Electron Microscope Study of the Human Neuromuscular Junction

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PLATES 3 TO 5

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ABSTRACT

A preliminary electron microscope study of human neuromuscular junction is presented.

The biopsy material was taken from the palmarus longus, and fixed routinely in osmium tetroxide and embedded in methacrylate. The structure of the motor endings and the relationship of the synaptic vesicles to the axolemmal membrane are described. The synaptic clefts are filled with an homogeneous material in continuity with the basement membrane covering the muscle fiber. The subneural apparatus is described, and special attention is paid to a vesicular component present in the sarcoplasm of the junctional area, which differs from synaptic vesicles and is presumed to be a derivate of the sarcoplasmic reticulum.

During the last few years the structure of the neuromuscular junction has been the object of repeated electron microscope study (5, 7, 8, 13, 14). Synaptic vesicles have been described as a fundamental component of the nerve ending. The physiological significance of these vesicles is not yet entirely clear, especially since Pease described similar vesicles in dendritic endings (11). On the muscular side of the neuromuscular junction, a profuse folding of the sarcolemma has been observed and this structure is recognized as responsible for the lamellar appearance seen with the light microscope. It is known as the "subneural apparatus" (4). This apparatus is present in all animal species where the motor impulse is transmitted through a motor end-plate; in reptiles, amphibians, birds, and mammals including man (1).

To our knowledge, the human neuromuscular junction has not been previously studied with the electron microscope. As a result of the development of highly precise biopsy techniques (2), such material is now available for electron microscopy. Although these neuromuscular biopsies were taken from diseased subjects, it is possible to obtain muscle samples with unaltered neuromuscular junctions. These samples were used for the present description of the normal neuromuscular junction in man.

Techniques

A neuromuscular biopsy of the palmaris longus was performed on two patients, one suffering from a fever of unknown origin and from anemia, the other from asthenia and mild muscular tenderness. Since the neuromuscular biopsy technique has been described in detail elsewhere (2, 3), only its main features will be described here. The muscle fascia is exposed and incised. The band of terminal motor innervation, where the motor end-plates are concentrated, is then localized by measuring the excitability threshold of this band, which is lower than that of the other parts of the muscle fasciculi. (For this purpose, a sterile metallic electrode connected to the cathode of an electric stimulator[1] is used, and 1 per second impulses of 1 to 2 milliamperes are applied to the muscle surface.) Thus, a very small muscle biopsy of 2 to 4 mm. in length may be obtained which provides a reasonable chance of finding neuromuscular junctions in the thin sections cut for the

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[1] Stanley-Cox Ltd., London.

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electron microscope. The muscle samples were immediately fixed for 1 hour in chilled 1 per cent OsO_4 buffered at pH 7.2 and containing 0.2 molar sucrose. After dehydration in 70, 90, and 100° methanol, the tissue was embedded in a prepolymerized mixture of 9 parts butyl and 1 part methyl methacrylate. The plastic was allowed to polymerize overnight at 45°C. Sections were obtained with a Porter-Blum microtome equipped with a diamond knife, and were examined with a Siemens Elmiskop I electron microscope.

OBSERVATIONS

In these biopsies, numerous neuromuscular junctions were observed. Light microscope investigation of these samples, using methylene blue vital staining and Koelle's method for cholinesterase, did not show any pathological alterations in the second case, which, therefore, provided an opportunity for studying (1) the motor endings, (2) the synaptic clefts, and (3) the subneural apparatus.

The motor endings have a diameter of 2 or 3 μ (Fig. 1). They are situated in depressions of the muscle fiber surface classically described as the synaptic gutters. They are surrounded by an axolemmal membrane 70 A thick. The axoplasm contains mitochondria and synaptic vesicles (Fig. 3). The mitochondrial profiles are frequently elongated, measuring approximately 1 μ in length and 0.1 μ in width. The few cristae present are most frequently oriented in the direction of the long axis of the mitochondrion. Synaptic vesicles are spherical or oval in shape. Their mean diameter averages 300 A and the thickness of their single limiting membrane averages 50 A. Their distribution in the axoplasm is irregular. They are particularly numerous at the periphery of the nerve ending in the region facing the muscle fiber, where they number up to 250 per square micron of thin (silver) section. At this level, synaptic vesicles are frequently in intimate contact with the axolemmal membrane, giving it an irregular outline. On the opposite side of the motor endings, *i.e.*, on the side away from the muscle fiber, synaptic vesicles are far less numerous, and are rarely seen in contact with the axolemma which is smoother and more regular.

