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The site and state of myosin in intestinal smooth muscle

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[Plates 25 and 26]

The longitudinal layer of the guinea-pig ileum represents a highly advantageous specimen for the study of vertebrate smooth muscle structure. In this muscle we regularly observed thick filaments, consisting presumably of myosin, in longitudinal as well as in cross-sections, if the samples were fixed at constant length, i.e. standing under mechanical tension. Thick filaments were regularly present also in muscles relaxed by atropine. On the other hand, thick filaments were absent in many cases in slack muscles in K^+ contracture. As a consequence, we regard myosin filaments as regular constituents of smooth muscle, independently of the functional state. Their absence in electronmicrographs taken from slack muscles seems an artefact due to processing. We observed the same artefact in bee-wing muscle, i.e. in a striated muscle, too. This fact indicates the importance of mechanical tension and polymer crystallization in the survival of myosin filaments. On the basis of a recent work of Ladik, Biczó and Garamvölgyi we discuss how tension may be exerted on the myosin filaments of the resting muscle. Anyway, the sliding model seems valid also for vertebrate smooth muscle, without any substantial modification.

Introduction

It seems now well established that the contractile system of vertebrate smooth muscle is also based on a sliding mechanism of two different sets of filaments. This means that the thick filaments consisting presumably of myosin represent regular structural constituents of the smooth muscle cells. This conclusion can be drawn from the work of at least four different laboratories and represents the result of a very extensive discussion lasting several years. Instead of reviewing the details of this discussion we shortly recapitulate our own work performed on this subject.

Our work started essentially in 1969, when one of us (E. S. V.) learned of the preparation of a highly advantageous specimen of smooth muscle at the Department of Pharmacology, Oxford. This is the longitudinal layer of the guinea-pig ileum. This muscle consists of a few layers of cells only, which are perfectly parallel. Pharmacological experiments have shown that this kind of muscle is extremely permeable. This is why we considerd this muscle to be suitable for electron microscopic investigations. We assumed that the fixatives will readily penetrate this muscle allowing no time to destroy the *in vivo* structure. On the other hand, we wanted to exploit the advantage of a rather parallel arrangement of cells in order to study the effect of tension on the system of filaments. Since a stretch exerted on a muscle strip is perfectly axial for all cells, we expected a uniform result.

Our observations were first presented at the meeting of the Hungarian Physiological Society, June 1970, and they were published in detail in January 1971 (Garamvölgyi, Vizi & Knoll 1971).

Materials and Methods

The muscle strips were usually fixed at constant length, from the slack length up to an elongation of 50 %. Other strips were fixed in their slack state. All muscles were prefixed in glutaraldehyde, postfixed in osmium tetroxide, block-stained in uranyl acetate, embedded in araldite and contrasted by uranyl acetate and lead citrate.

Some muscles were pretreated in a Krebs–Ringer solution containing 0.25 % atropine sulphate. The cessation of mechanical activity was indicated by a transducer. In other muscles contracture has been evoked by potassium-rich Krebs–Ringer solution.

Observations

In muscles fixed at a constant length higher than their excised length, we observed thick filaments with an astonishing regularity. Among several hundred cells there were only a few in which no thick filaments were present. These cells were most probably torn.

It is to be pointed out that the majority of samples were not seriously stretched. For demonstration we used exclusively the moderately stretched muscles (figures 1, 2) in which the individual thick filaments can be distinguished more readily, due to the greater lateral separation. In muscles fixed at their excised length (to avoid any stretch), there were not an appreciable number of thick filaments.

In the meantime Kelly & Rice (1969) arrived at a similar result on guinea-pig taenia coli; they attributed, however, the existence of thick filaments in stretched muscles to the activation induced by stretch. In order to check this very important statement muscles were relaxed by atropine when they were contracting isometrically, in a stretched state. In these relaxed muscles we found thick filaments as well (figures 3 and 4) (Garamvölgyi *et al.* 1971). Contrary to this, in relaxed slack muscles we failed to demonstrate the regular occurrence of thick filaments, in total agreement with Kelly & Rice (1969). This fact indicates that the existence of thick filaments is independent of the functional state, but mechanical tension is necessary for their preservation.

Essentially the same fact was shown in muscles depolarized in solutions containing an increased concentration of K-ions. Muscles prevented from shortening always contained thick filaments (figure 5), while in muscles shortened freely there is usually one single kind of thin filaments (figure 6) (Vizi & Garamvölgyi, unpublished). It is to be noted that we do not attribute any significance to the lack of thick filaments in slack muscles of either functional state. According to our opinion both kinds of filaments belong to the normal structure of smooth muscle as well.

Description of plate 25

Figure 1. Longitudinal section of a cell of the longitudinal layer of guinea pig ileum fixed at constant length. The thick filaments are clearly visible.

Figure 2. Cross-section of a cell of guinea-pig intestinal muscle fixed at constant length.

Figure 3. Cross-section of a cell as in figure 2 but pretreated by atropine.

