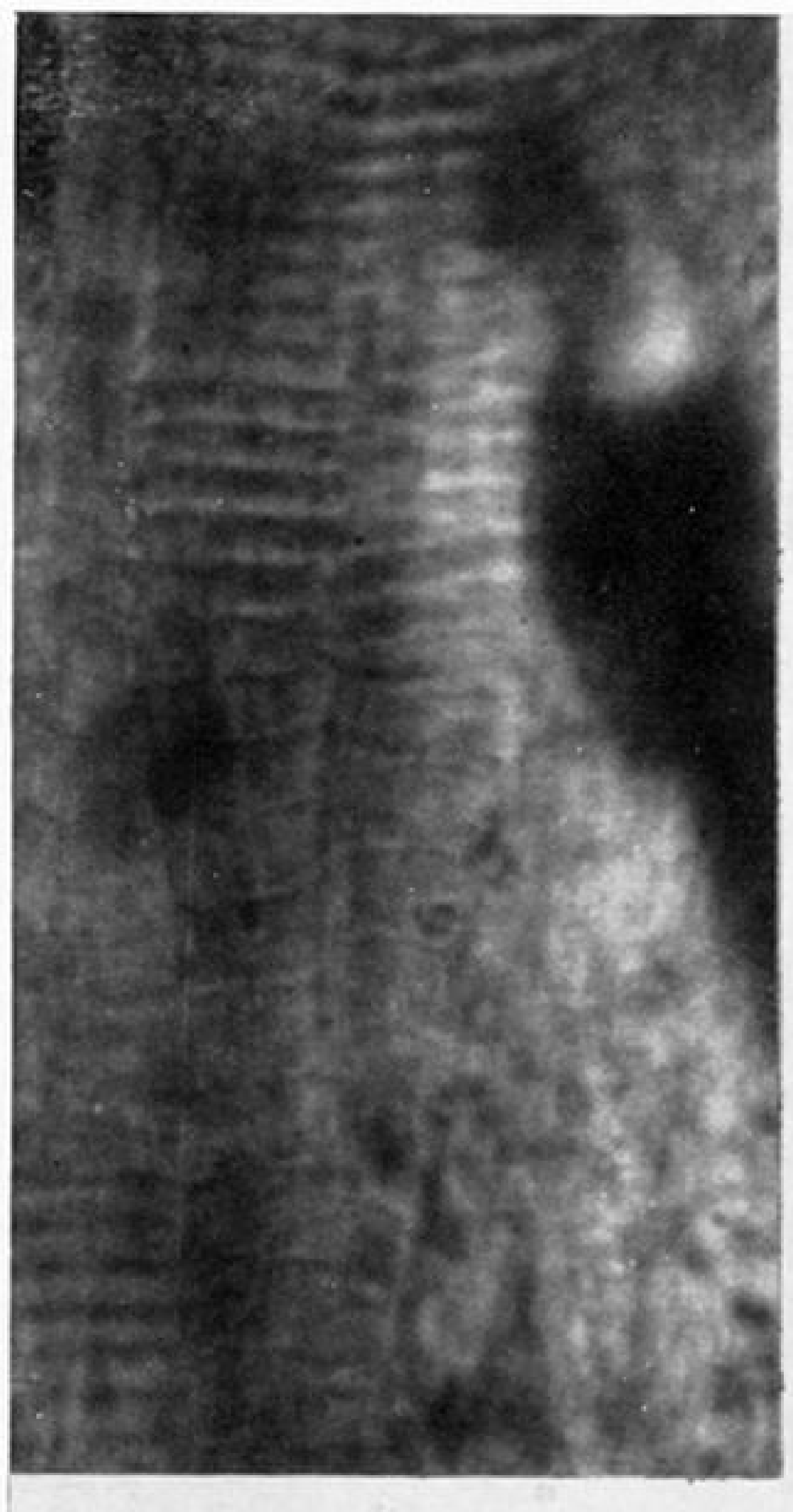
Hürthle, K. 1909 *Pflüg. Arch. ges. Physiol.* **126**, 1.

Rollet, A. 1891 *Arch. mikr. Anat.* **37**, 654.

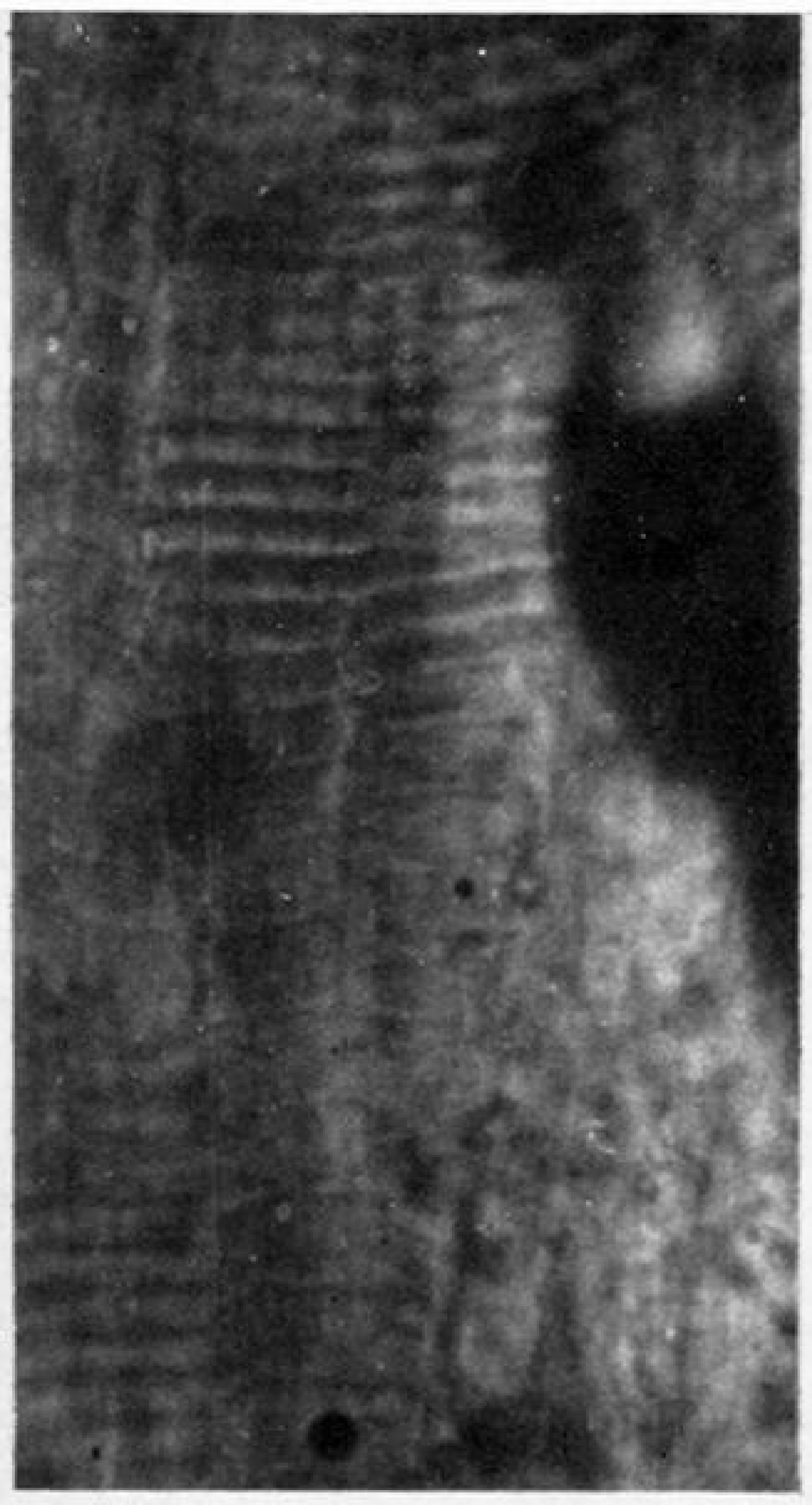
Tiegs, O. W. 1932 *J. Anat., Camb.*, **66**, 300.