In accordance with Palay's designation (10), the name "synaptic cleft" will be used to describe the space between the axolemma and the sarcolemma. This space, however, can be subdivided into primary and secondary clefts (Figs. 1 and 2). The secondary clefts are perpendicular extensions of the primary clefts. The primary clefts border the axolemma, whereas the secondary clefts plunge into the muscle fiber and are limited from it only by the infolded plasma membrane of the muscle fiber. The secondary clefts measure 1 or 2 μ in length. Their extremities reach the level of the myofibrils only in the central part of the junction. Those situated more peripherally are in contact with the numerous mitochondria which are crowded in the sarcoplasm of the motor end-plate (Fig. 1). Primary and secondary clefts are ~600 A in width, and are filled with a basement membrane–like substance which is homogeneous and moderately dense. This substance is in complete continuity with the basement membranes which cover the whole sarcolemma and the teloglial cells. These basement membranes merge near the synaptic gutter. No synaptic vesicles were seen crossing the synaptic clefts. Unidentified dense bodies, (Figs. 2 and 3), averaging 280 A in diameter, were, however, infrequently observed in the secondary clefts.

The subneural apparatus is formed by lamellae of sarcoplasm interdigitating with the secondary clefts and separated from them by a continuous folded plasma membrane 70 A thick, the sarcolemma. These lamellae do not contain any myofibrils and show at least two different types of cytoplasmic organelles: vacuole profiles, and small dense granules. The vacuole profiles (Fig. 2) are not regular in shape, and their mean diameter is 500 A. They are frequently arranged in single rows along the sarcolemma limiting the secondary clefts. These vacuole profiles seem to be a direct continuation of short double membrane systems also present in the sarcoplasm and are probably parts of the endoplasmic reticulum or sarcoplasmic reticulum of the fibre (Fig. 3). There is, consequently, a marked difference between these vacuole profiles and those of the synaptic vesicles of the motor endings. Dense granules with a mean diameter of 150 A are also found in clusters in the sarcoplasm of the subneural apparatus (Fig. 3). Granules of similar appearance and dimension were described recently in insect neuromuscular junctions and were called "aposynaptic granules" (7). The small granules observed in the present material can be designated in the same way. These aposynaptic granules do not seem to be in contact with the sarcolemma, the mitochondria, or the endoplasmic reticulum. Numerous small mitochondria lie between the different junctions of

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the same motor end-plate (Fig. 1). These mitochondria are irregular in shape and orientation. Their cristae are frequently transversely oriented, and the mitochondrial matrix contains numerous dense granules averaging 230 A in diameter.

DISCUSSION

According to a recent publication by De Robertis (6), it can be argued that the electrical stimulation applied to localize the motor end-plate may induce some changes in the number of the synaptic vesicles. It is most unlikely, however, that the low frequency, and low intensity current used in the present study could have produced this kind of modification.

With due reservation for the small amount of material thus far examined, the main differences between the human neuromuscular junctions and those previously described in other species are: (1) the more elaborate subneural apparatus, with numerous secondary clefts penetrating deeply into the sarcoplasm; (2) the complex vacuolar system in the sarcoplasmic lamellae; and (3) the homogeneous appearance of the material filling the primary and secondary clefts, which are occupied by a basement membrane-like substance. This homogeneous substance is continuous with the two basement membranes covering the sarcolemma and the teloglial cells. These two basement membranes merge near the synaptic gutter and extend into all the synaptic clefts. This basement membrane material is presumably responsible for the positive PAS reaction of synaptic membranes. Layers of different densities like those described by Robertson (14) have not been observed.