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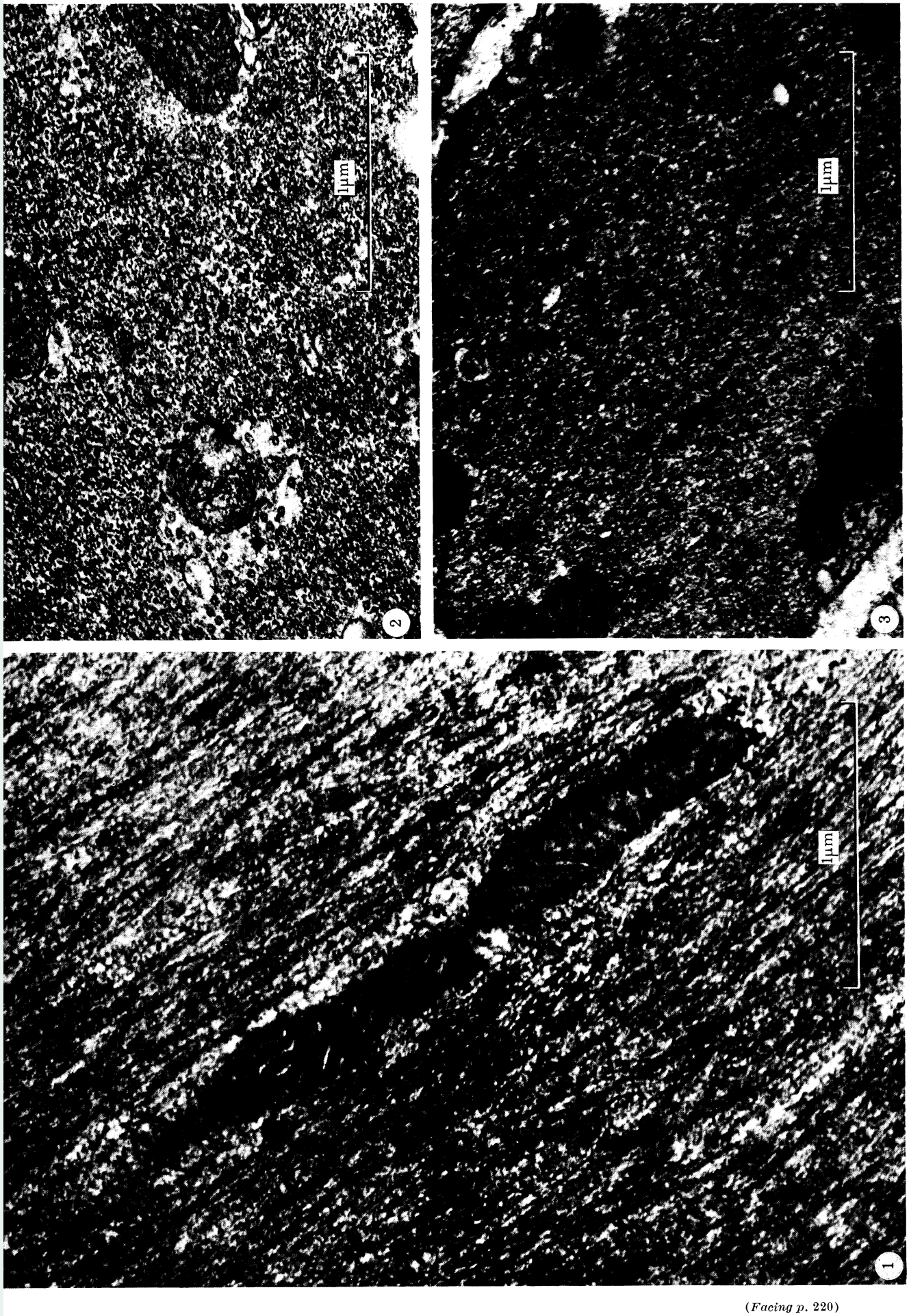

FIGURES 1 TO 3. For legends see facing page.

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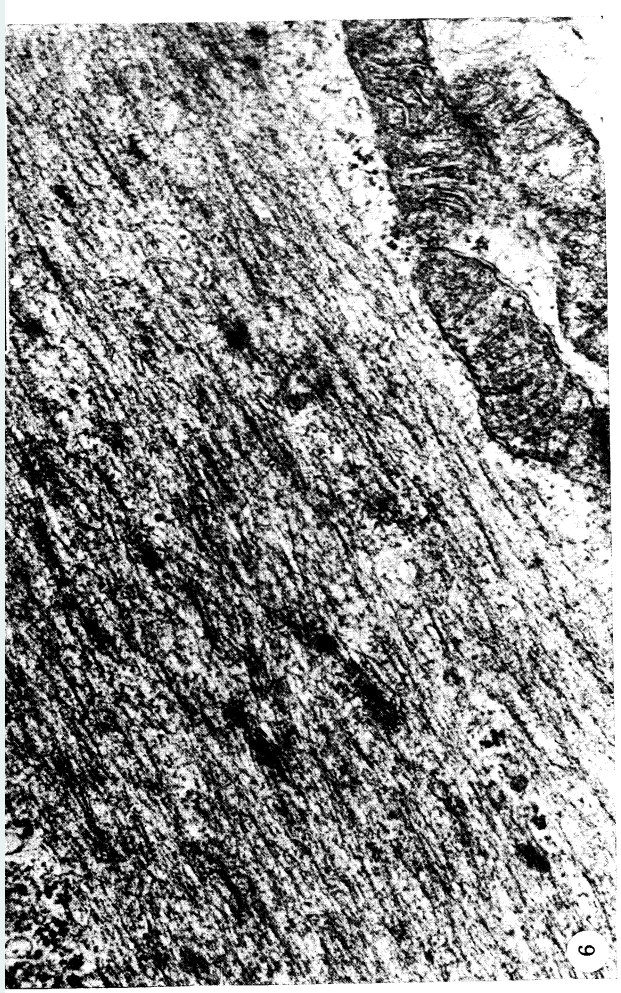