Tiegs, O. W. 1934 *Proc. Roy. Soc.* B, **116**, 38.

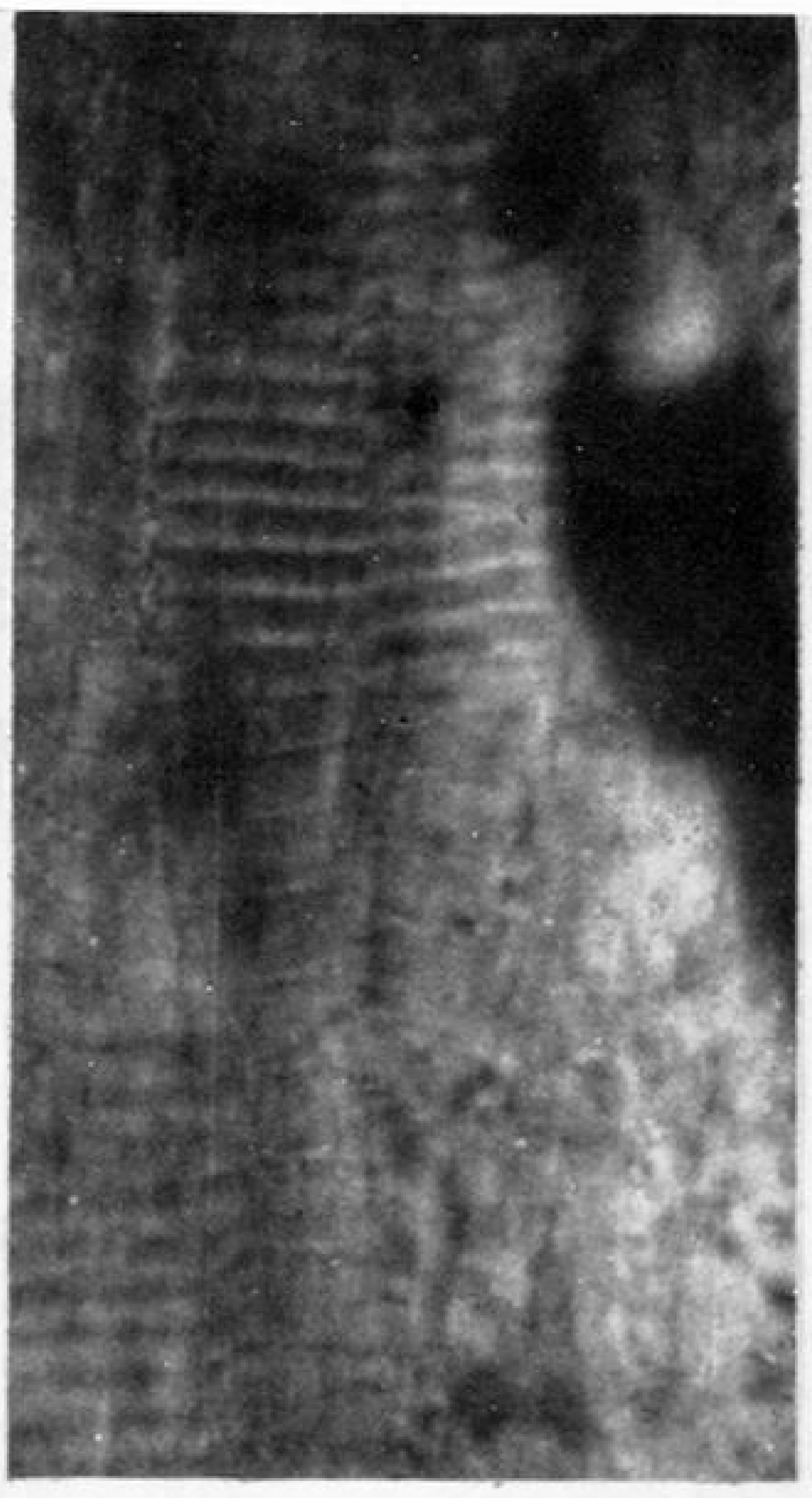
Tiegs, O. W. 1955 *Phil. Trans.* B, **238**, 221.



