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Changes in the fine structure of fast-twitch rat muscle after reinnervation by slow nerve

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Patologia. — *Changes in the fine structure of fast-twitch rat muscle after reinnervation by slow nerve* (*). Nota di STEFANO SCHIAFFINO e EUGENIO VISONÀ, presentata (**) dal Socio M. ALOISI.

RIASSUNTO. — La reinnervazione crociata del *m. extensor digitorum longus* (muscolo rapido) del ratto con il nervo del *m. soleus* (muscolo lento) induce una radicale trasformazione del muscolo rapido, le cui fibre assumono caratteristiche istochimiche e ultrastrutturali proprie del muscolo lento. Le modificazioni ultrastrutturali nelle fibre reinnervate riguardano le miofibrille, e particolarmente la struttura delle bande Z e M, la quantità e la distribuzione dei mitocondri, e lo sviluppo del reticolo sarcoplasmico, che appare sensibilmente ridotto in estensione rispetto ai muscoli di controllo. Queste osservazioni dimostrano la notevole plasticità strutturale delle fibre muscolari scheletriche del ratto e confermano l'importanza dell'innervazione nel differenziamento dei tipi di fibre. I risultati vengono discussi in rapporto a precedenti dati biochimici e fisiologici sui muscoli etero-innervati.

Cross-reinnervation experiments have shown that motoneurones, in addition to a generic trophic influence responsible for the survival of skeletal muscle fibres and maintenance of fibre size, exert a more specific control on the differential fibre properties. Cross-union of the nerves to fast-twitch and slow-twitch mammalian muscles produces reciprocal changes in the muscle contraction velocity, myosin ATPase activity and metabolic profile [1, 2, 3, 4, 9, 17, 18, 26]. Whether and to what extent this functional conversion is accompanied by structural changes in the muscle fibres has not yet been determined. In the chicken the fine structure of slow muscle was found to change to a fast type only when cross-reinnervation was performed at an early stage of development [28], whereas no change was seen when cross-reinnervation was performed in the adult fowl [11].

We report here the results of a correlated histochemical and electron microscopical investigation on the effect of reinnervation of a fast-twitch rat muscle by a slow nerve. The extensor digitorum longus muscle was used in this study since the morphological characteristics of its constituents fibres are known [22] and the effect of cross-reinnervation on its contractile properties and myosin structure have been analyzed in detail [1, 4, 24].

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Materials and methods

Cross-reinnervation of extensor digitorum longus (EDL) muscle by soleus nerve was performed on one month old Wistar rats essentially as described by Barany and Close [1] for their experimental group IV. The EDL and soleus nerves were cut and the proximal stump of the soleus nerve was sutured with the distal stump of the EDL nerve. In order to avoid reinnervation of EDL also by nerve fibres from the common peroneal nerve this was transected, reflected and tied back over the thigh. The tibialis anterior and other muscles innervated by the common peroneal nerve were thus denervated and underwent atrophy. We assume that the functional overload on EDL resulting from the incapacitation of the synergistic tibialis anterior did not influence the results of the experiment. In fact, we have shown that the histochemical profile of EDL is practically unchanged after extirpation of tibialis anterior in one or two month old rats [23].

Eight to ten months after surgery cross-reinnervated (X-EDL), contralateral unoperated (C-EDL) and self-reinnervated (S-EDL) muscles were removed and sectioned transversely through the midbelly with a razor blade. The two halves of each muscle were separately pinned on plastic supports under light stretch and processed for histochemistry and electron microscopy, respectively. For histochemistry, the muscles were frozen in liquid nitrogen and cross-sectioned in a cryostat. Sections were cut at the level of the midbelly, but in some case a second series of sections were also cut towards the tendon. Staining for myosin ATPase after alkaline or acid preincubation [7], succinate dehydrogenase [15] and α-glycerophosphate dehydrogenase [10] was performed on serial sections.

For electron microscopy the muscles were fixed in 5 % glutaraldehyde in 0.1 M phosphate buffer. During subsequent washing in buffer with added 0.2 M sucrose the muscles were dissected into thin bundles of fibres which were postfixed in 1 % phosphate-buffered osmium tetroxide, dehydrated in alcohol and embedded in Epon. Samples from different groups of fibres were selected for thin sectioning. The sections were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop 1 A electron microscope. Calculations of sarcotubular system volume were performed by point counting analysis on slightly oblique transverse sections, randomly sampled from different blocks and levels [27].