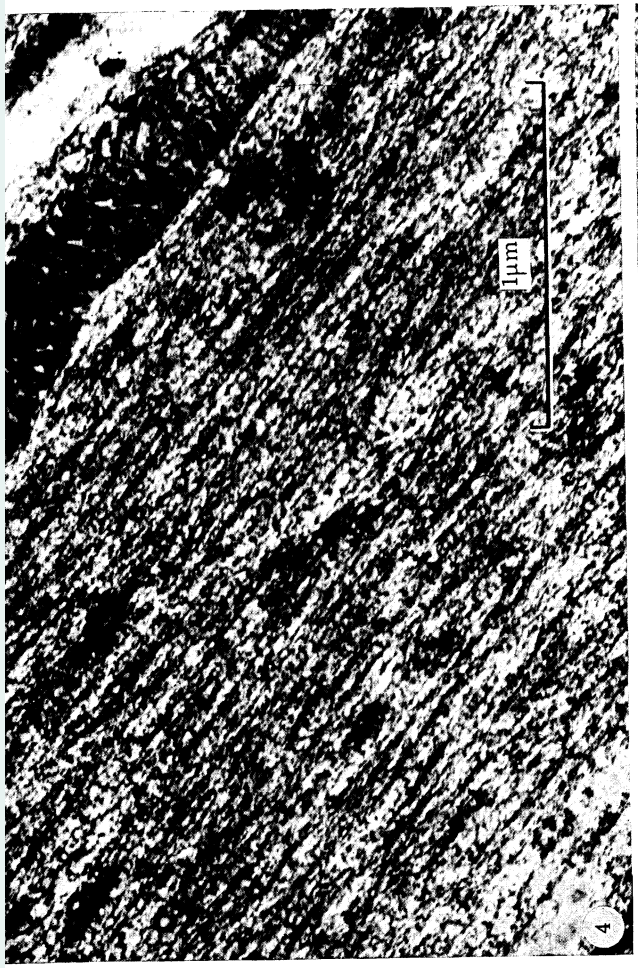

FIGURES 4 TO 7. For legends see facing page.

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Discussion

In our first detailed paper on smooth muscle (Garamvölgyi *et al.* 1971) we wrote: 'We assume that myosin filaments are more regular constituents of vertebrate smooth muscle cells than supposed by Kelly & Rice (1969), but for their reliable preservation a certain degree of mechanical tension seems necessary'. Essentially the same statement was made independently of us by Devine & Somlyo (1971) on the basis of a work performed on vascular smooth muscle. They wrote: 'The results suggest that demonstration of the normally present thick filaments in mammalian smooth muscle is primarily a problem of adequate preservation for electron microscopy'. It seems unnecessary to discuss any more the former assumption of Kelly & Rice (1969), since in a more recent work (Rice, McManus, Devine & Somlyo 1971) the constant existence of thick filaments has been recognized. The lack of thick filaments in electronmicrographs can thus be regarded as an artefact.

The fact that electron microscopic processing may selectively annihilate myosin (i.e. thick) filaments has been shown by one of us (N.G.) in glycerinized bee-wing muscle, i.e. in a striated muscle, too. The muscles were extracted in a highly stretched state for two months, subsequently they were released and fixed. There is a total lack of myosin filaments, though Z-lines, actin filaments and even traces of the M-lines are preserved (figure 7) (Garamvölgyi 1971). This is an obvious artefact without any functional significance and the same artefact may occur in vertebrate smooth muscle as well, due to the well-known high extractibility of smooth muscle myosin. Stretch may, however, exert a certain stabilizing effect on glycerinized bee muscle fibrils, too, because in muscles fixed in their original stretched state myosin filaments seem intact.

Recently Somlyo, Devine & Somlyo (1971) claimed to have regularly found thick filaments in completely unstretched mesenteric vein muscles ('excised and dropped into the fixative'). With the same conditions we also observed thick filaments in vas deferens muscle of the guinea-pig (J. Knoll & N. Garamvölgyi, unpublished). Neither of these results conflicts with our assumption on the significance of mechanical tension. The cells of these muscles are not arranged in a parallel manner and thus the individual cells following different courses may exert tension and compression on each other, particularly when the cells become activated by the effect of the fixative. On the other hand, we are convinced that by improved technics of fixation both kinds of filaments will be demonstrated in all smooth muscles quite regularly.

It is a still unanswered question, how mechanical tension can be exerted on the myosin filaments, when the crossbridges are detached in a relaxed muscle. Since it seems now probable that the basic structural mechanism of both smooth and striated muscle is essentially the same, a recent theoretical work (Garamvölgyi, Biczó, Ladik & Eöry, in preparation) based on the

Description of plate 26

Figure 4. Atropine treated smooth muscle cell in longitudinal section.

Figure 5. Longitudinal section of a cell of guinea-pig intestinal muscle depolarized by a K-rich Krebs–Ringer solution. Thick filaments are present. This muscle was fixed at constant length.

Figure 6. The same as figure 5, but fixed in a slack state. Only thin filaments are visible.

Figure 7. Longitudinal section of a fibre from the wing muscle of the bee glycerinized for 2 months, in a highly stretched state. Released and immediately fixed. The myosin filaments have been selectively annihilated by the process of fixation.

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structural parameters of frog striated muscle should be mentioned shortly. According to this computation the lattice forces acting between the myofilaments may result in an axial elastic force. By means of this axial component the tension of the resting muscle increasing with the length can be (theoretically) attributed to the muscle substance, in spite of the decreasing amount of the overlap. It is true that near the resting length the tension of the inactive muscle is insignificant in relation to the active force development at the same length. Nevertheless, it seems possible that this small amount of tension, which causes the shortening of the inactive muscle to the equilibrium length, is sufficient to bring about an increased stability of smooth muscle myosin filaments, presumably by polymer crystallization.