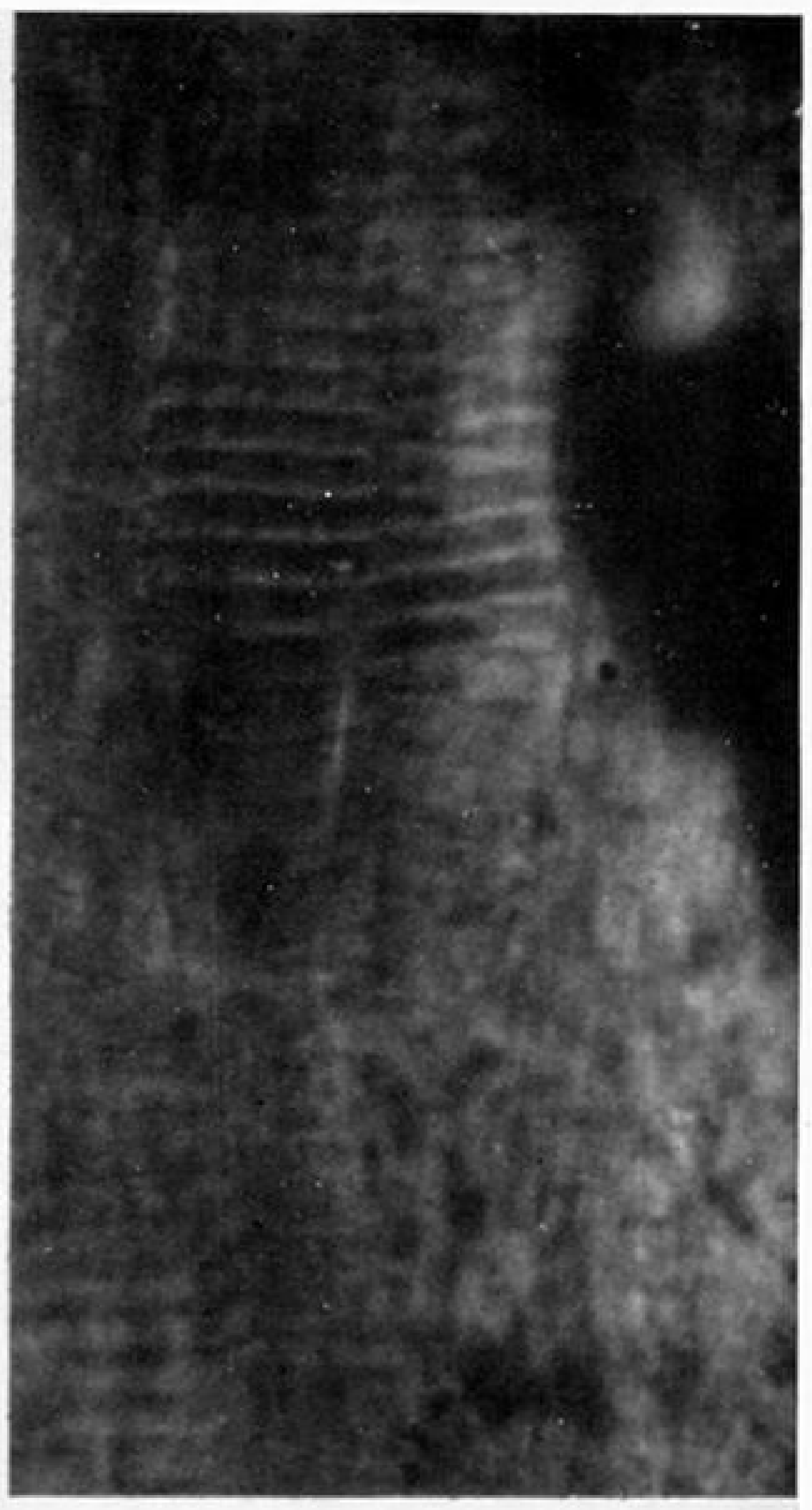
1



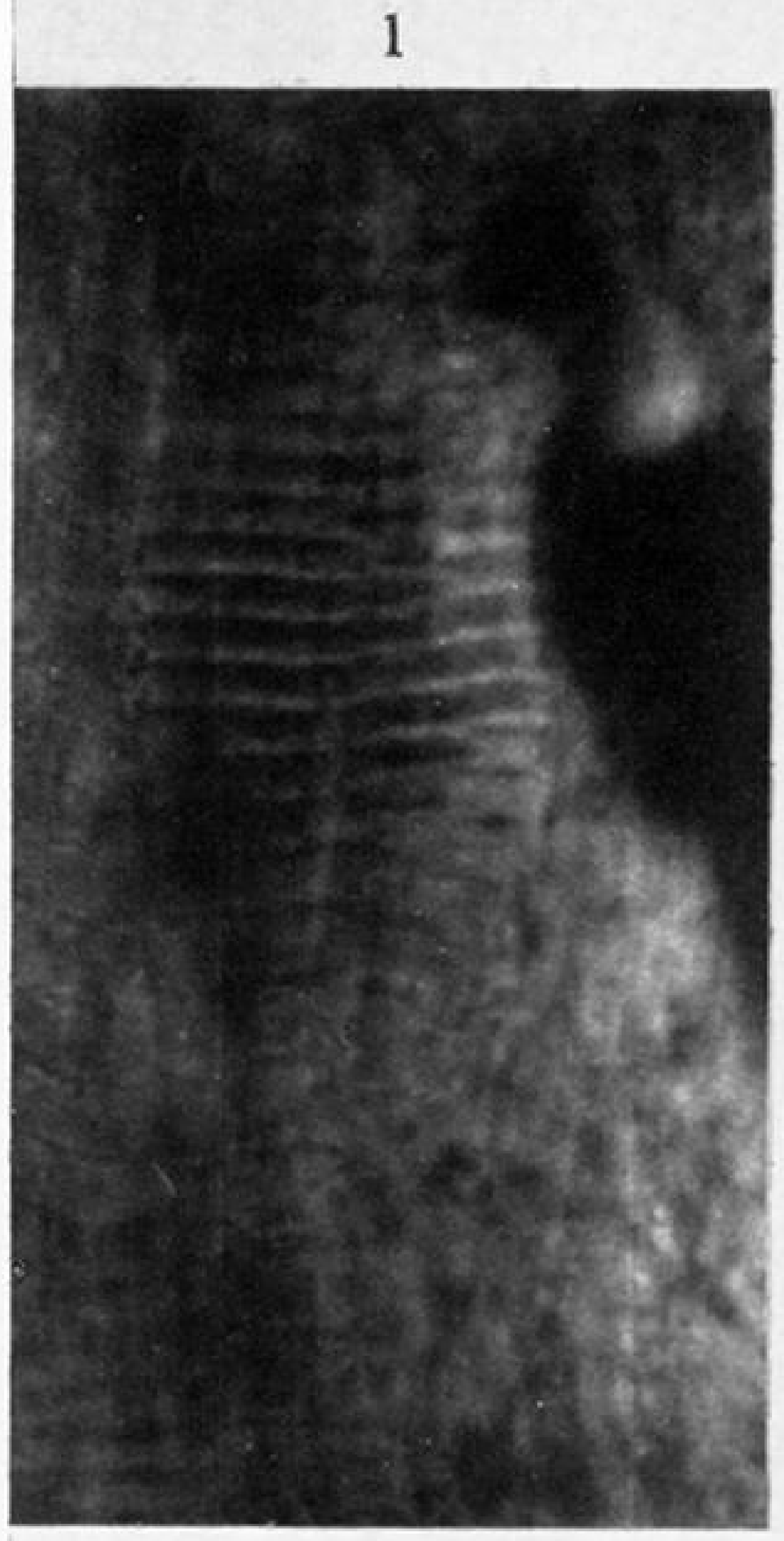
2



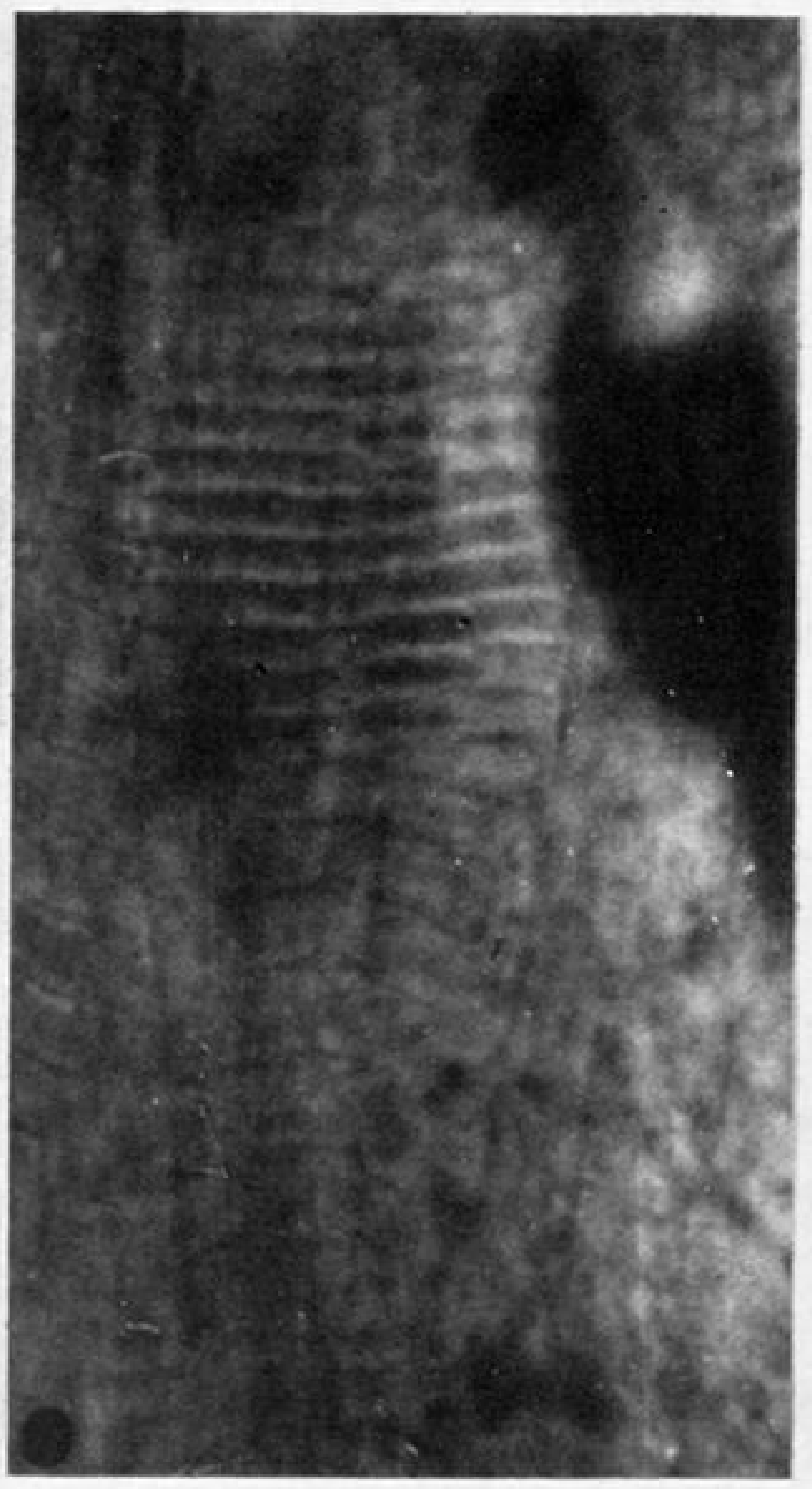
