J Physiol. (1960), **150**, *pp.* 134–144
With 6 *plates and* 3 *text-figures*
Printed in Great Britain

THE FINE STRUCTURE OF THE NEUROMUSCULAR JUNCTION OF THE FROG

BY R. BIRKS, H. E. HUXLEY AND B. KATZ
From the Department of Biophysics, University College London

(*Received* 20 *July* 1959)

In recent years fairly detailed information has been obtained on the physiological events which occur at the vertebrate neuromuscular junction. Experiments on frog and mammalian muscle have shown that the transmitter, acetylcholine, is released from the axon terminals in discrete multi-molecular packets, and attention has been directed to the presence of submicroscopic 'vesicles' inside the nerve endings and their possible significance in the quantal distribution of the transmitter agent (de Robertis & Bennett, 1954, 1955; del Castillo & Katz, 1956; Robertson, 1956*b*; Palay, 1956; de Robertis, 1958; Reger, 1958; Katz, 1958). The purpose of the present work was to examine in more detail the submicroscopic structure of the myoneural junction in the frog from which the greater part of the physiological evidence had been derived. The basic information on the structure of this synapse is already available, as a result of the remarkable microscopic and histochemical studies of Couteaux (1947, 1955, 1958).

METHODS

For most purposes junctional regions of muscle fibres situated at the edge of the frog's sartorius were selected. The muscles were carefully dissected, mounted spirally on Perspex rods and examined in transmitted light with a dissecting microscope, in the way normally used for physiological experimentation (see del Castillo & Katz, 1957*a*). The innervation of the edge fibres of the muscle can be recognized without much difficulty even in the fresh state and shows up more clearly after fixation in osmic acid, enabling one to cut out a small junctional region, about 0·5 mm square, for further examination. The use of fibres on the edge of the muscle has the further advantage of providing suitably thin specimens for fixation and embedding. If potassium permanganate is used as a fixative, previous marking of the required area is necessary, e.g. by attaching a small ligature to the connective tissue at the edge. An alternative method is to dissect a fine bundle of fresh muscle fibres (from the adductor longus) whose innervation zone can be easily recognized. However, selection of junctional portions from the edge of the sartorius was so simple and successful that it was adopted throughout.

The usual procedure was to cool the muscle and then to fix it in ice-cold OsO_4 solution, 1% (w/v), buffered with acetate-veronal (Palade, 1952), for 30 min. After transferring it to 70% ethyl

alcohol, the junctional regions were cut out, dehydrated and stained during the night with 1% phospho-tungstic acid (PTA) in alcohol. Some muscles were fixed in 0·6% buffered $KMnO_4$ solution (Luft, 1956) for 6 hr at 0° C, others in OsO_4 without additional PTA stain. The specimens were embedded, after preliminary soaking for at least 24 hr, in 'Araldite' mixture (Glauert & Glauert, 1958), in gelatine capsules or polythene moulds shaped so that they could be conveniently mounted for longitudinal or transverse sectioning.

The majority of specimens were examined in longitudinal section, which was found to give the more useful information. The disadvantage of this procedure was that, because of the characteristic longitudinal spread of the amphibian nerve endings along the surface of the muscle fibre (Kühne, 1887), more time had to be spent in finding sections which contained them. For final examination in the electron microscope (Siemens, Elmiskop I, used at 60 or 80 kV), the microtome was set to provide sections approximately 400–800 Å thick.