According to the group of Lowy (e.g. Lowy & Small 1970) the myosin forms large ribbons in smooth muscles, instead of individual thick filaments. We never observed ribbon-shaped structures and the thick filaments were always circular as seen in cross sections. We are inclined to accept the argument of Somlyo, Somlyo, Devine & Rice (1971) explaining the formation of ribbons by aggregation of thick filaments.

At any rate, vertebrate smooth muscles represent a two-filament system. In terms of polymer physics smooth muscle is essentially a nematic system, instead of a smectic system as is a striated muscle the elements of which are in register. This means that functionally smooth muscles also consist of 'sarcomeres' of contractile units randomly distributed alongside the cell axis. The cross-sectional areas lacking thick filaments can thus be regarded as 'I-bands'. If this is true, the model of the sliding filaments is valid for all types of muscle, without any exception.

We are indebted to the organizers of this discussion meeting, and to the Royal Society for covering all costs of one of us (N.G.).

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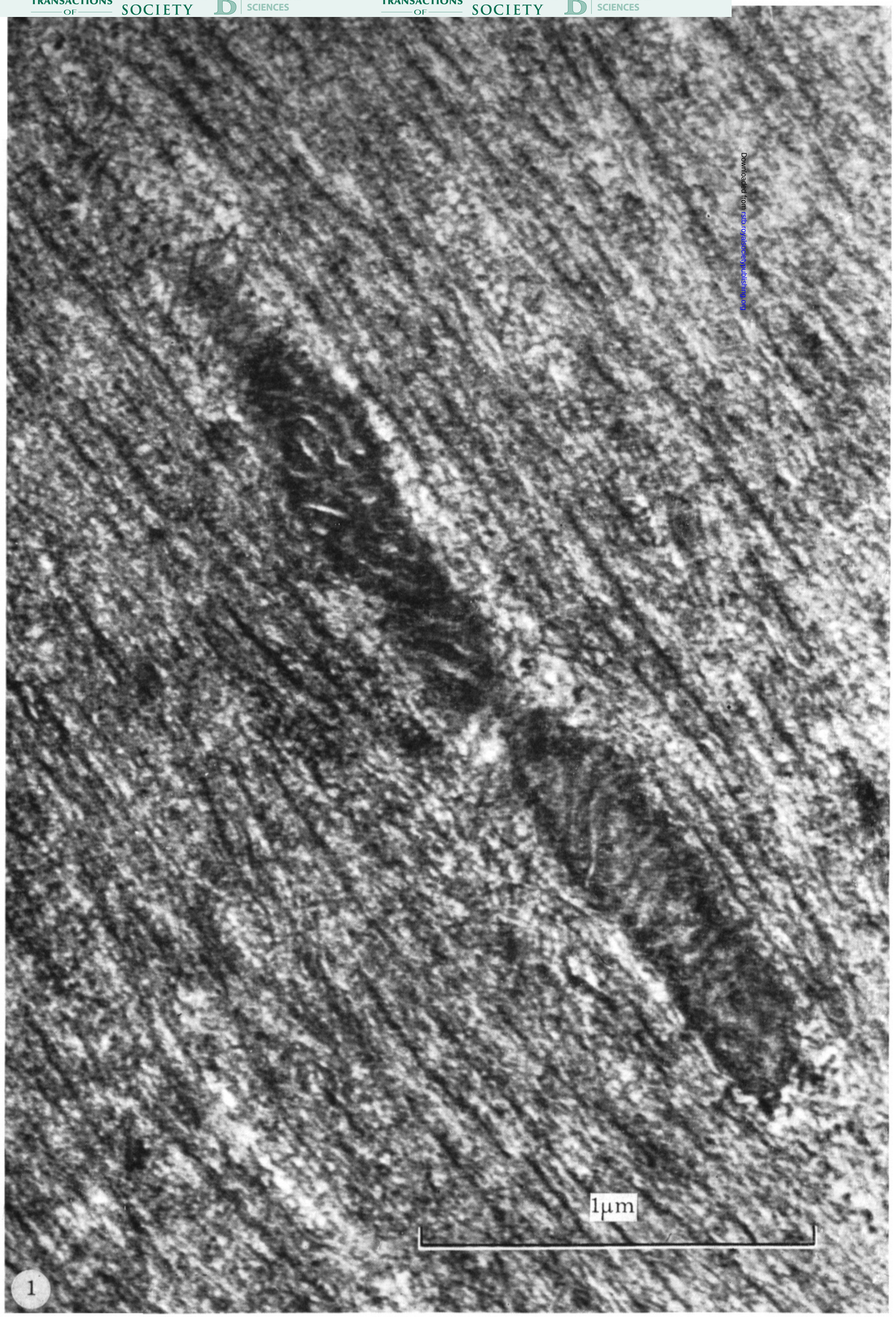