3



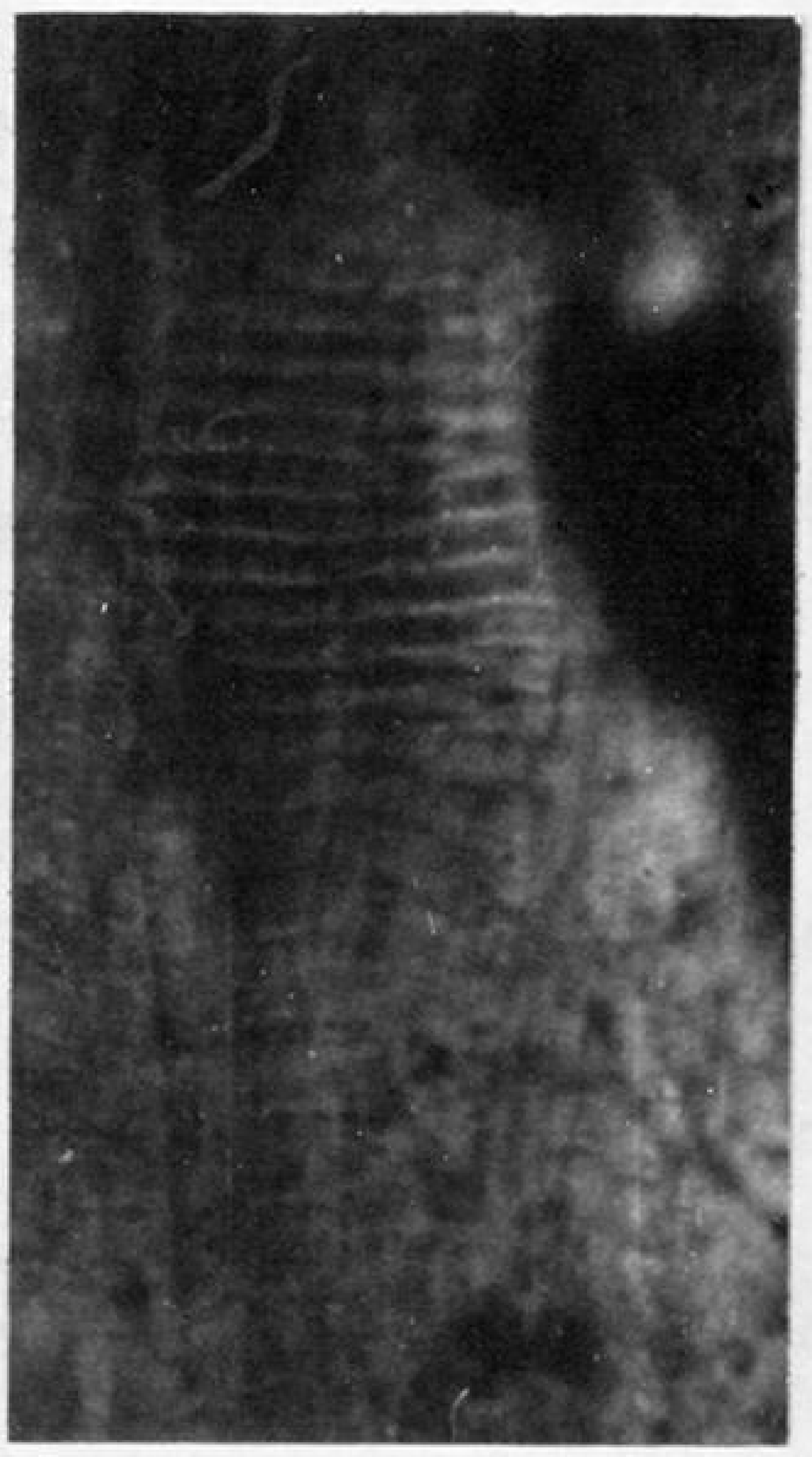
4



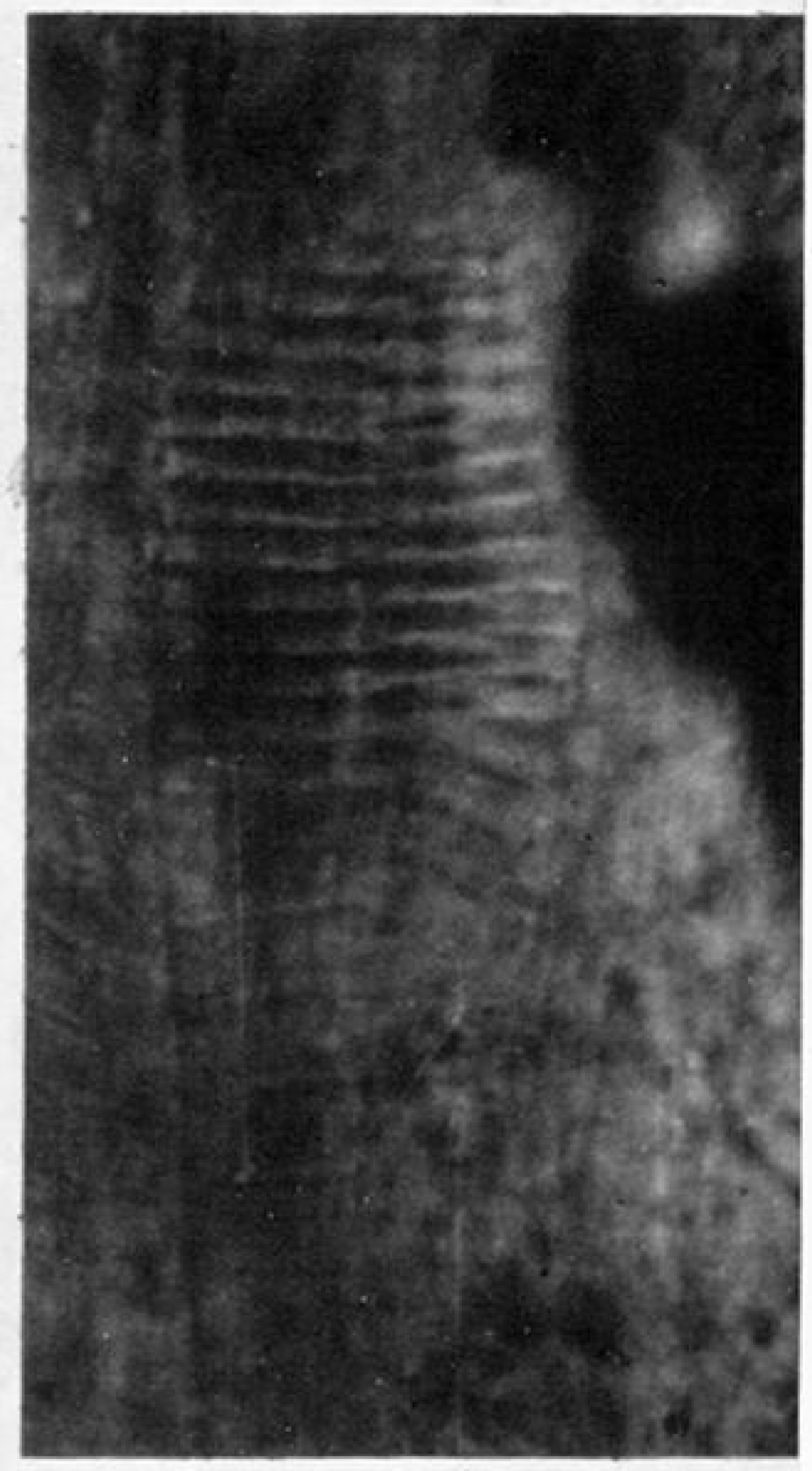
5