RESULTS

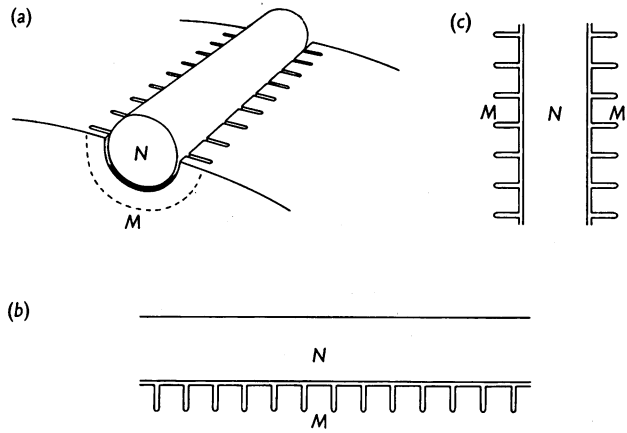
To appreciate the details seen in electron micrographs of the myoneural junction, it is necessary first to give a brief summary of its general lay-out as revealed by optical microscopy. The following description applies to the innervation of 'fast' skeletal muscle fibres in the frog. The myelinated motor axon approaches the muscle fibre and gives off an array of non-myelinated terminal branches which spread along the fibre surface in both directions in the shape of an elongated 'tree' or 'antler' (Kühne, 1887). Its total length may amount to a few hundred micra. The terminal branches run parallel to the axis of the muscle fibre and lie in shallow 'gutters', i.e. depressions formed by the fibre surface. In Text-fig. 1 a diagram of a small part of such a nerve–muscle junction is shown. An additional feature is seen in this diagram, that the superficial gutters of the muscle fibre are depressed at regular intervals into deeper local 'trenches', the so-called junctional folds (Robertson, 1956*b*) which run at a right angle to the fibre axis and communicate with the external space at both sides of the nerve terminal.

It may be recalled that this system of trenches shows up strikingly with specific cholinesterase-staining methods (Couteaux, 1955), and because of its solid appearance was at one time regarded as a palisade-like structure consisting of a series of lamellae, rather than of folds containing, and communicating with, the aqueous extracellular phase. There is, of course, no conflict between this latter view which is based on electron-microscopic examination (Palade, 1954; Robertson, 1956*b*) and the histochemical result, for the structure of the folds cannot be resolved with the light microscope.

In Text-fig. 1 three principal planes of section are illustrated which determine the aspect of the myoneural junction seen in the electron micrograph; a transverse section and two longitudinal sections through the muscle fibre, one of which is called tangential because it grazes the muscle at the level of its junctional folds while the others pass more deeply through the fibre.

Plates 1, 2 and 5 show typical electron micrographs from a myoneural junction of the frog sartorius in longitudinal section through the muscle fibre. If one compares them with the diagram (Text-fig. 1), the most interesting

difference, apart from the wealth of structural detail, is the presence of a third cellular layer which separates the nerve ending from the extraneous connective tissue. This layer is made up of nucleated cells which form the terminal Schwann-cell cover of the axon. Plate 2*A* shows a longitudinal section which passes through the nuclear region of this 'satellite cell'. The triple-complex, Schwann cell, nerve ending, muscle fibre, is seen equally in permanganate-fixed preparations (Pl. 4*A*) and in the transverse sections in Pls. 2*B*, 3 and 4*B*.



Text-fig. 1. Diagram of neuromuscular junction. (*a*) Showing small portion of a terminal axon branch (*N*) lying in a 'gutter' formed by the surface of the muscle fibre (*M*), and the array of semi-circular, post-junctional, folds. The frontal view shows cross-sectional aspect of the junction. The dotted line indicates the recessed border of the muscle fibre in the junctional fold. (*b*) Viewing the junction in longitudinal section; (*c*) in 'tangential' section.

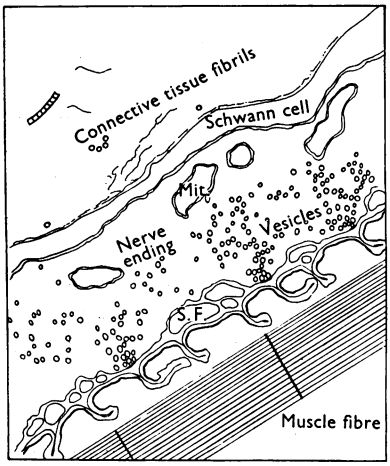
Much of the structural detail shown in the present pictures has been described before (Robertson, 1956*b*; Palay, 1958; Andersson-Cedergren, 1959) in connexion with other neuronal or myoneural junctions, and there is no need here to point out more than a few special features.

