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Smooth Muscle: Contraction Hypothesis Based on the Arrangement of Actin and Myosin Filaments in Different States of Contraction

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Smooth muscle: contraction hypothesis based on the arrangement of actin and myosin filaments in different states of contraction*

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[Plates 23 and 24]

Electron microscopical studies were made of the changes occurring during contraction of mouse taenia coli. In relaxed fibres actin filaments were found clearly ordered into bundles. Within the bundles the filaments were often arranged in rows or in a hexagonal lattice. In the areas between the filament bundles thick filaments were detected. The shortened fibres showed a different appearance. Thick and thin filaments were intermingled in a random fashion. By comparing these findings with observations made on invertebrate smooth muscles, a model for smooth muscle contraction is proposed. According to this hypothesis the contractile apparatus of smooth muscles is composed of small contraction units of interdigitating bundles of thick and thin filaments. They seem to be irregularly shaped and randomly arranged. During the contraction the sets of filaments slide into each other. The result is the intermingling of the thick and thin filaments found in contracted fibres.

Introduction

The reports about the types of filaments and their arrangement in smooth vertebrate muscles are rather contradictory. Thin actin-like filaments are always found, but thick ones only by some of the investigators (Nonomura 1968; Heumann 1969, 1971; Rice *et al.* 1970; Devine & Somlyo 1971; Garamvölgyi, Vizi & Knoll 1971; Cooke & Fay 1972*b*). Others failed to find thick filaments (Panner & Honig 1967, 1970) or could detect them only after treatments of the muscles thought to be not very physiological (Kelly & Rice 1968; Cooke, Chase & Cortes 1970; Rosenbluth 1971). Lowy & Small (1970) claimed that the myosin exists in very long ribbon-like structures. Thus doubts were raised about the state of aggregation of myosin in the living muscle cell. In this study it is demonstrated that round, thick filaments can be seen in the intestinal muscle of the mouse when various electron microscopical techniques are applied. Furthermore, from changes found in the contractile apparatus of relaxed and contracted fibres a model for a contraction mechanism is proposed.

Materials and methods

The tissue studied was the circular muscle of the intestine of the white laboratory mouse. For freeze-etching the technique recently described was applied (Heumann & Speth 1971). For freeze-substitution small pieces of the muscle layer were frozen with isopentan and substituted with 70% glycol at −50 °C. After dehydration the strips of muscle were embedded without any fixation in Epon. For the contraction experiments the intestine was cut in rings about 1 cm long which were placed for 1 to 2 min in warm Krebs solution containing Suprarenin (for relaxation) or acetylcholine (for contraction). The tissue was usually fixed with glutaraldehyde and postfixed with osmium tetroxide, but formol prefixation or osmium tetroxide fixation alone were also carried out. Sections were stained with uranyl acetate in methanol and lead citrate and viewed in a Siemens Elmiskop IA.

* Dedicated to Professor F. Duspiva on the occasion of his 65th birthday.

Results

Thick filaments were found in contracted fibres of the intestinal muscle of the mouse in different types of preparation (see table 1).

Table 1. Data of thick and thin filaments from the intestine muscle of the mouse

	thick filaments			thin filaments
preparation	diameter nm	length μm	density μm²	diameter nm
osmium tetroxide	—	—	—	9
formol/osmium tetroxide	16	1.0	80–100	—
glutaraldehyde/osmium tetroxide	17	1.2	95–120	7
freeze-etching technique	17	[0.1]	80–100	—
freeze-substitution technique	15	1.3	100–150	—

Only in muscle fibres fixed with osmium tetroxide were thick filaments not detectable. They were easily identified in glutaraldehyde- or formol-prefixed muscle cells (figure 7). Thick filaments were also observed in muscles which were unfixed but first frozen and then either fractured and replicated (freeze-etching technique, Moor 1964) (figure 1) or after freeze dehydration with glycol (freeze-substitution technique, Pease 1968) (figure 2). In all types of preparations, the filaments showed identical dimensions (diameter 15 to 17 nm), length up to 1.3 μm) and a density of 80 to 150 μm².

The thin actin filaments (diameter 7 to 9 nm) could be clearly demonstrated only in the glutaraldehyde-prefixed or osmium tetroxide-fixed preparations, not, however, in the freeze-etched or freeze-substituted muscle cells. Since these small filaments are very sensitive to mechanical influences I assume that most of them are destroyed during freezing. However, the *in vivo* existence of thin actin-like filaments in vertebrate smooth muscles is beyond doubt.

A third type of filaments observed in the fixed preparations should be mentioned. They are about 10 nm in diameter and run along the longitudinal fibre axis or cross it obliquely. According to Cooke & Fay (1972*a*) their function is to link together the dense bodies into a network.

To examine the filament arrangement during contraction, experiments were undertaken, in which muscles were fixed in relaxed and shortened states. Figure 3 shows a transverse section through a portion of a relaxed muscle fibre.

The striking features in the cytoplasm are the profiles of thin filaments. They are clearly arranged into bundles of different sizes leaving areas free of filaments. Within these filament fields, the degree of order is very high. Often the arrays are composed of several rows of thin filaments; occasionally, a hexagonal lattice can be observed (figure 4). In these regions neighbouring filaments are separated only by small spaces of 4 to 5 nm. In other areas, especially at the border of the filament groups, the filaments are distributed more irregularly and are more widely separated from each other.

In the actin-free areas different cell organelles are seen: mitochondria, endoplasmic reticulum, microtubules and glycogen granules. Even more interesting is the fact that thick filaments can be observed in these areas, occasionally (figure 5). They are easily distinguished from the glycogen granules or microtubules by their smaller and more irregular diameter, and are usually separated from the areas of the thin filaments.

Randomly dispersed in the cytoplasm are dark areas often called 'dense bodies'.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