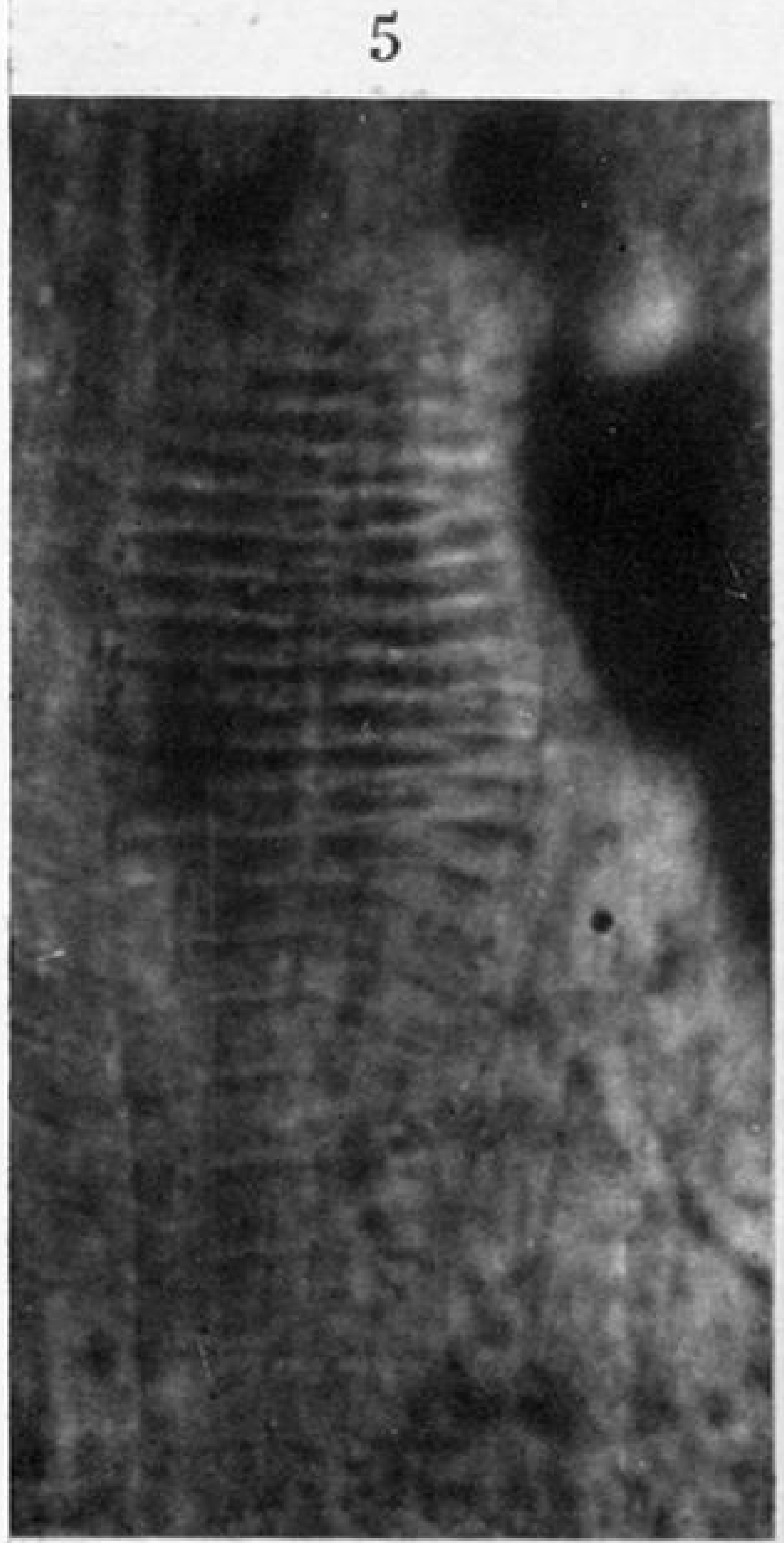
6



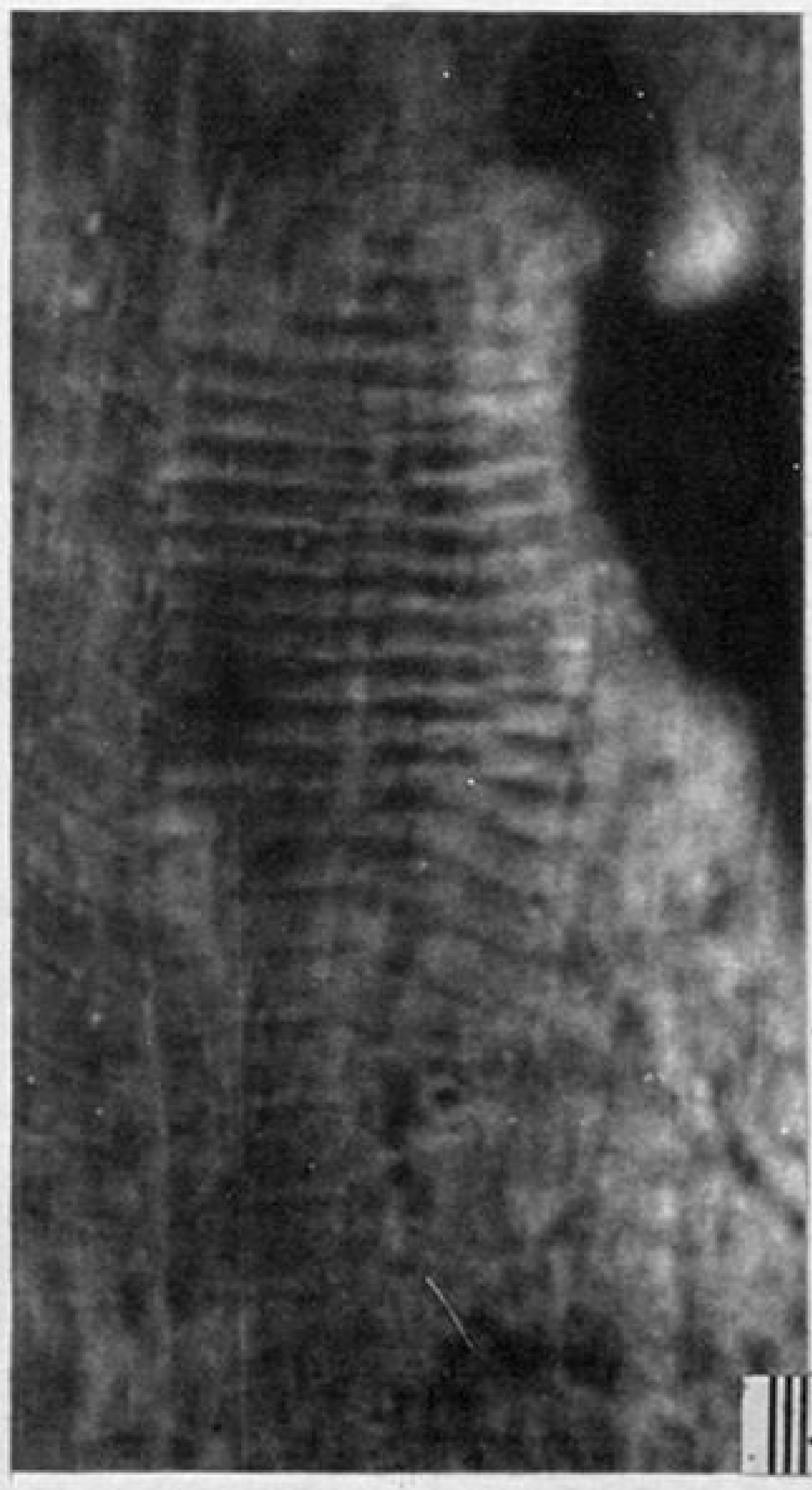
7



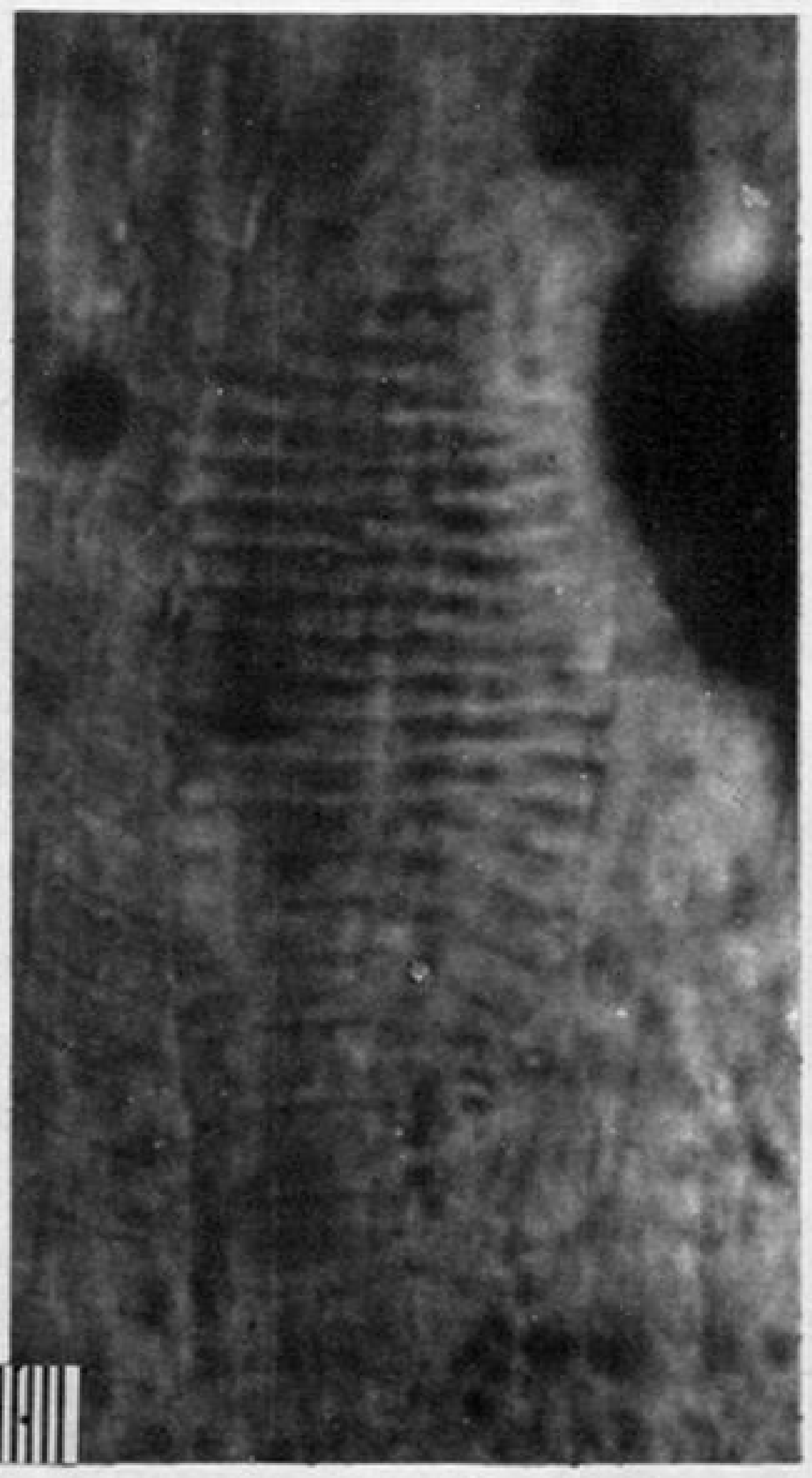