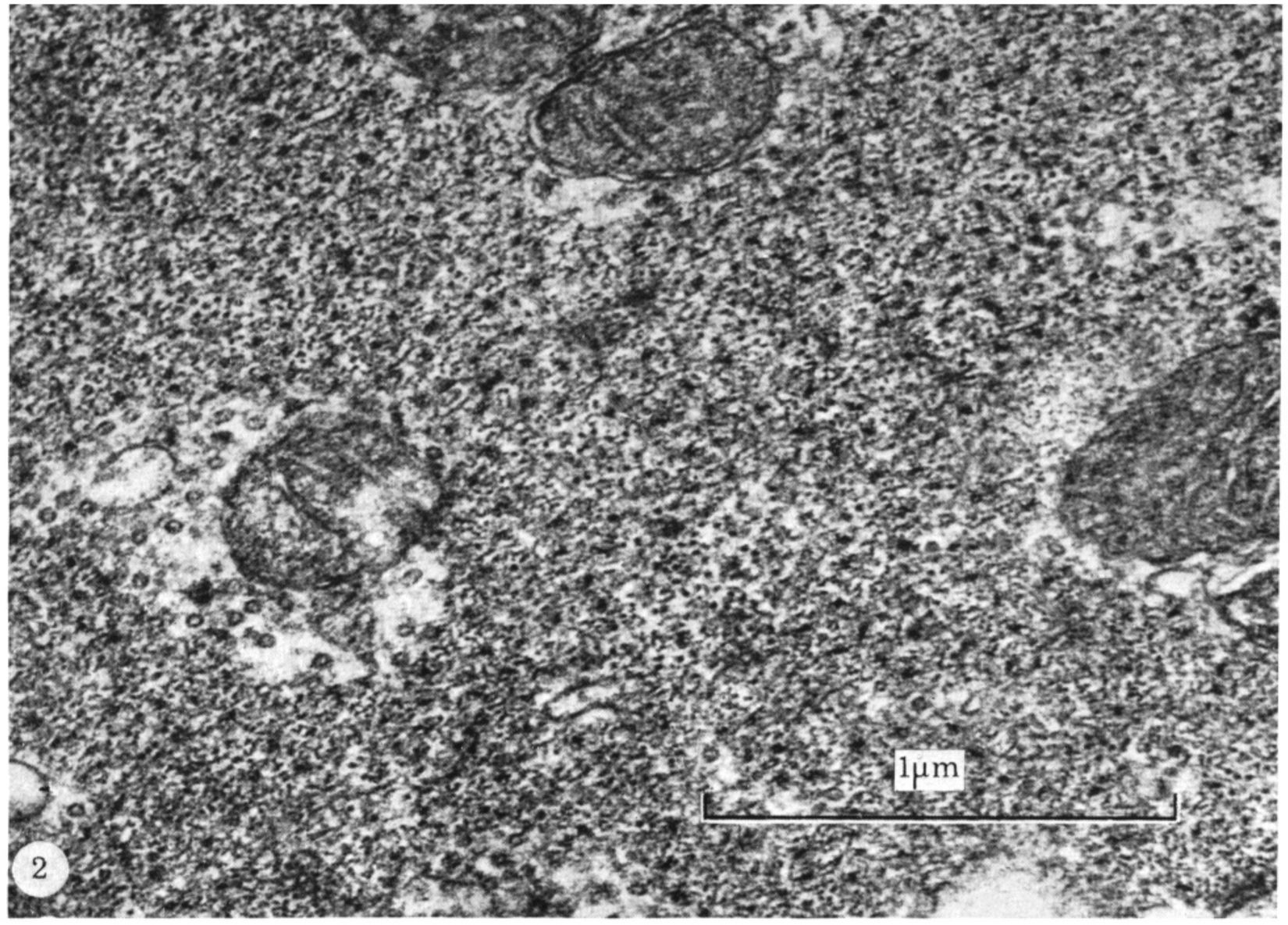

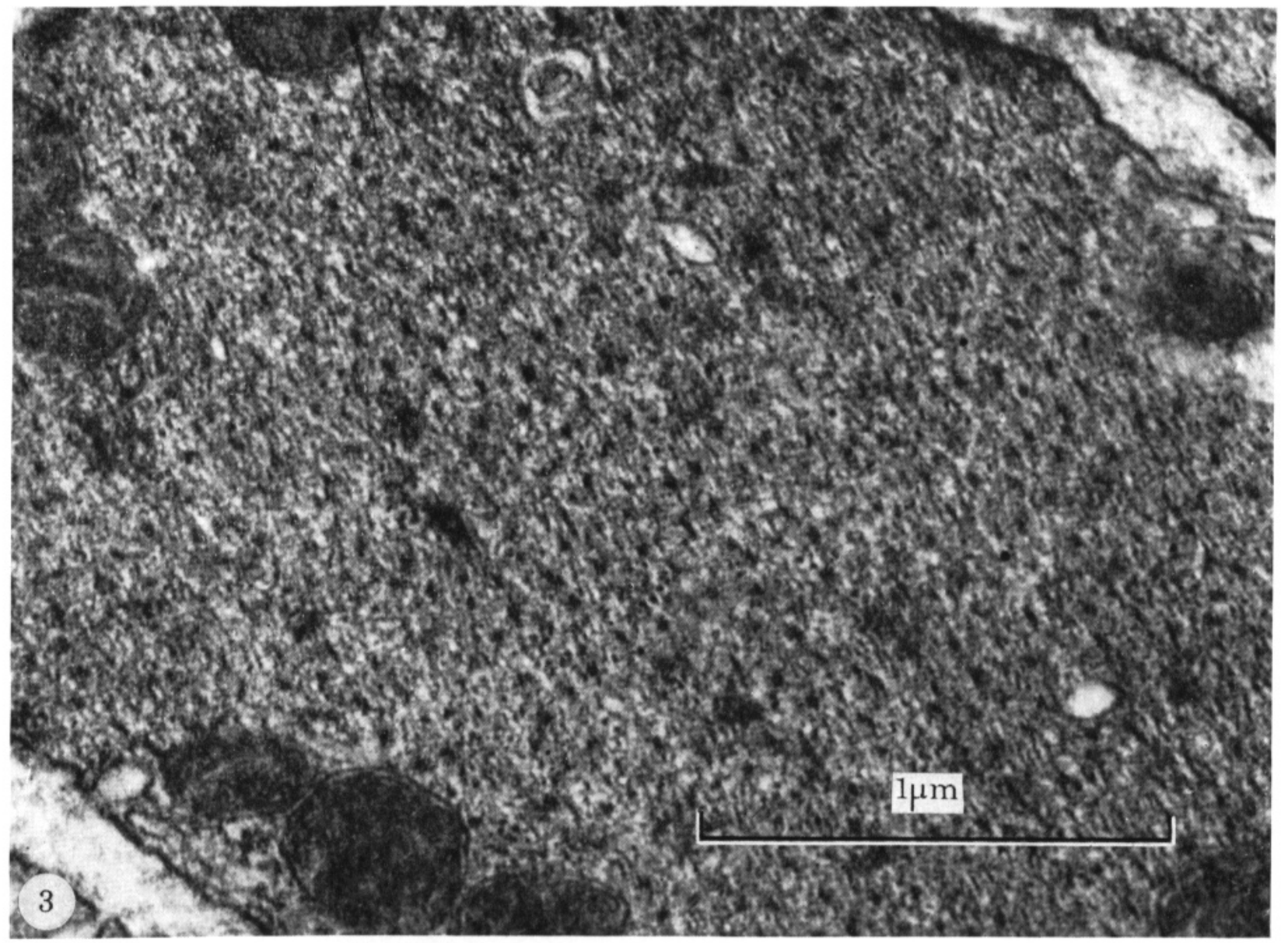