Histochemical techniques have shown that a junctional cholinesterase is located at the level of subneural lamellae (2, 3). If the enzyme is actually linked to a sarcoplasmic component, it would be of great interest to find out which structure is involved. Aposynaptic granules were assumed to be involved in the chemical process of neuromuscular transmission in insects (8). Similar aposynaptic granules were present in the human material used for this study. Up to now, however, there is no evidence that an enzymatic function can be attributed to the aposynaptic granules. Moreover, it should be stressed that the RNP particles of Palade have been described in the sarcoplasm (9). These granules, however, have a diameter of 200 to 300 A, and are thus larger than the asposynaptic granules described in the present paper. Nevertheless, the possible identity between aposynaptic granules and the Palade particles should be kept in mind, despite the difference in size.

The secondary synaptic clefts are in close contact with a complex vacuolar system. The hypothesis presented here states that these vacuole profiles differ from synaptic vesicles, and are closely related to the endoplasmic reticulum. Porter (12) and Ruska (15) assumed that the endoplasmic reticulum could act as an internal conduction system. According to this hypothesis, the possible relationship observed in the present study between secondary clefts and endoplasmic reticulum may be involved in the propagation processes of muscular contraction.

The study of human material, particularly in cases of neuromuscular disease and disorders of contraction will, perhaps, shed some light on the physiological significance of the complex structure of the neuromuscular junction. Further studies in this direction are in progress.

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BIBLIOGRAPHY

1. Coërs, C., *Arch. Biol.*, 1953, **64,** 133.
2. Coërs, C., *Acta Neurol. et Psychiat. Belg.*, 1955, **55,** 741.
3. Coërs, C., and Woolf, A. L., The Innervation of Muscle, a Biopsy Study, Blackwell Scientific Publications, Oxford, 1959.
4. Couteaux, R., *Rev. Canad. Biol.*, 1947, **6,** 563.
5. De Robertis, E. D. P., and Bennett, S. H., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 47.
6. De Robertis, E. D. P., *Exp. Cell Research*, 1958, suppl. **5,** 347.
7. Edwards, G. A., Ruska, H., and de Harven, E., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 107.
8. Edwards, G. A., Ruska, H., and de Harven, E., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 251.
9. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 59.
10. Palay, S. L., *Exp. Cell Research*, 1958, suppl. **5,** 275.
11. Pease, D. C., and Quilliam, T. A., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 331.
12. Porter, K., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 163.
13. Reger, J. F., *Exp. Cell Research*, 1957, **12,** 662.
14. Robertson, J. D., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 381.
15. Ruska, H., *Exp. Cell Research*, 1958, **5,** 560.

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Explanation of Plates

Key to Abbreviations

A, Axolemma.
BM, Basement membrane.
E, Cytoplasmic sheet of endomysial cell.
ER, Endoplasmic reticulum.
ME, Motor ending.
MM, Mitochondria of the motor ending.
P, Sarcoplasmic mitochondria.
PSC, Primary synaptic cleft.
S, Sarcolemma.
SG, Small sarcoplasmic granules.
SSC, Secondary synaptic cleft.
SV, Synaptic vesicles.
VP, Sarcoplasmic vacuolar profiles.

Plate 3

Fig. 1. Two junctional areas of the same motor plate are shown. They are separated by a swarm of small mitochondria (*P*). The general organization of primary and secondary clefts is seen. The continuity between the basement membranes (*BM*) and the material within the synaptic clefts (*PSC* and *SSC*) is evident. It is clear, also, that the secondary synaptic clefts located in the central part of each junction extend close to the myofibrils. × 18,000.