Results

Histochemistry. The histochemical profile of EDL was profoundly transformed by cross-reinnervation. In contrast with C-EDL and S-EDL which were almost entirely (> 95 %) composed of type II fibres, i.e. fibres which stain darkly for myosin ATPase after alkaline preincubation and lightly

after acid preincubation, X-EDL was predominantly composed of type I fibres, which display a reversed pH dependence (Plate I). The degree of histochemical conversion varied from about 60 to 80 % in different muscles, but was constant in the same muscle at different levels. Fibres with intermediate staining properties comprised only a minor proportion of the fibre population in cross-reinnervated muscles. Parallel changes were observed in sections incubated for α-glycerophosphate dehydrogenase. Succinate dehydrogenase activity was moderate in the type I fibres of X-EDL, like in the corresponding fibres in soleus, and consistently high in the type II fibres persisting in cross-reinnervated muscles: type II-white fibres, which were numerous in C-EDL and S-EDL, were absent in X-EDL. In agreement with previous reports [1, 5, 24] X-EDL was always relatively atrophic when compared with control muscles. Fibre size was variable in X-EDL, but there was no consistent difference in size between type I and type II fibres. Extremely atrophic, presumably non reinnervated, muscle fibres were occasionally seen, isolated or in small groups, in cross-reinnervated muscles.

Electron microscopy. The ultrastructure of muscle fibres in C-EDL and S-EDL was similar to that previously described for normal EDL [22]. All fibres in these muscles were characterized by a richly developed sarcoplasmic reticulum (SR) both at the A- and I-band level (Plate II). By contrast, the mitochondrial content varied from fibre to fibre: the large "white" fibres showed only few elongated mitochondria extending transversely in the intermyofibrillar spaces at the I-band, whereas the small "red" fibres contained also subsarcolemmal accumulations of mitochondria and longitudinal chains of large mitochondria running along the myofibrils. Parallel variations were observed in the Z-band which was wider in the "red" fibres.

The fine structure of most fibres in X-EDL did not conform to that of either "white" or "red" fibres in control muscles. The most striking difference concerned the relative amount of SR, which was best evaluated in transverse sections. As shown in Plate III, only sparse profiles of SR were seen in cross-reinnervated muscle fibres both at the A- and I-band level. In this respect X-EDL fibres appeared similar to soleus muscle fibres. In one X-EDL which showed the greatest histochemical transformation (78 % type I fibres) the estimate volume of the entire sarcotubular system (SR plus T system) was 3.6 ± 0.4 %; the corresponding value for S-EDL was 8.1 ± 0.5 %. Due to the small contribution of the T system to the sarcotubular system volume these values should reflect mainly differences in SR volume. In longitudinal sections enface views of the SR were rarely encountered in X-EDL fibres. The area of contact between SR and T system was also apparently reduced, as T tubules flanked only along one side by SR cisternae were often seen. The myofibrils were largely confluent at the A-band; at the I-band they were separated by elongated branched mitochondria connected in an extensive transverse network with longitudinal projections in the A-band. This distribution of mitochondria is similar to that observed

in soleus muscle fibres. A minor proportion of fibres in X-EDL showed a more abundant mitochondrial complement and also a more extensive SR. By contrast, the mitochondria-poor fibres with thin Z-band, which represented a large proportion of the fibre population in control EDL, were completely absent in X-EDL. Most fibres in X-EDL displayed a peculiar M-band consisting of four M lines. This M-band pattern, which is typical of most soleus muscle fibres (Sjöström and Schiaffino, in preparation), was never seen in control EDL fibres whose M-band was composed by three or five M lines.

Discussion

The fast-twitch EDL muscle of the rat is changed to a slow-twitch muscle by the soleus nerve and its myosin becomes similar to the soleus myosin [1]. The present study shows that the histochemical and ultrastructural properties of EDL fibres are also changed to a slow type by the new innervation. The degree of histochemical conversion of X-EDL was greater than that found previously in other cross-reinnervated fast-twitch muscles [6, 8, 9, 18, 19], presumably because reinnervation by original fibres has been more effectively eliminated. The absence of type II-white fibres and the presence of a minor proportion of type II-red fibres in X-EDL is consistent with the absence and presence, respectively, of corresponding motor units in soleus [12]. Ultrastructural remodeling in X-EDL fibres involved the myofibrils, with changes in the Z-band and M-band, the mitochondria, with changes in both their total mass and distribution, and the SR, which was drastically reduced in amount. These findings indicate that, in the rat, mature fast-twitch muscle fibres maintain a considerable plasticity not only at the molecular level but also at the level of substructures and organelles. The changes in SR are of particular interest since they may be related to the modified time course of the isometric twitch of X-EDL. The duration of the active state of mammalian muscle fibres is probably determined by the rate of uptake of calcium by the SR, and the latter is in turn dependent on the total amount of active SR and on the specific activity of SR membranes [5].