8



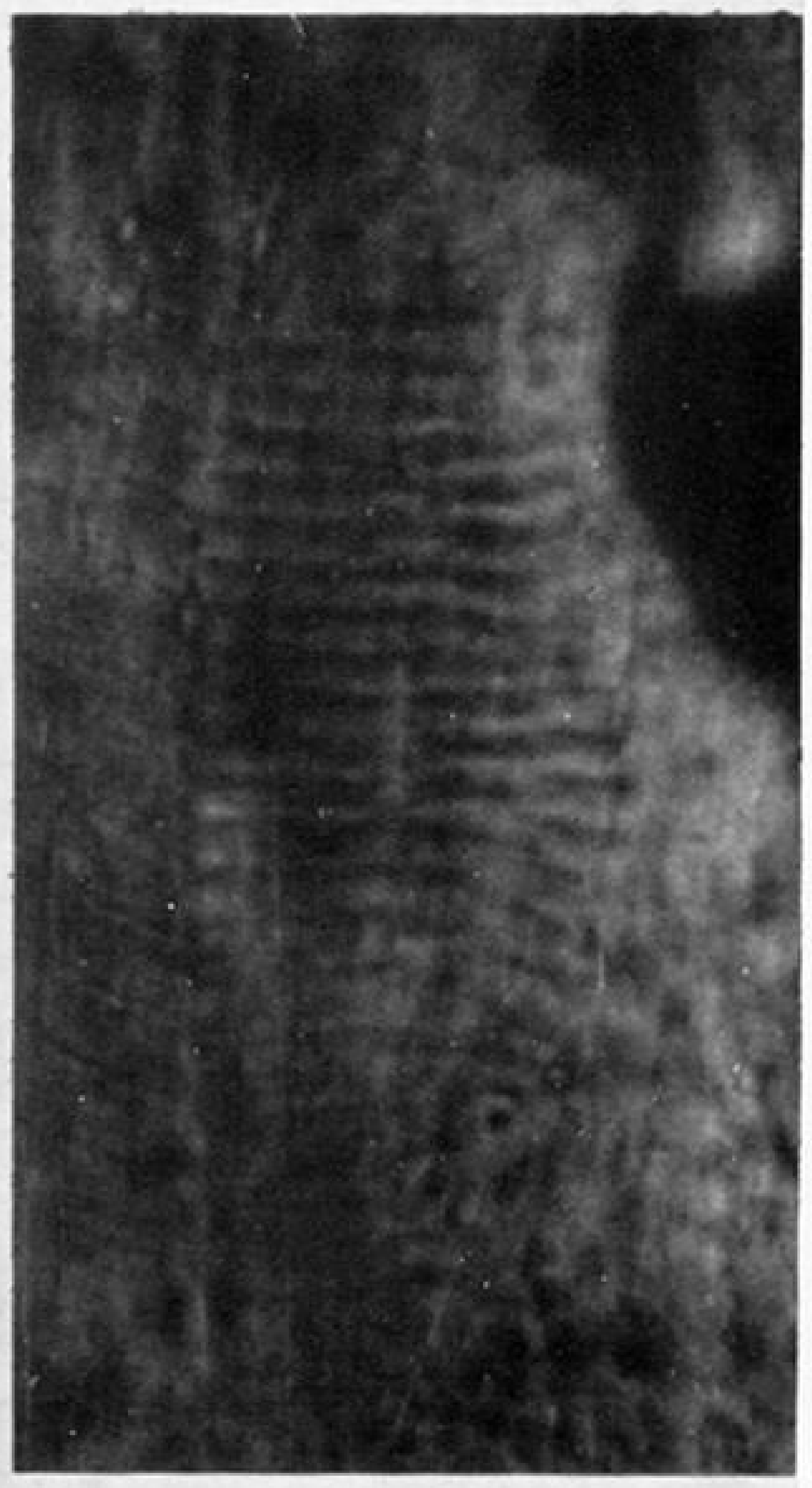
9



10

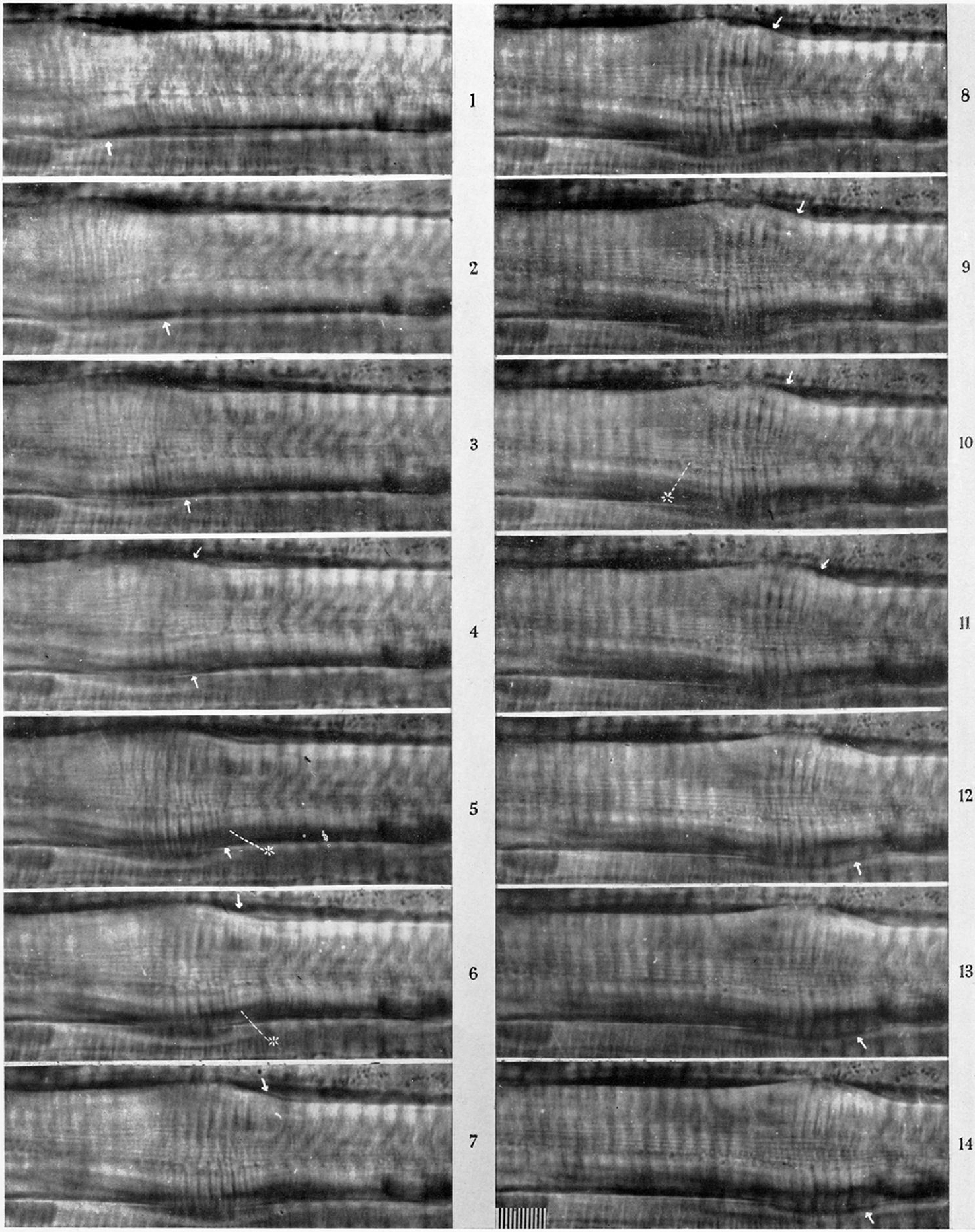


11