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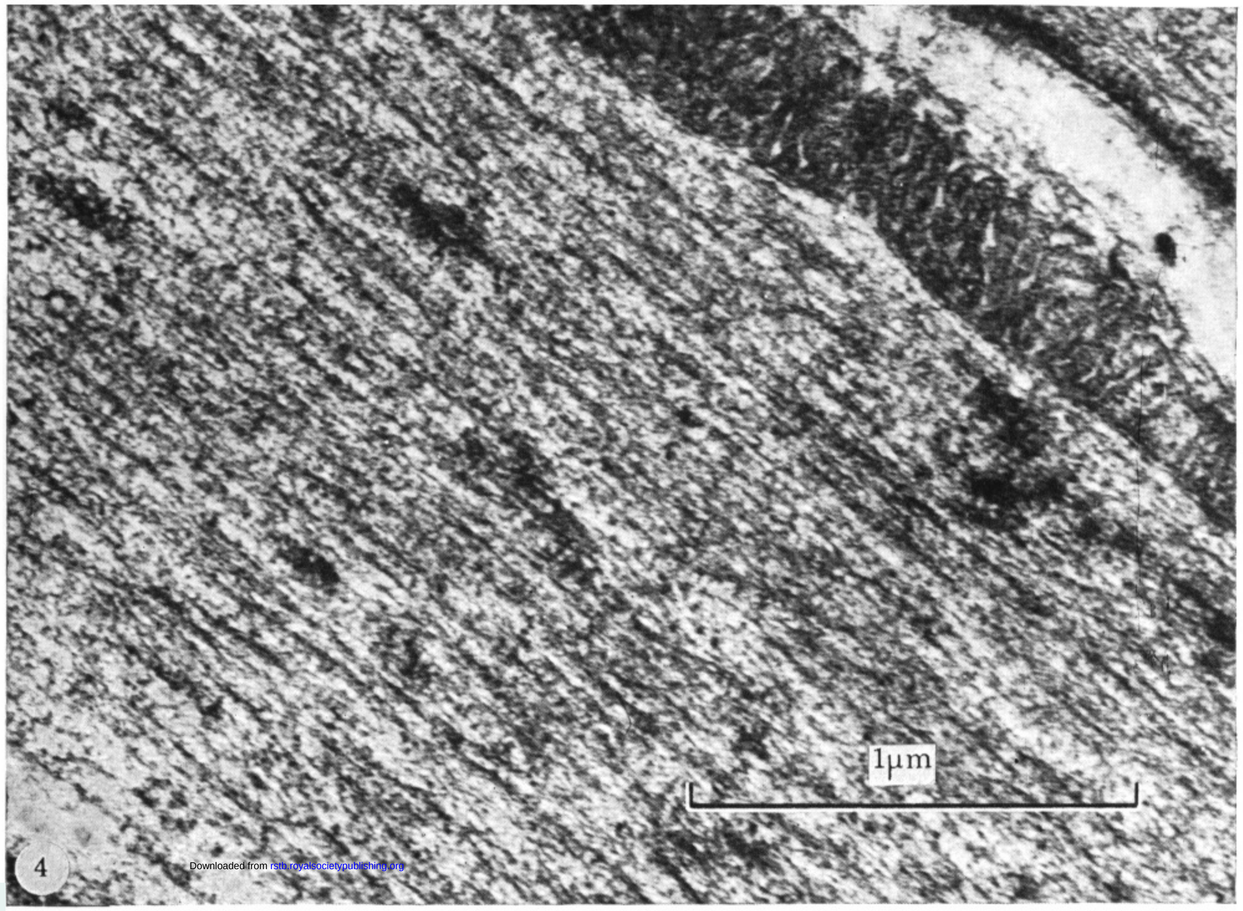