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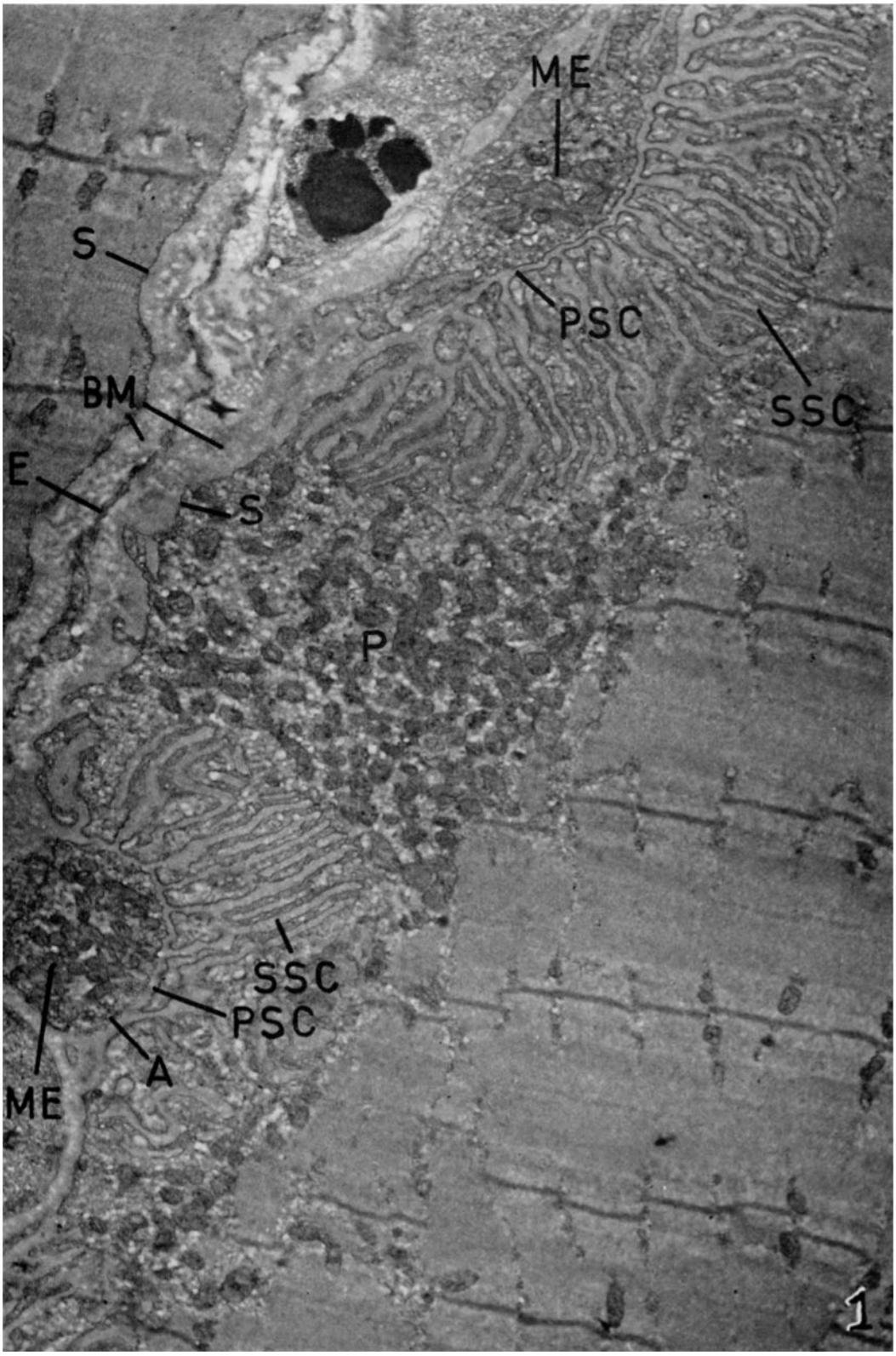

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PLATE 4

FIG. 2. Higher magnification of one of the junctions seen in Fig. 1. The axoplasm of the motor ending (*ME*) contains synaptic vesicles (*SV*) and mitochondria. The synaptic vesicles are particularly numerous in the region facing the primary synaptic cleft. There is no recognizable filamentous structure in the axoplasm. The synaptic clefts are filled with an homogeneous material which is continuous with the basement membranes. Unidentified granules are seen in two secondary synaptic clefts (arrows). Single rows of vacuole profiles (*VP*) are situated in the sarcoplasmic lamellae between secondary clefts. The numerous mitochondria surrounding the junction are irregular in shape, and their matrix contains numerous, dense granules averaging 230 A in diameter. (The mitochondria in the motor endings have no such granular component in their matrices.) × 44,000.