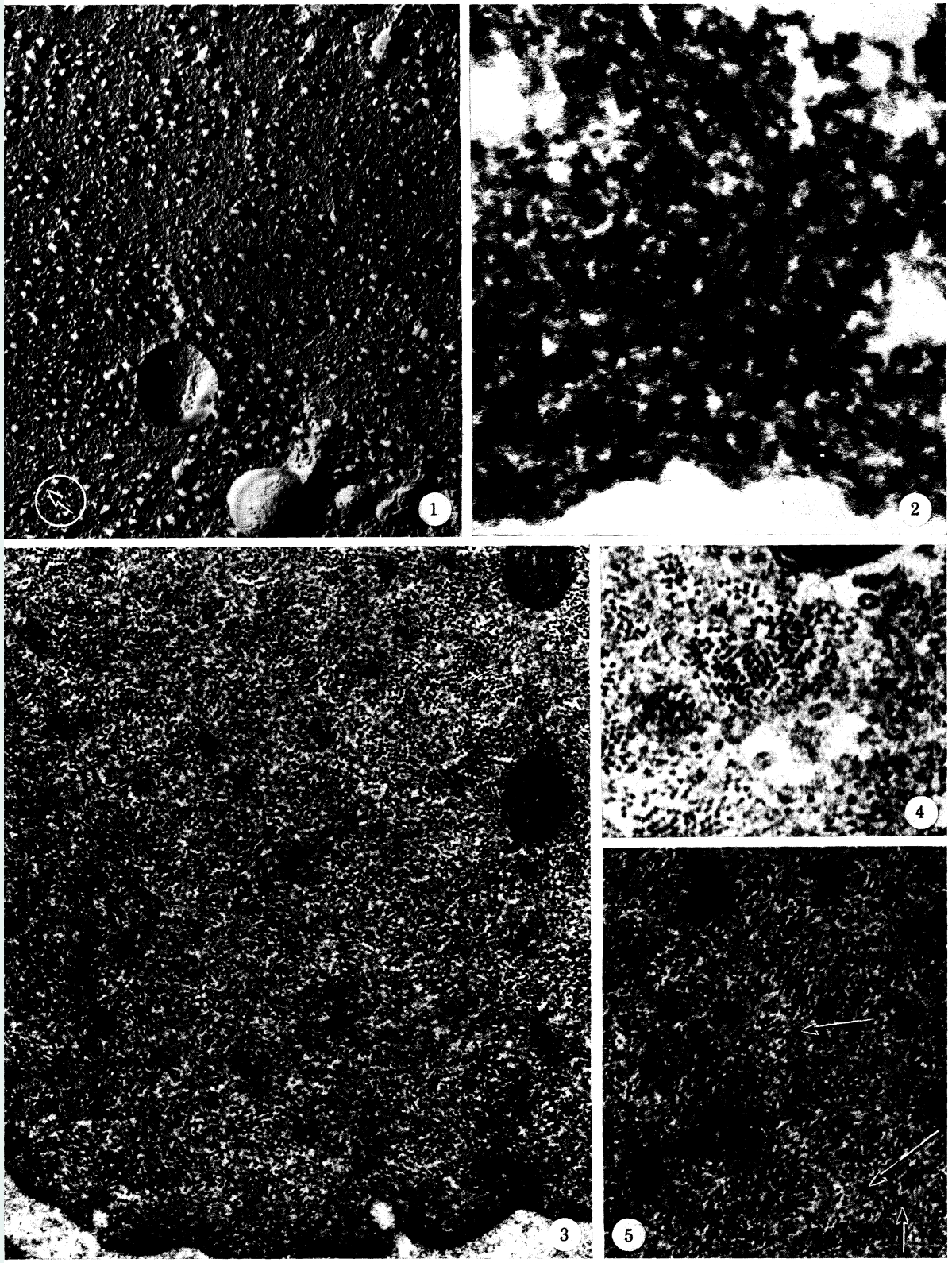

FIGURE 1. Transverse fracture of a muscle cell of the mouse intestine. The circular electron-dense profiles with white shadows represent broken ends of thick filaments. The encircled arrow indicates the direction of metal shadowing. (Magn. ×45000.)

FIGURE 2. Transverse section through a muscle fibre prepared by freeze-substitution. (Magn. ×45000.)

FIGURE 3. Transverse section of the intestinal muscle relaxed before fixation with Suprarenin. The thin filaments are arranged in bundles. (Magn. ×60000.)

FIGURE 4. Detail of a filament area demonstrating a hexagonal lattice of the thin filaments. (Magn. ×100000.)

FIGURE 5. Between the thin-filament bundles irregularly formed profiles of thick filaments are seen. (Magn. ×65000.)

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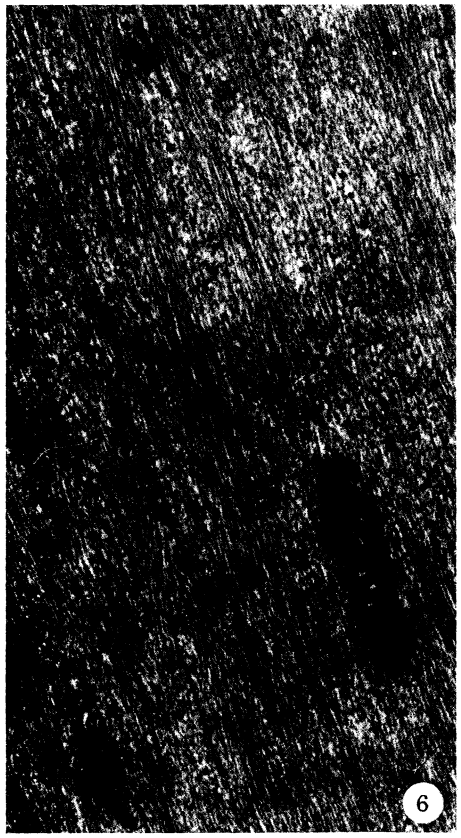
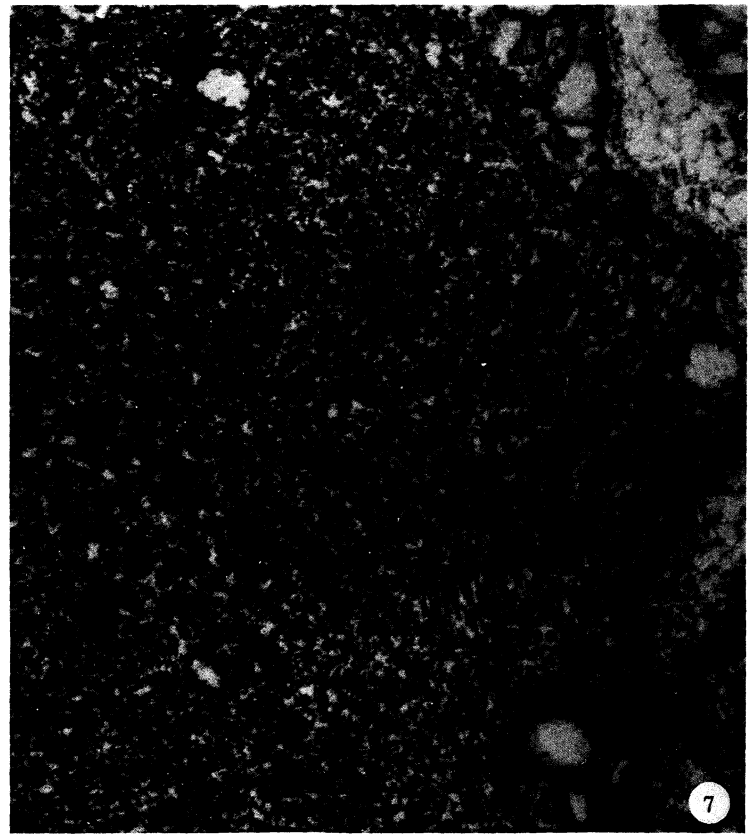
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES





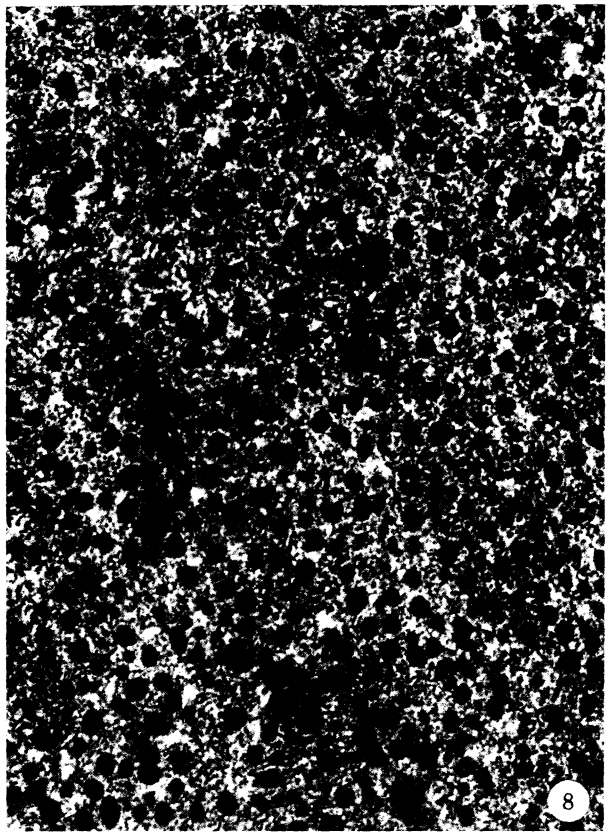


FIGURE 6. Longitudinal section through a relaxed muscle fibre. It contains bundles of thin filaments and some thick filaments. (Magn. × 60000.)

FIGURE 7. Contracted muscle fibre. Thick and thin filaments appear randomly distributed. (Magn. × 60000.)

FIGURES 8, 9. Transverse sections through the byssus retractor muscle of *Mytilus* in the contracted (figure 8) and relaxed state (figure 9). (Magn. × 60000.)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

In longitudinally sectioned muscle fibres the bundles of the actin filaments are visible also as the thick filaments (figure 6). However, they are not so exactly arranged that a location of both ends of the thick and thin filaments in the same section is possible.

The appearance of shortened muscles differs from that of relaxed ones (figure 7). Even at low magnification the fibres appear to be dense. This is due to the dense packing of their filaments. The three types of filaments mentioned can always be distinguished on the basis of their diameters and their arrangement. Thick and thin filaments are not found in separate areas but seem to be intermingled in a random fashion. Dense bodies are distributed throughout the cytoplasm.

Discussion

The existence of thick filaments in the intestinal muscle of the mouse can be demonstrated by various electron microscopical techniques. In their dimensions they resemble the myosin-containing filaments of the cross-striated muscles and are therefore also thought to contain myosin. The presence of the filaments is affected by the state of contraction in which the muscle was fixed. In contrast to the contracted state, in which thick filaments are always visible, they are often only poorly preserved in the relaxed state. From similar observations Rice *et al.* (1970) supposed that the thick filaments aggregate before contraction and disaggregate during relaxation. The results obtained with the polarizing microscope (Fischer 1944) argue against this assumption as do also those obtained by the X-ray diffraction technique (Lowy, Poulsen & Vibert 1970). A 14.3 nm meridonal reflexion was found with the latter method in the relaxed taenia coli. This is probably due to a structural organization of the myosin molecules. The reflexion is identical with that assigned in cross-striated muscles of Huxley & Brown (1967) to the helically arranged myosin heads. From the width of the reflexion the authors deduced that the myosin filament diameter is larger than in other muscles and recently Lowy & Small (1970) published electron micrographs showing ribbon-like myosin structures. These results could not be confirmed by other investigators who only found round thick filaments (Devine & Somlyo 1971; Garamvölgyi *et al.* 1971; Heumann 1971; Cooke & Fay 1972*b*).

An explanation for these discrepancies may be found in the high solubility of the myosin structures. Especially in the relaxed state they may disaggregate to single molecules hardly visible with the electron microscope or may aggregate to artificial structures. It is possible that ATP and divalent ions (Shoenberg 1967, 1969) or mechanical stretch (Garamvölgyi *et al.* 1971) may stabilize the filaments. Since in my preparations thick filaments are present at least occasionally in the relaxed fibres and always in the contracted ones, the existence of thick and thin filaments in the contractile apparatus of smooth vertebrate muscles can be considered as physiological.

The differences in the filament distribution of relaxed and contracted muscle fibres are striking. In the relaxed state thick as well as thin filaments are mainly dispersed into separated areas. A characteristic feature of this state seems to be the lattice-like arrangement of the thin filaments. A similar pattern was recently seen in electron micrographs of various vertebrate smooth muscles (Lowy & Small 1970; Rice *et al.* 1970; Cooke & Fay 1972*b*) and also noted by X-ray diffraction (Elliott & Lowy 1968). However, a determination of the three-dimensional construction of the filament bundles and their relationships to each other was prevented by the fact that the bundles are not properly oriented and the thick filaments often only poorly preserved.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

In this connexion it might be useful to compare the appearance of vertebrate smooth muscle fibres with that of invertebrate smooth muscle fibres. In the latter, the analysis of the three-dimensional reconstruction of the contractile apparatus is facilitated by the fact that the thick filaments are always easily identified. The similarity between the two muscle types can be seen when comparing a cross-section through the anterior byssus retractor muscle of *Mytilus edulis* (figure 9) (for details see Heumann & Zebe 1968). In accordance with the arrangement in mouse intestinal muscle, the thin filaments are organized into groups. Within these filament areas the thin filaments are arranged in a lattice-like pattern. This is not so well preserved in electron micrographs as shown here, but was clearly demonstrated with X-ray diffraction by Lowy & Vibert (1967). Thick filaments of variable diameters – due to their spindle shape – are seen in separated areas. They are partly surrounded by thin filaments especially in the regions of their smaller diameters. Dense bodies packed with thin filaments are dispersed in the cytoplasm.