Both factors can apparently be influenced by the type of innervation, as shown by this study and by previous reports on changes in the calcium-transporting activity of isolated sarcotubular vesicles [13, 14]. Comparable biochemical changes in SR properties can be induced in fast-twitch muscles by electrical stimulation at a frequency imitating the rate of discharge of slow motoneurones [25]. The contractile and metabolic characteristics of fast-twitch muscle fibres and the properties of their myosin are likewise modified by chronic stimulation at low frequency [16, 20, 21]. In the light of these findings it would be of interest to investigate whether the structural differences between skeletal muscle fibres are also established and maintained by specific patterns of impulse activity.

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EXPLANATION OF PLATES I-III

PLATE I

Figs. 1–2. – Transverse serial sections of a block composed of cross-reinnervated (X) and contralateral (C) EDL muscle of the rat. The sections were incubated for myosin ATPase after alkaline (fig. 1) and acid (fig. 2) treatment. Note the almost complete histochemical conversion of the cross-reinnervated muscle. Type grouping of persisting type II fibers in X-EDL is also apparent. ×20.

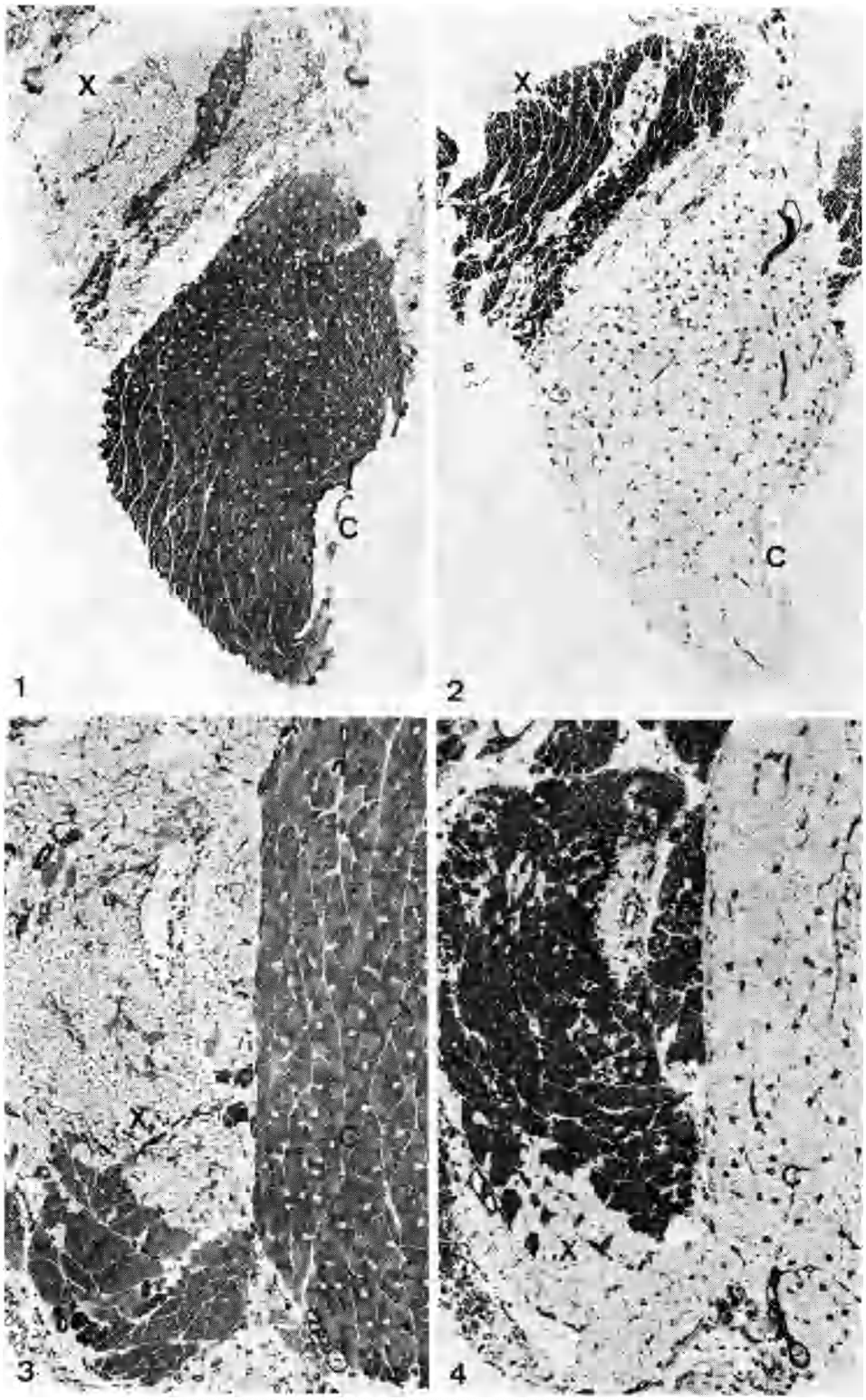
Figs. 3–4. – The figures illustrate the histochemical conversion of another cross-reinnervated (X) EDL muscle, compared with contralateral control (C). Sections incubated for myosin ATPase after alkaline (fig. 3) and acid (fig. 4) treatment. ×30.