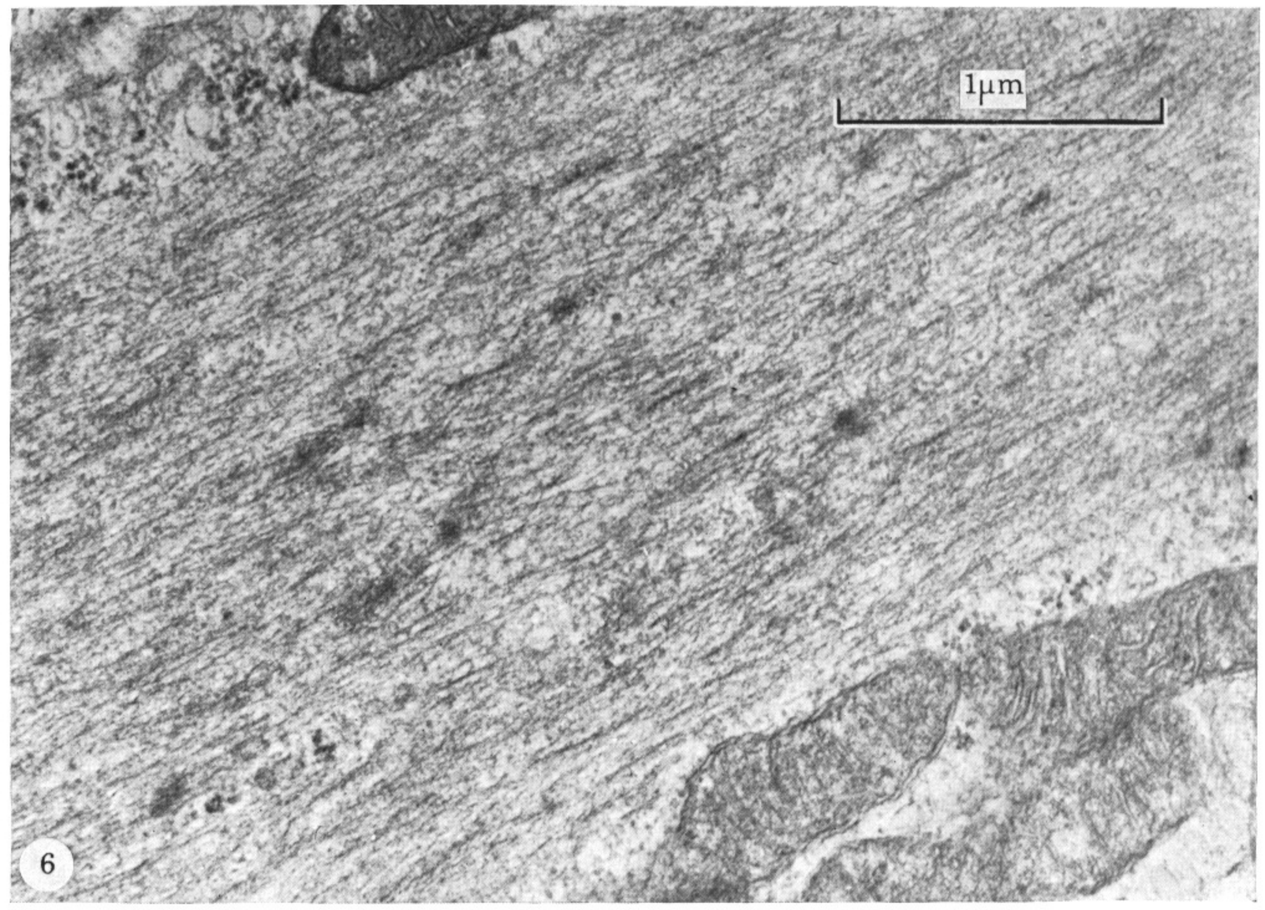

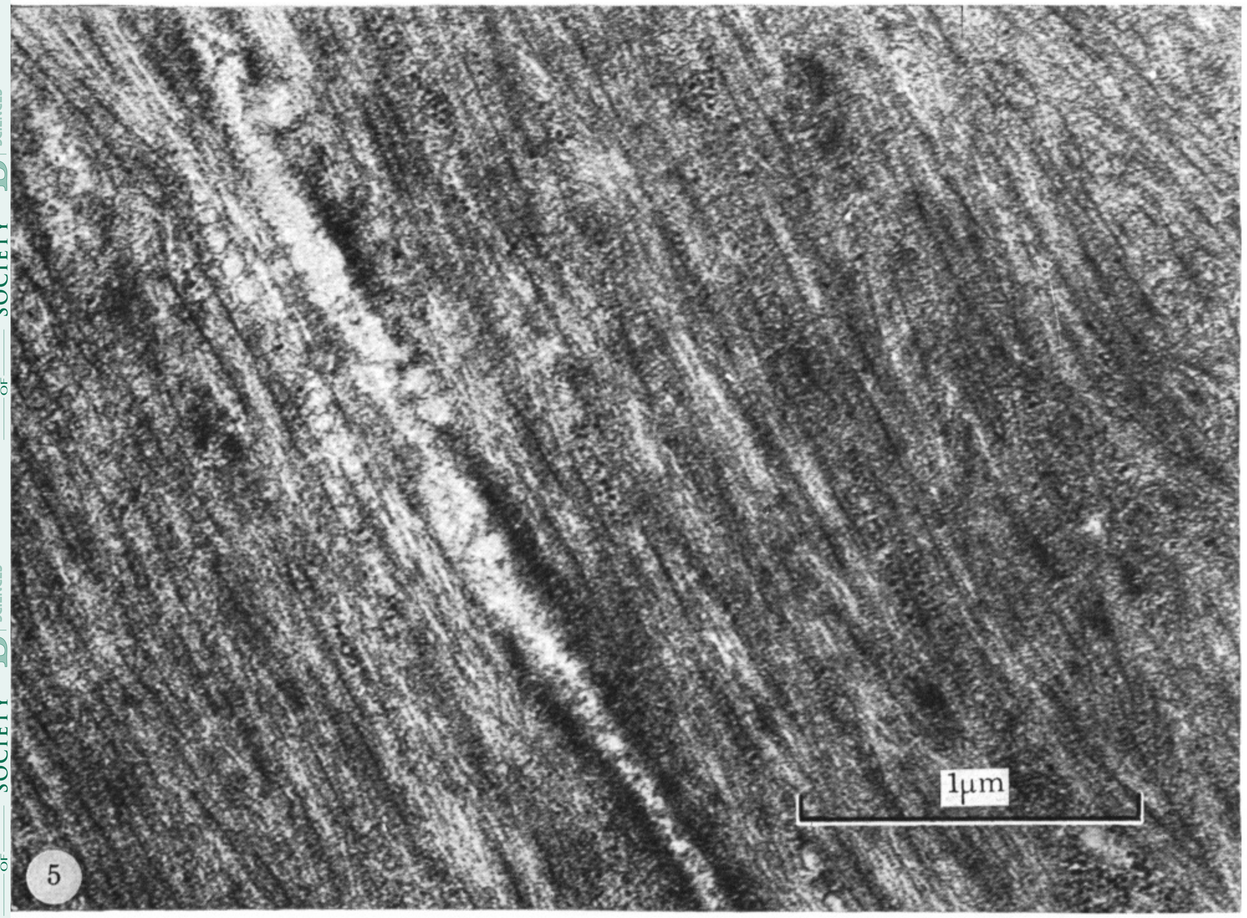