From these observations I would draw the conclusions that the structure of the contractile apparatus of the byssus retractor muscle as that of the mouse intestinal muscle consists of interdigitating bundles of thick and thin filaments, staggered against each other. Therefore, in longitudinal section only loosely arranged bundles of thick and thin filaments are seen. In cross-section, on the other hand, neighbouring bundles are sectioned in different zones. Consequently, we find groups of filaments of different composition side by side. In homology to the cross-striated muscles we can call these areas I-zones, A-zones, H-zones and Z-elements. In spite of the fact that the degree of order of the filaments is here much smaller than in cross-striated muscles a sliding mechanism may operate between thick and thin filaments in smooth muscles as in striated muscles.

This assumption of the structure of the contractile apparatus of invertebrate and vertebrate smooth muscles is supported by the pictures of shortened fibres (cf. figure 8). In both cases the separation of the two types of filaments, seen in the relaxed fibres is replaced in the contracted fibres by a more random arrangement of intermingled thick and thin filaments. According to the proposed model these changes are due to a sliding of the filament bundles deeper into each other, until I- and H-zones have disappeared. Since in the smooth muscles no lateral alinements of large units of filaments are developed, as in the cross-striated muscles, any regularity is lost in the contracted state.

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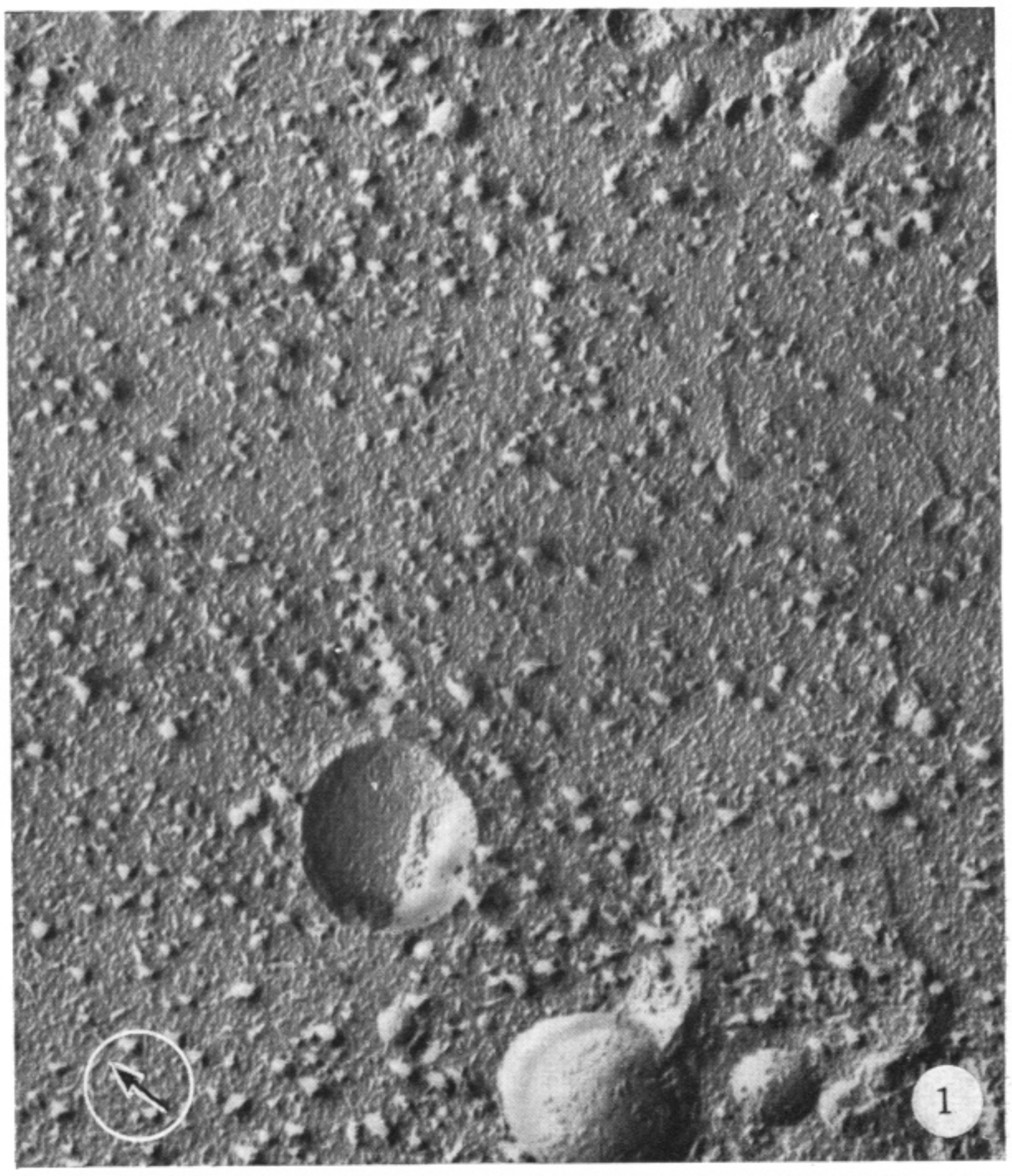