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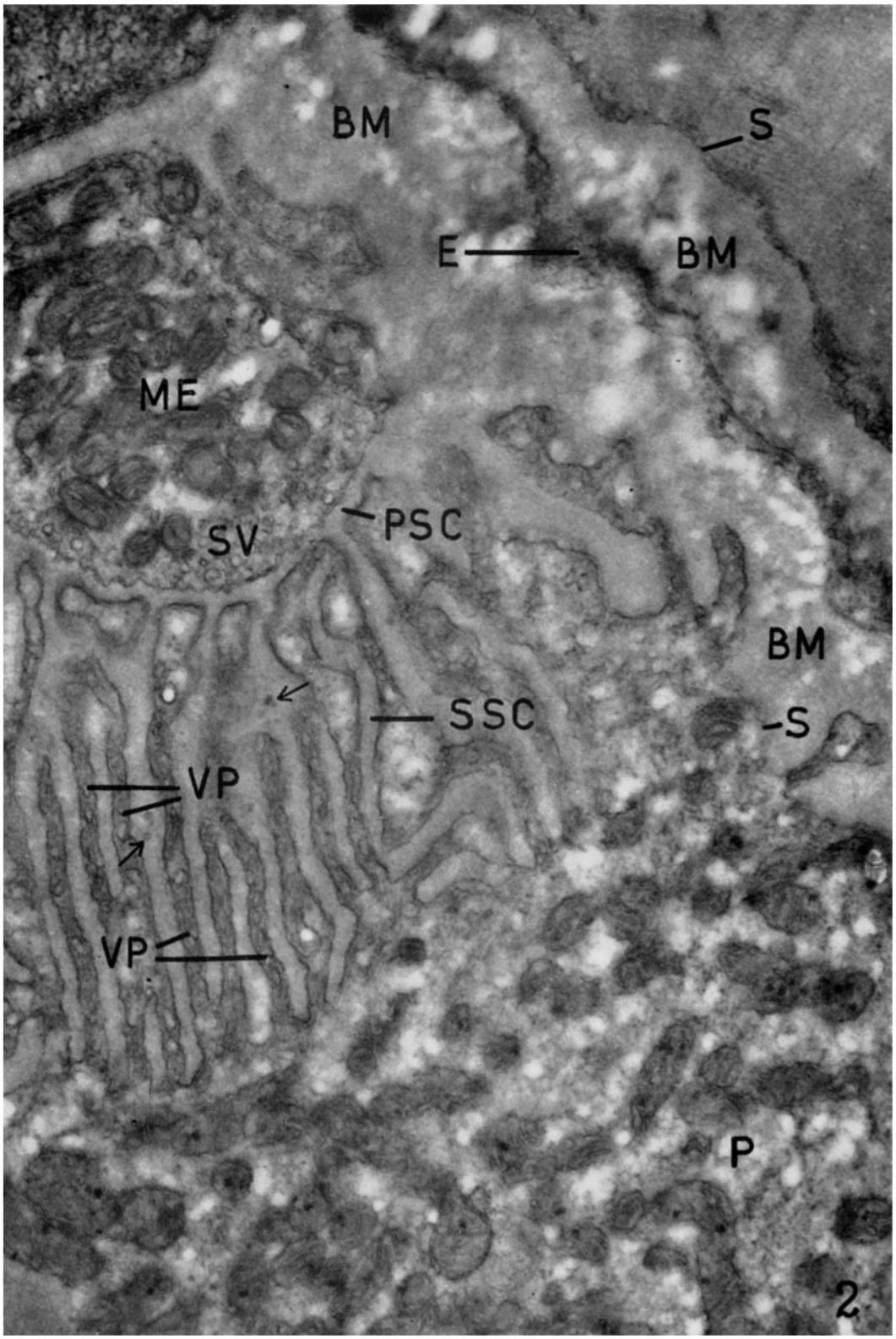

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PLATE 5

FIG. 3. The mitochondria of the motor ending (*MM*) are characterized by the longitudinal orientation of their cristae. Synaptic vesicles (*SV*) are regular in shape and in dimension. No continuity between these vesicles and other axoplasmic structures is observed. The crenated outline of the axolemmal membrane (*A*) is evident, contrasting with the smoothness of the sarcolemmal membrane (*S*). The synpatic clefts are filled with an homogeneous substance. The primary synaptic cleft seems to be particularly large in this micrograph, but this may be the result of the obliquity of the section. One unidentified granule is present (arrow). In the sarcoplasmic lamellae, clusters of small dense granules (*SG*) are recognizable. One vacuole profile (*VP*) is apparently continuous with the endoplasmic reticulum (*ER*). × 98,500.