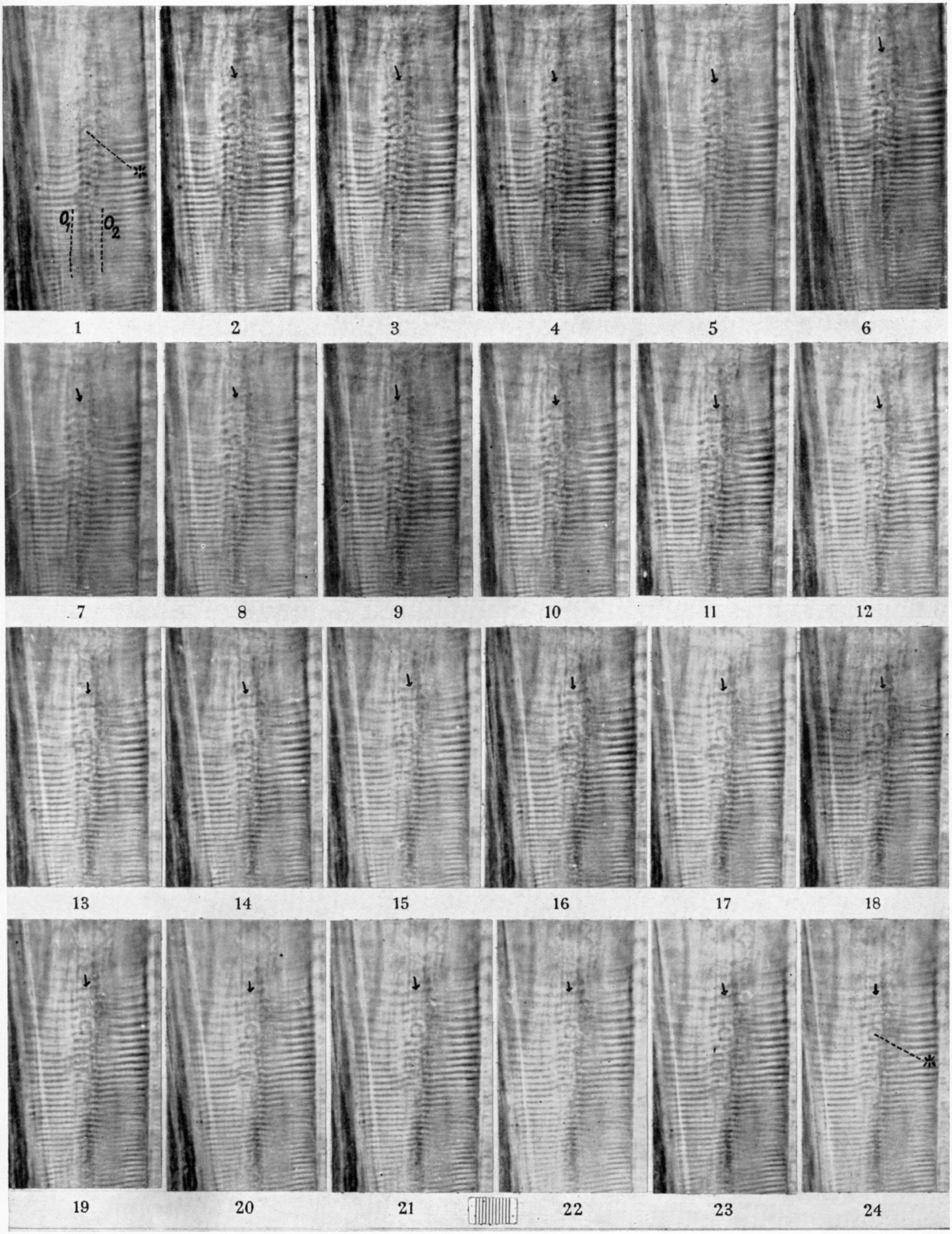


12

Series A. Scale: 1 division = 1μ.



Series B. Scale: 1 division = 1μ.