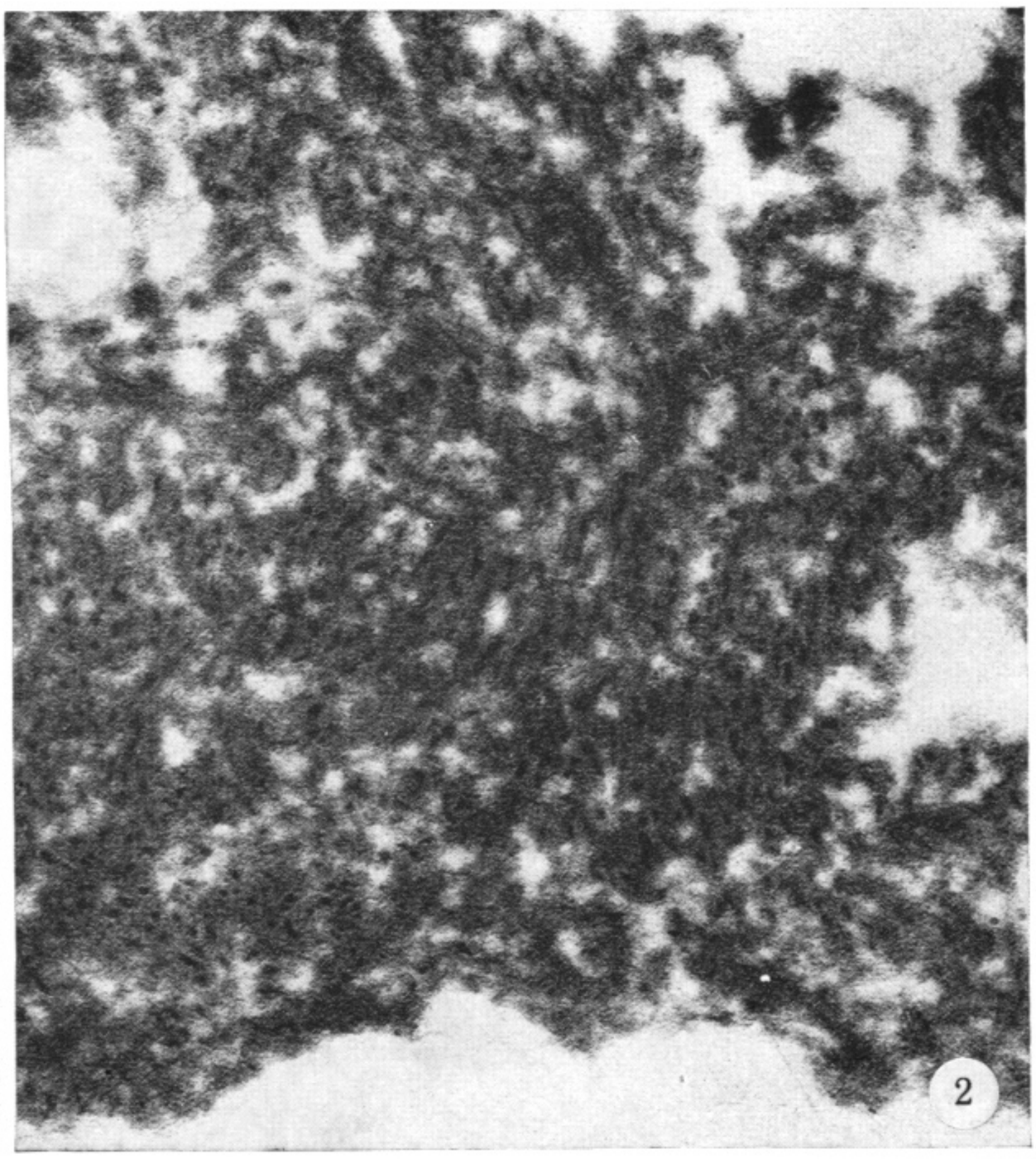

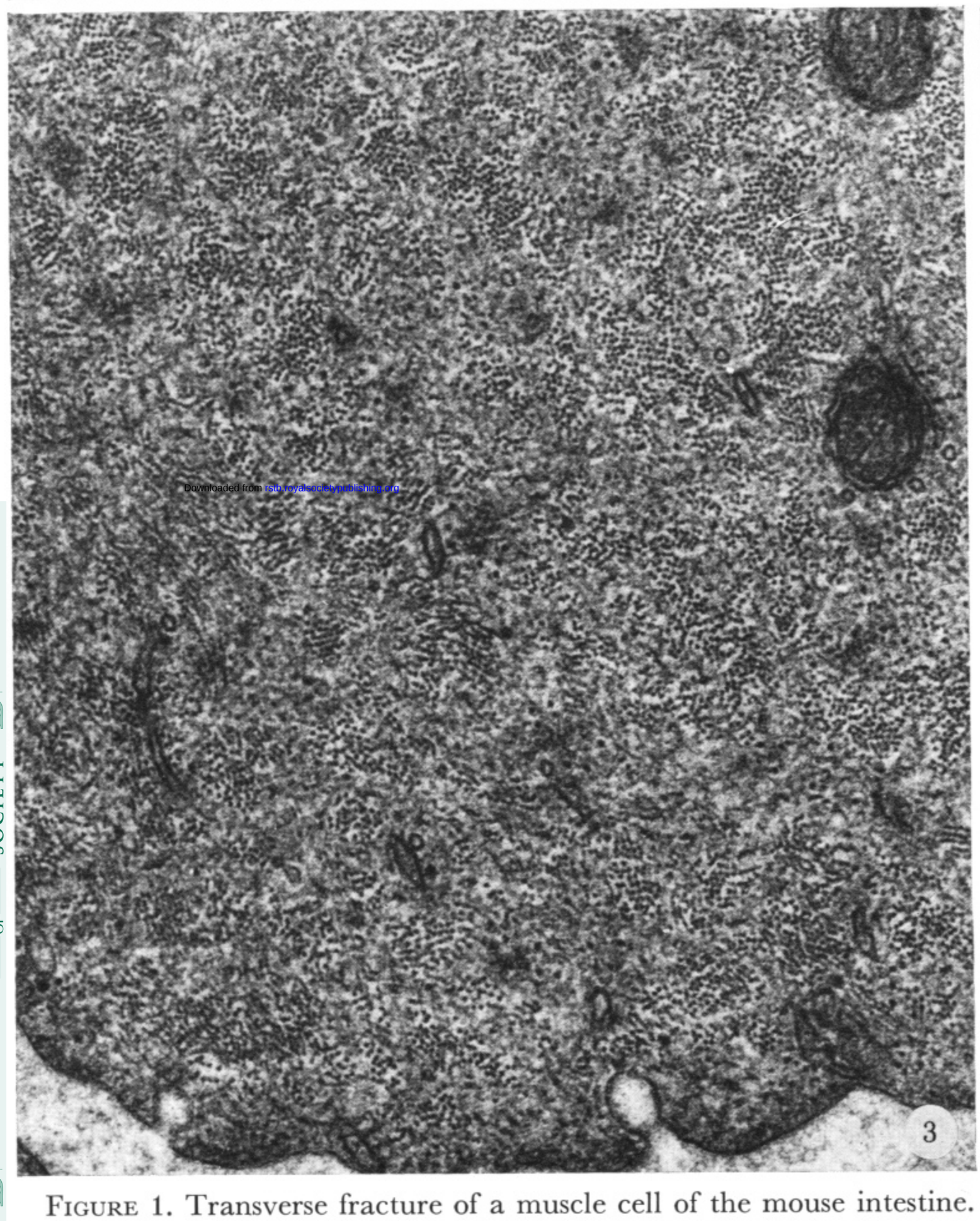

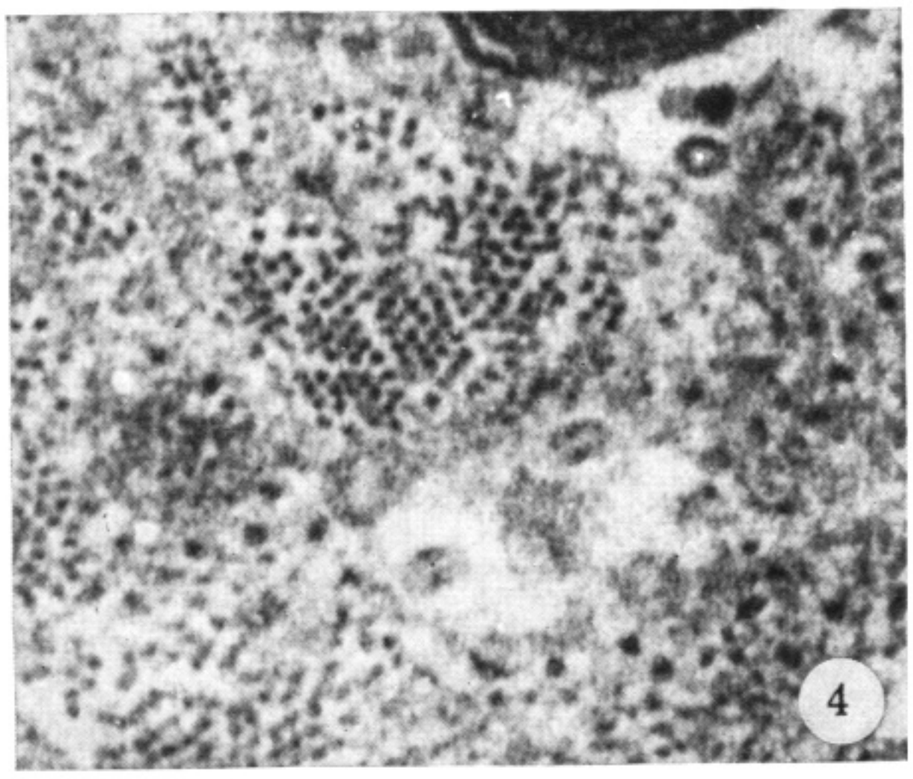