Terminal Schwann-cell layer

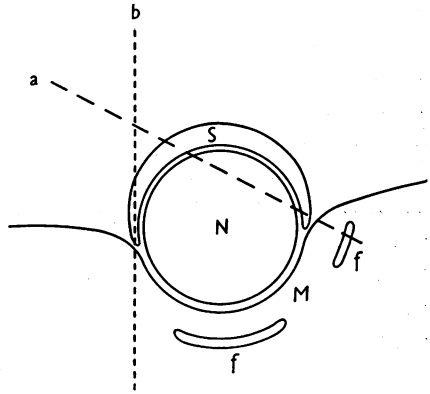
In the majority of the micrographs the Schwann-cell coating, which separates the axon terminal from collagen and finer connective tissue fibrils, was seen quite clearly. In some sections the Schwann cytoplasm formed a very thin layer and its border lines were at times obscured, presumably as a result of oblique sectioning. But in only one out of several hundred micrographs did we find a nerve ending which was 'naked' and clearly lacked a Schwann cover over an appreciable length.

A point of interest is the extent to which the satellite cell intrudes into the synaptic space between nerve and muscle. In cross-section (Pls. 2–4) the

Schwann cell is seen to engulf the nerve ending, but it never surrounds it completely, and its contact with the muscle fibre is restricted to a short stretch at either side of the axon. In longitudinal section (Pls. 1, 2, 5; Text-fig. 2), one often finds cellular segments of various shapes interposed between nerve and muscle. These are probably parts of the same Schwann cytoplasm whose 'fingers' protrude in an irregular way into the synaptic region. It is clear, at any rate, that a longitudinal section through the structures shown transversely in Text-fig. 3 may pass through the Schwann cell twice, and show its edge interposed between nerve and muscle surfaces.



Text-fig. 2



Text-fig. 3

Text-fig. 2. A tracing of some characteristic components of the myoneural junction, from Pl. 1. *Mit.*: mitochondria of axon terminal; *S.F.*: a 'Schwann finger' (cf. Text-fig. 3, line *a*).

Text-fig. 3. Schematic cross-sectional view showing Schwann cell (*S*), nerve ending (*N*), muscle fibre (*M*) with junctional folds (*f*). *a* and *b* indicate planes of longitudinal section in which the Schwann cell may be seen in contact with the muscle fibre (and, in *a*, interposed between nerve and muscle).

It will also be evident that there is a chance of missing the nerve ending and obtaining a longitudinal aspect in which only a layer of Schwann cytoplasm is seen in contact with the synaptic (i.e. folded) region of the muscle membrane. However, such occurrences would be rare, and we have, in fact, never found a section in which the nerve ending did not show up at some place of the junctional region.

In spite of this complication, the use of longitudinal rather than cross-sections has one definite advantage, namely that it enables one to identify the synaptic region in normal as well as denervated muscle by the very regular disposition of the junctional folds which survive nerve degeneration (Birks, Katz & Miledi, 1960). Because of their regular and approximately transverse alignment, junctional folds are much less conspicuous in cross-sections of frog muscle. In this respect the situation in the frog differs from the arrangement in reptiles and mammals, as has been fully explained by Couteaux (1958).

The structural detail of the Schwann cytoplasm varies greatly from one area to the next. In some sections no inclusions can be seen apart from a few fine granules and filaments. In other regions there is an accumulation of 'vesicles' of various sizes and of mitochondria. Occasionally complicated deep foldings of the cell membrane are observed. The nucleus is surrounded by a 'perinuclear bag', a few hundred Ångström units wide, with a thin membrane separating it from the cytoplasm.

Membrane relations

The surface membrane of the Schwann cell shows the characteristics described by Robertson (1959). It is separated from the axon membrane by a space of about 150 Å width (this measurement applies to preparations fixed with OsO_4; in those fixed with $KMnO_4$ (Pl. 4*A*) the space appears to have shrunk considerably). On the other side of the Schwann cell, facing the connective tissue space, there is the familiar external dense layer separated from the Schwann cell membrane by a space *ca.* 200–300 Å across. This layer can be followed around the surface of the Schwann cell up to the place where it contacts the muscle fibre (Pls. 3, 4*B*, 5*B*). At this point, the external layer fuses with a similar dense layer which envelops the muscle membrane. It is noteworthy that this layer can be traced continuously along the whole of the muscle fibre membrane right into the depths of its synaptic folds, while it does *not* penetrate at any point into the spaces between Schwann cell and axon.