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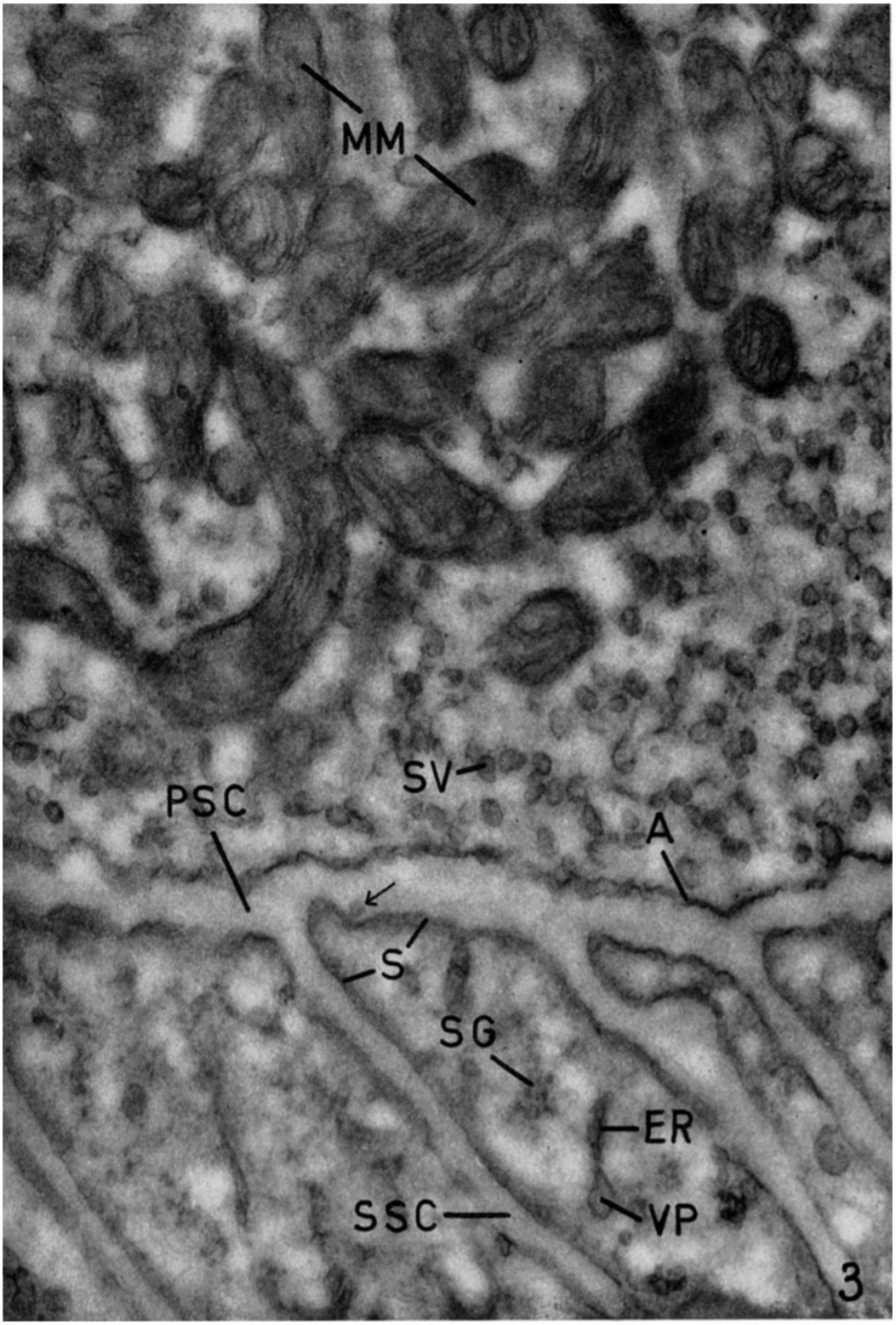

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