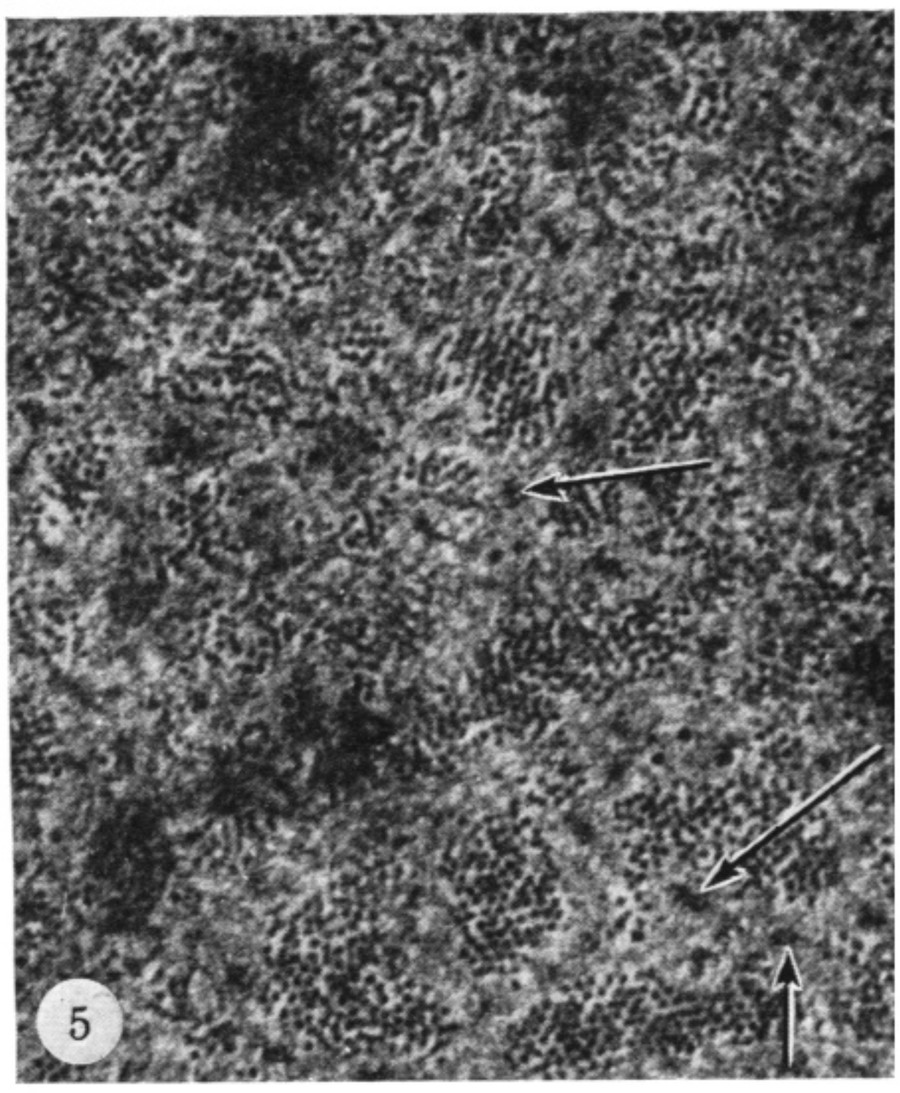

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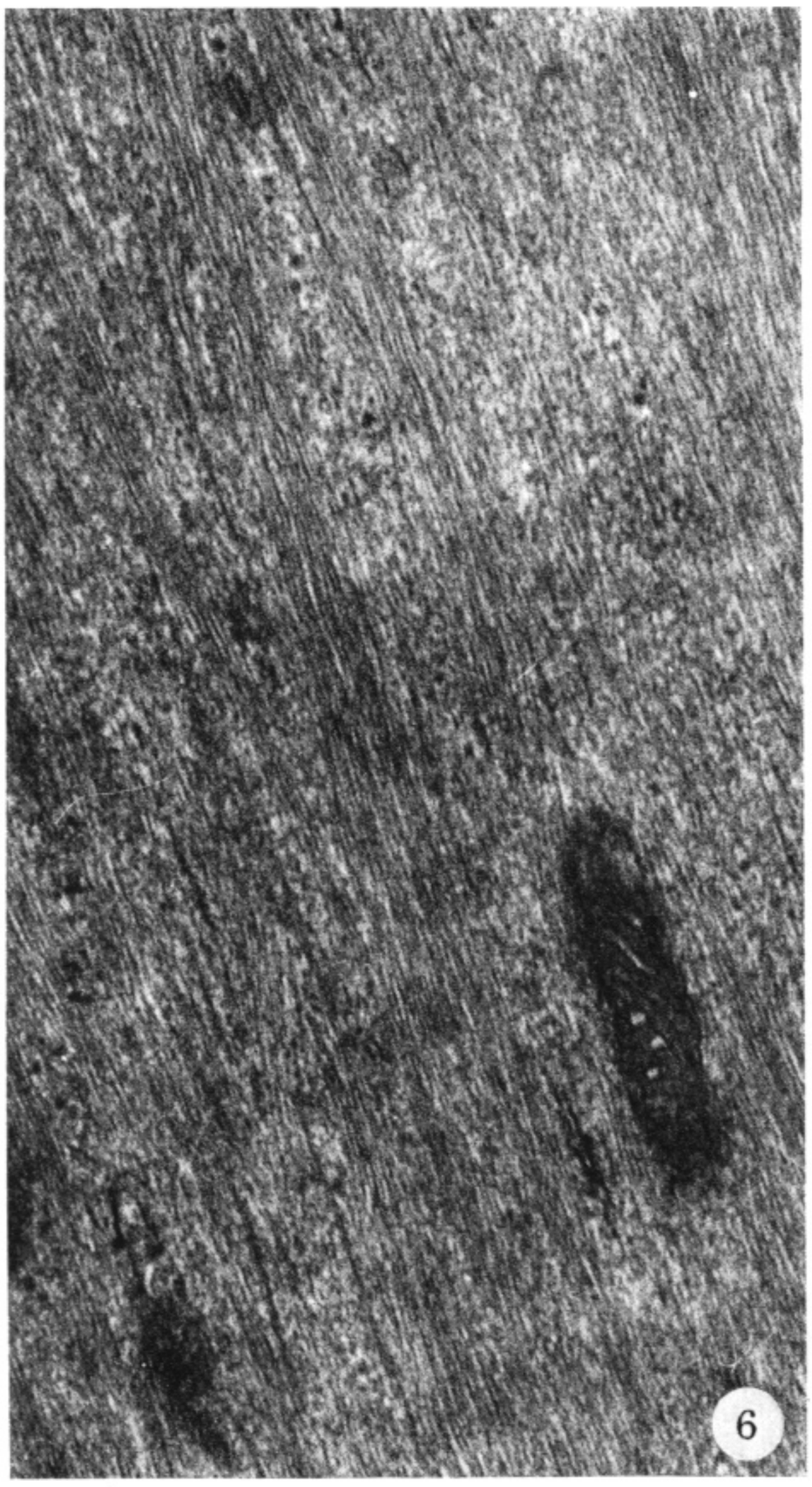