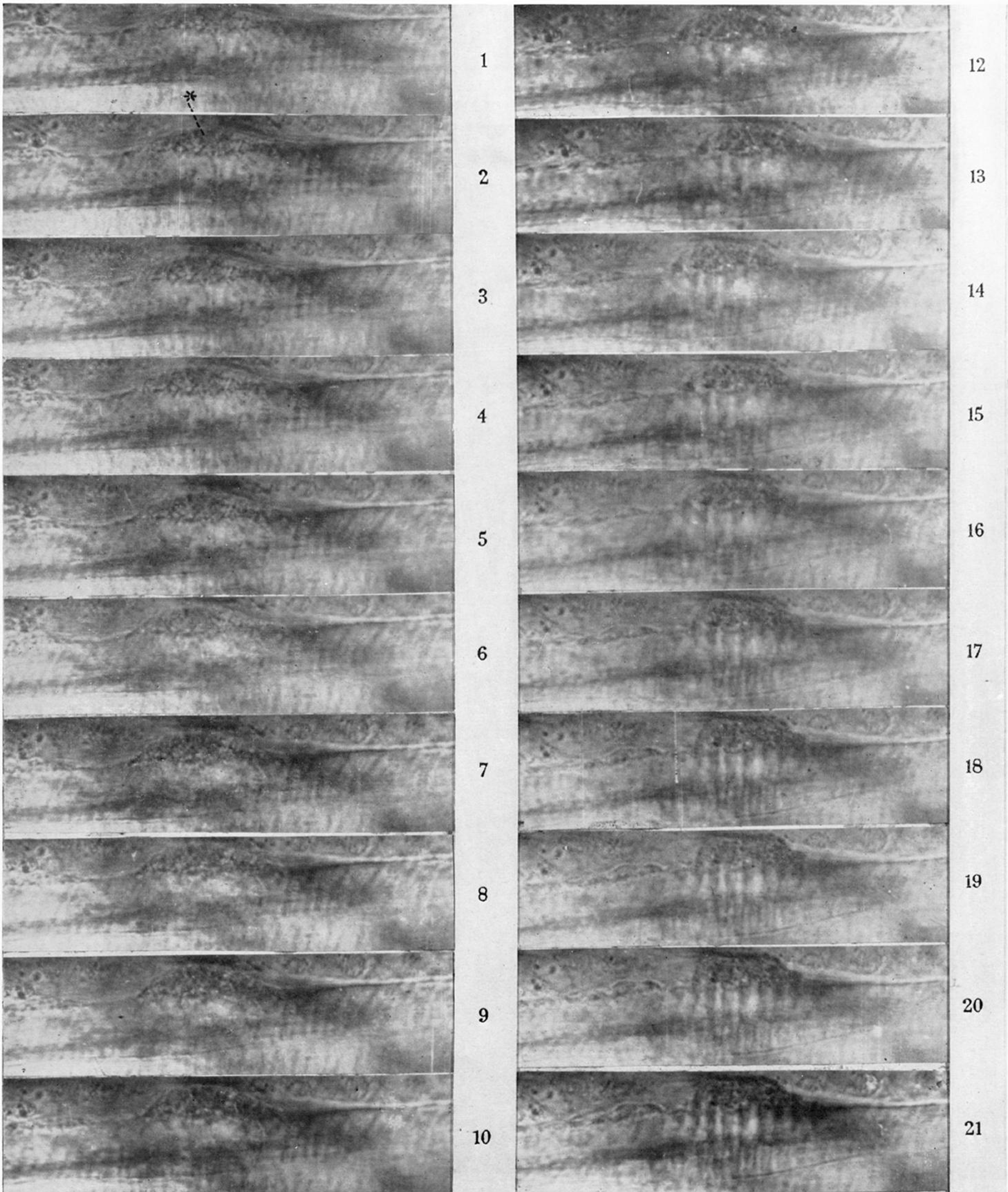
1 2 3 4 5 6

7 8 9 10 11 12

13 14 15 16 17 18

19 20 21 22 23 24

Series C. Scale: 1 division = 1μ.



SERIES D. Origin of contractile wave from nerve ending. 1 division = 1μ.

23

24

25

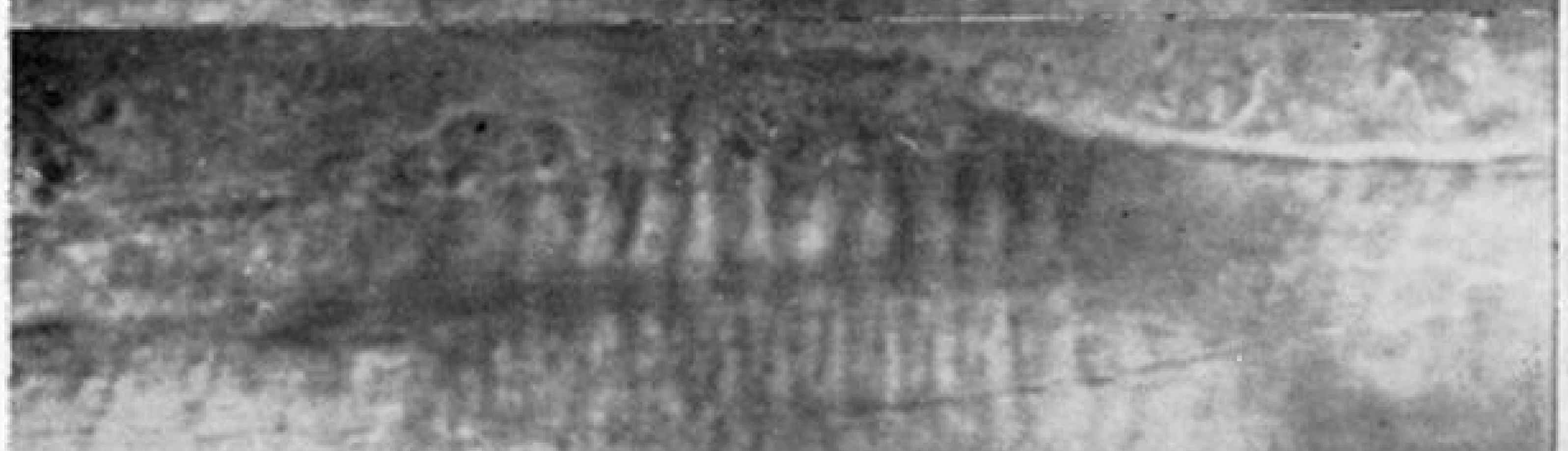
26

27

28

29

30

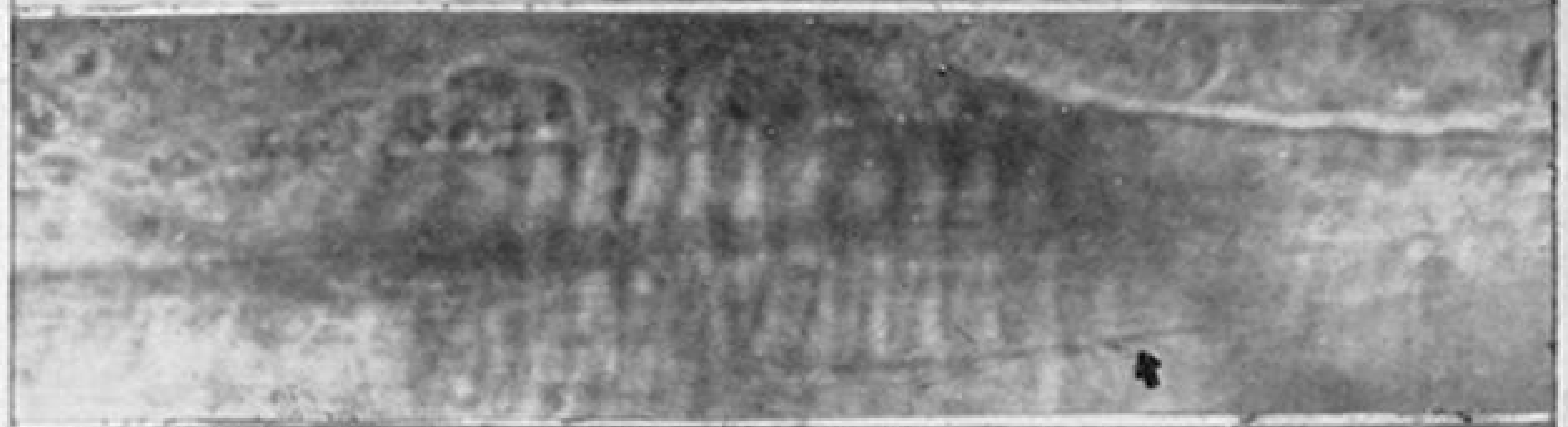


31

32

33

34



35

36

37

38

39

40

41

42

43

44

SERIES D (continued)