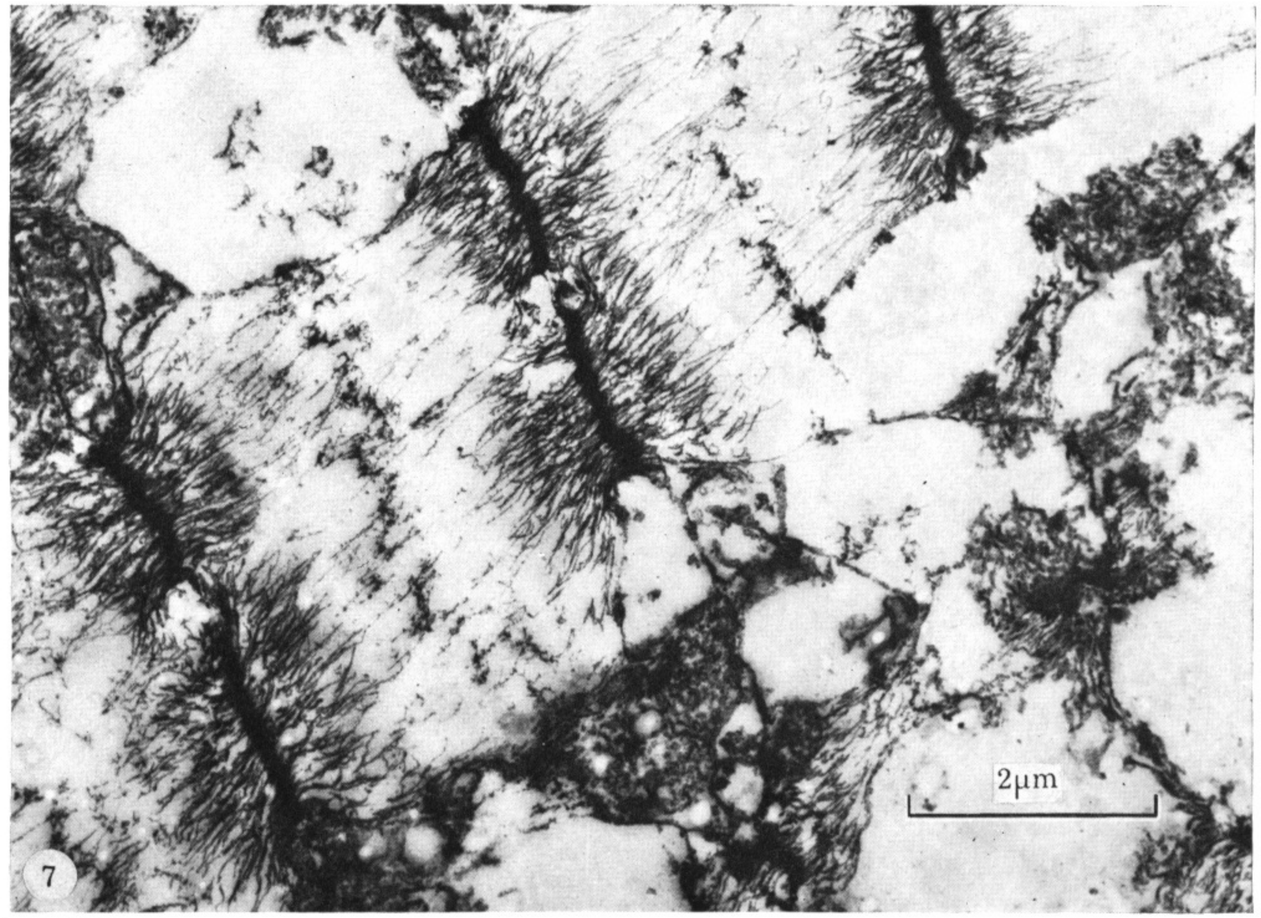

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