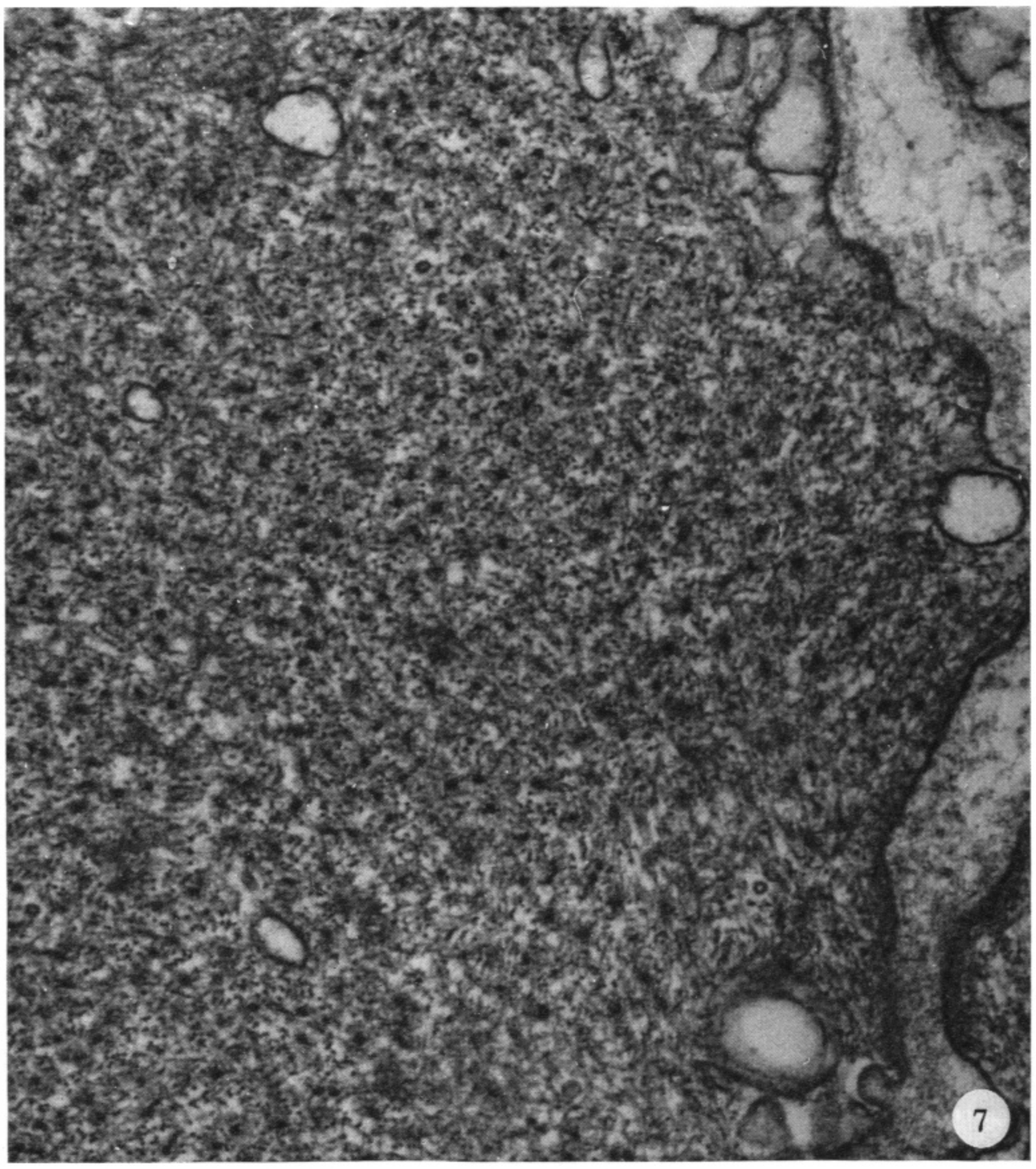

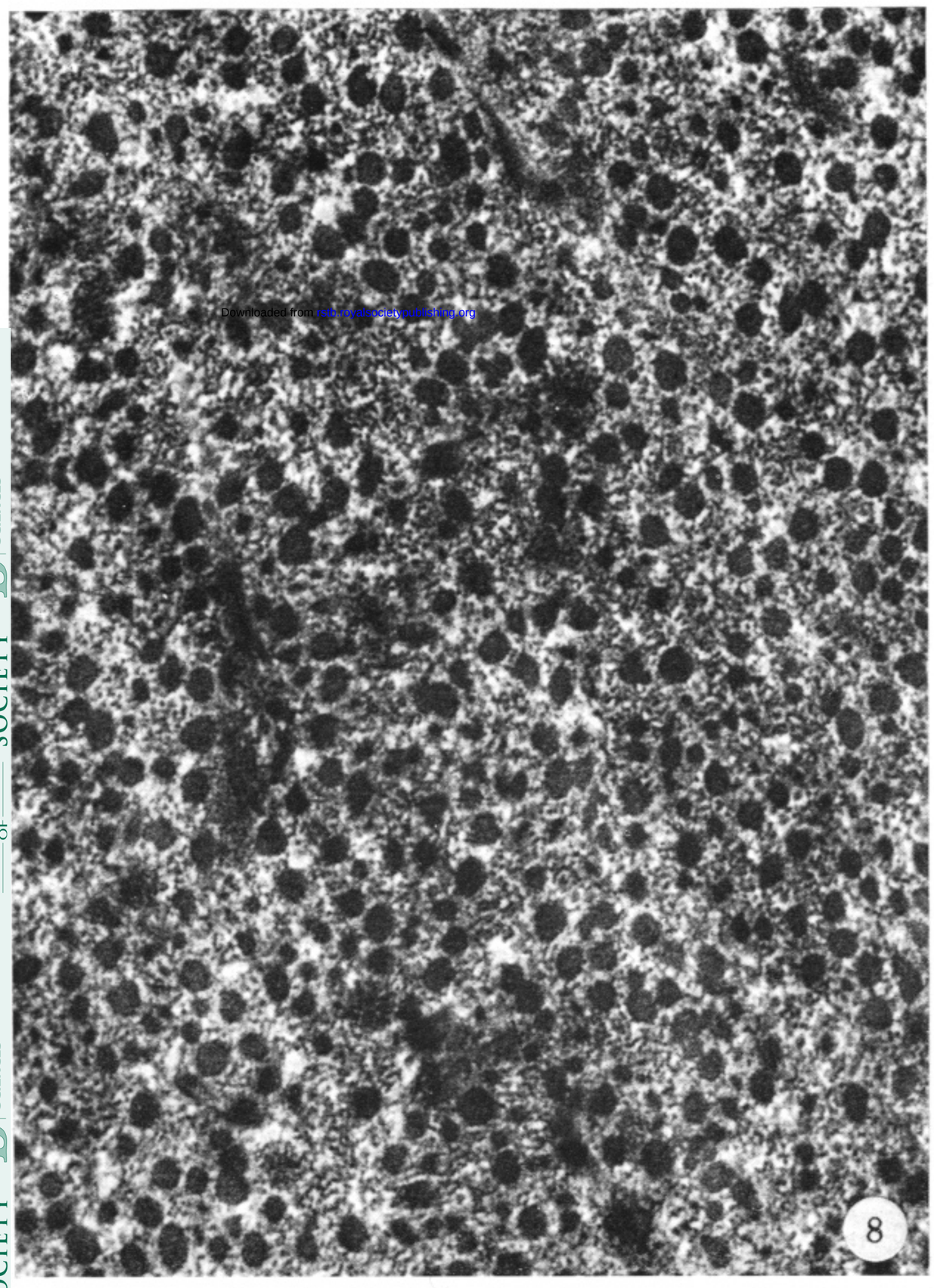