The functional significance of this external layer is obscure. It is clearly not an integral part of any individual cell membrane (see also Robertson, 1959), and its intermediate position in the synaptic space between nerve and muscle makes it difficult to suppose that it can act as a diffusion barrier with the properties envisaged for the cell membrane itself. There is some additional evidence which distinguishes this dense layer from the membranes enclosing the individual cells and some of their internal particles. The electron-microscopic density and 'staining properties' of this layer were found, under different treatments, to be closely related to those of the muscle proteins and not those of the cell membrane: for example, in $KMnO_4$-fixed preparations (Pl. 4*A*), neither the myofibrils nor the external layer show up, while the membranes of the three cells, and of the intracellular vesicles, nuclei and mitochondria are observed in good contrast. In other $KMnO_4$-treated specimens the external layer was just visible, though at much less contrast than in OsO_4 + PTA preparations; in the same specimens the myofibrils showed up rather more densely than in Pl. 4*A*. Although these observations throw little light on the chemical properties of the external layer, they do at least support the idea that its composition differs from that of the cell membranes, and are not in conflict with what must be postulated on physiological grounds.

Axon terminals

The nerve endings are characterized by well-known cytoplasmic inclusions, notably an accumulation of 'synaptic vesicles' which have been described by many authors (de Robertis & Bennett, 1954, 1955; Palade, 1954; Palay, 1954; Robertson, 1956*b*).

There has been some question (Robertson, 1959) concerning the dimensions of these 'vesicles' and whether they do not really represent a cross-sectional aspect of a system of tubes. Such tubes, however, would have to be highly oriented to give the observed regularity of oval outlines, and their tubular aspect should be revealed by changing the plane of sectioning. In fact, the same population of vesicles is seen independent of the plane of sectioning (e.g. Pl. 2, *A–C*). Furthermore, in thick sections vesicles were seen whose contours overlapped, an observation which cannot be explained on the basis of a tubular structure. Moreover, whenever it was possible to examine serial or near-by sections of one specimen, with a sufficiently sparse vesicular distribution, it was found that the individual vesicles had dropped out in successive slices, while cylindrical structures of similar diameter (e.g. cross-sectioned groups of collagen fibrils) could be traced without difficulty. Nevertheless, on a few occasions numerous short 'tubes' or a meshwork of branching tubes were seen quite apart from mitochondria and vesicles. The diameter of these tubes was somewhat smaller than that of the vesicles (the latter being of the order of 500 Å).

Vesicles were counted in a series of sections from two specimens and their average 'volume density' was estimated on the assumption that each section covered effectively 800 Å in depth. The result was approximately 1000 vesicles/ μ^3 of axon terminal. If the whole of one terminal arborization occupies 1000 $\pi\mu^3$ (this being the volume of a cylinder of 1 μ radius and 1 mm length), the total number of vesicles present at such a junction would be approximately 3×10^6.

Another typical feature of the axon terminal is its mitochondria. They possess the characteristic twin-membrane of *ca.* 150 Å separation but their internal structure differs in appearance from the muscle mitochondria (Pl. 2*A*) which show a much more regular parallel system of cristae.

Within the terminal axoplasm the different particles are distributed, in general, not at random but in a consistent orderly pattern. Frequently one can divide the nerve terminal longitudinally into two main portions, a 'mitochondrial region' adjacent to the Schwann cover, and a 'vesicular region' near the muscle surface. Even within the latter region one often sees the vesicles concentrated in certain well defined areas (Pls. 1, 2*A* and 5) focused on a dense zone of the axon membrane directly opposite a post-synaptic fold. This interesting feature recurs much too frequently to be dismissed as fortuitous,

and it reminds one of somewhat similar observations on other types of synapses (Palay, 1958; Edwards, Ruska & de Harven, 1958).