PLATE II

Figs. 5–6. – Transverse sections through a " white " [5] and a " red " [6] fibre of control EDL muscle. The figures illustrate the rich development of sarcoplasmic reticulum in both kind of fibres. Mitochondria are more abundant in the " red " fibre. I: I-band; A: A-band. 5) ×20,000; 6) ×37,500.

PLATE III

Figs. 7–8. – Transverse sections through the same cross-reinnervated EDL muscle shown in figs. 1 and 2. Note the scarcity of sarcoplasmic reticulum both at the A-band [7] and at the I-band [8] and the extensive, transversely oriented mitochondrial network at the I-band. ×37,500.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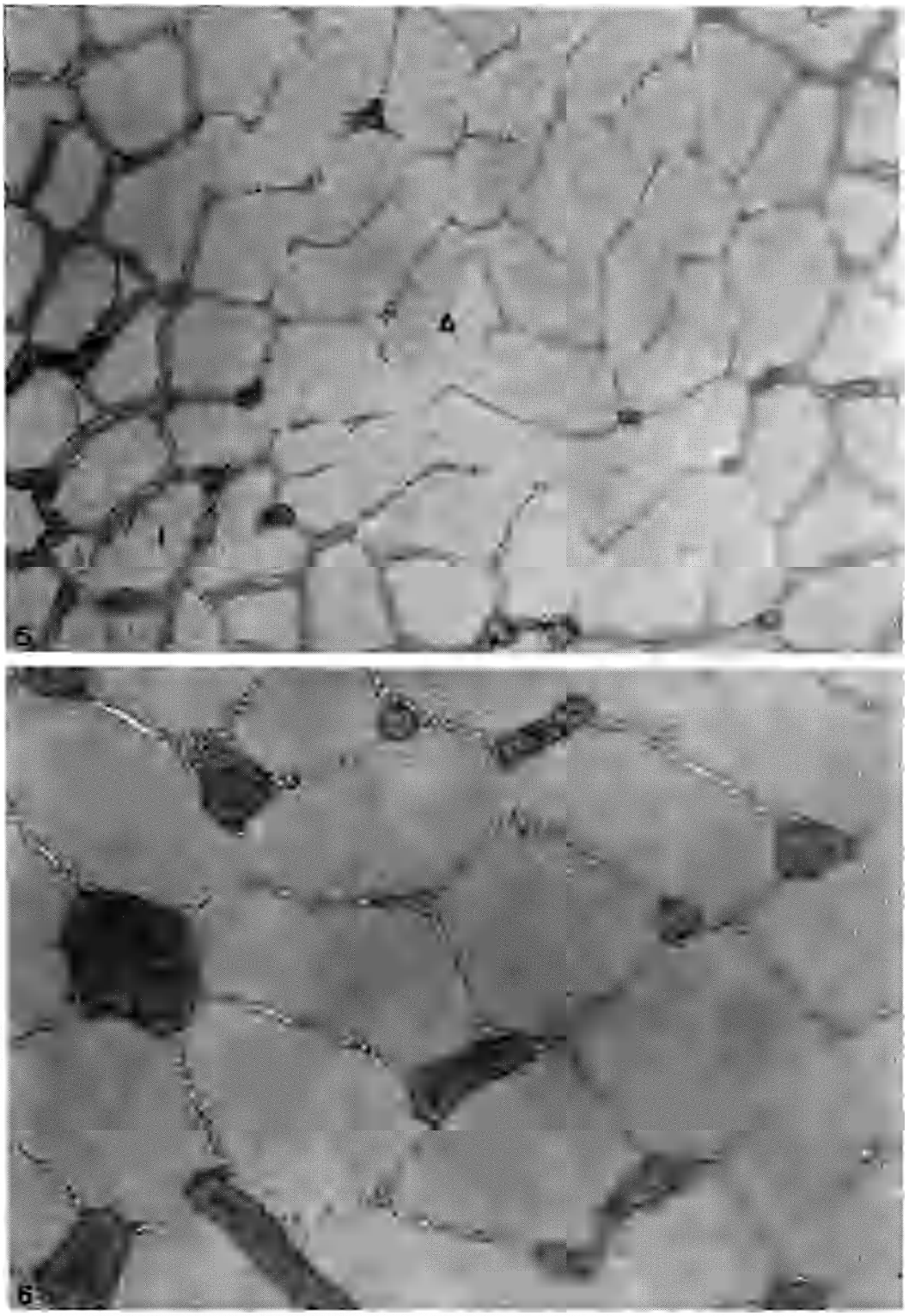