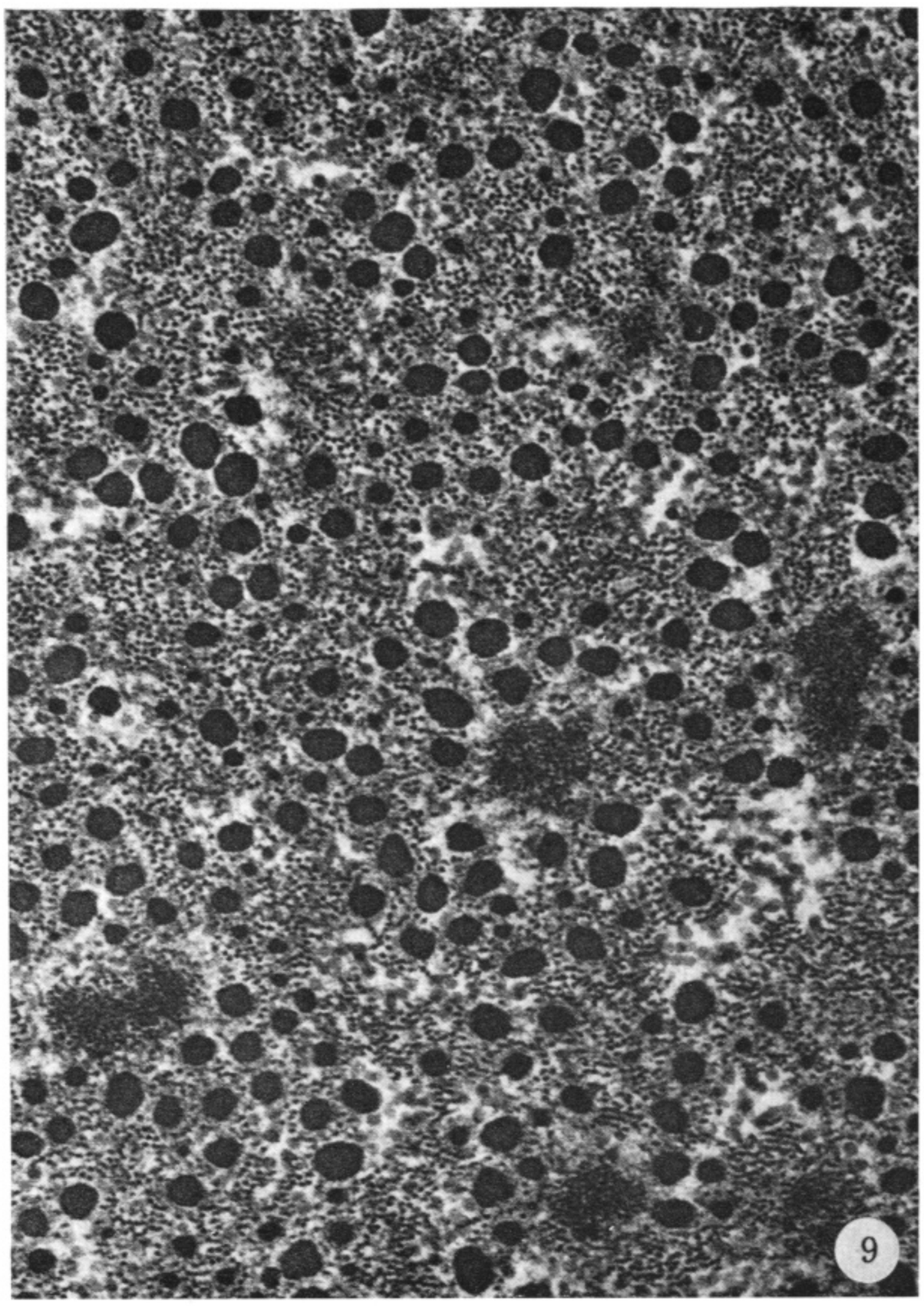

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