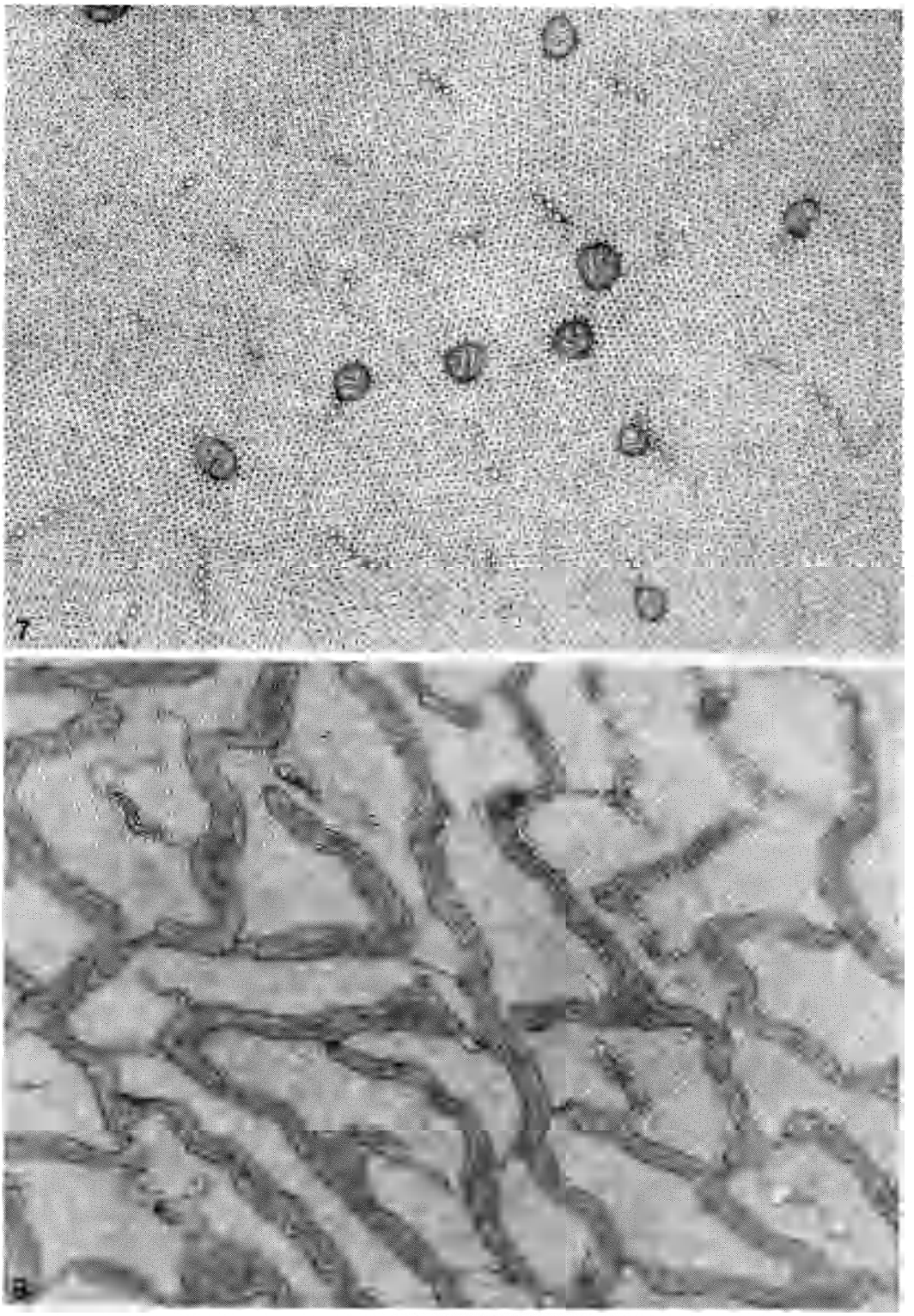
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