The differentiation of the terminal axoplasm is at times made more obvious by the presence of bundles of very fine longitudinal filaments (less than 100 Å thick, with dense spots recurring a few hundred Å apart), which appear to separate the vesicular region from the rest of the axon terminal, for instance in Pl. 2*A*.

Finally some unusual observations should be mentioned which are illustrated in Pl. 6. The nerve terminal shows here an even greater variety of structural detail than is generally found. Some of the 'vesicular islets' (in the upper part of Pl. 6*B*) are probably axon processes which protrude into the Schwann cell. But there are also various concentrations of vesicles within the axon itself, each bounded by a twin membrane; and the smallest of these enclosures are difficult to distinguish from terminal 'mitochondria' whose internal structure has often a tubular or vesicular appearance. Without serial reconstruction it is, of course, not possible to decide whether the various vesicular aggregates are completely enclosed in separate compartments, or formed by complicated invaginations of the axon terminal (whose membrane shows indeed some deep folds, e.g. Pl. 6*A*).

The post-synaptic folds

These have been first described by Palade (1954) and Robertson (1954, 1956*b*), and it is only necessary to point out some peculiarities of the amphibian junction. The longitudinal spacing of the folds is rather regular: approximately 3–4 per sarcomere. The width of the folds varies between about 500 and well over 1000 Å, the central core of their lumen being occupied by the dense external layer discussed on p. 138. The deeper parts of the folds bend and run obliquely with respect to the fibre axis; they often branch and anastomose with adjacent folds.

In cross-section the chances of including a substantial portion of any single fold are not high, and one usually sees only fragments somewhere below the level of the myoneural contact. Occasionally, the section passes through the fold tangentially (e.g. Pl. 4*B*) and then shows a dense post-synaptic patch, and facing it a concentration of vesicles in the nerve terminal lined up against a dense portion of the axon perimeter (cf. p. 139).

The post-synaptic membrane itself is distinguished by an exceptionally high density of its superficial contours. These are always more heavily 'stained' than the ordinary membrane of the rest of the muscle fibre or of the axon or Schwann cell. It is noteworthy that this special contrast shows up very clearly in specimens treated with OsO_4 or with OsO_4 plus PTA, but not in those treated with $KMnO_4$. On the lines of the argument presented on p. 138 this might be taken to indicate the presence of a special protein layer attached to

large parts of the post-synaptic membrane. There is a further interesting differentiation within this area, seen in all OsO_4 + PTA preparations (e.g. Pl. 1), namely that the region of high density does not extend into the deeper parts of the folds; but this could conceivably arise from inadequate penetration of the stain.

DISCUSSION

The fine structure of synapses is of interest in so far as it may be related to physiological events. Unfortunately such relations are difficult to establish experimentally, and their discussion is at present based on little or no direct evidence. Some promising observations have been made by de Robertis (1958), which show that it is possible, at least in certain types of neuronal junctions, to produce significant structural changes by periods of stimulation or imposed inactivity. We have not been successful in obtaining conclusive evidence from muscles which had been subjected to even quite drastic synaptic 'stimulation' (e.g. exposure to high potassium concentrations and hypertonic media).

A reduction in number of the terminal vesicles was seen on some such occasions, but was not convincing because of the wide range observed 'normally'. A more striking change was a swelling and internal break-up of mitochondria in the nerve endings, which, however, could be interpreted as an unspecific sign of cell damage. Much more significant alterations are found during the early stages of neuromuscular degeneration, which will be described in a subsequent paper.

There are certain features of synaptic structure which have figured prominently in recent physiological discussions (see Eccles, 1957; Katz, 1958). Among these are the absence of cytoplasmic continuity, i.e. the completeness of the membrane separation at the synapse; the size of the extracellular space between the contacting cells; and the function of the pre-synaptic vesicles.

These features have been discussed, and plausible roles can be allotted to them, in the light of what is known about chemical transmission at certain synapses. Thus, the presence of separating membranes and an extracellular space between them is more readily compatible with chemical transmission than with direct 'core-conduction' (provided we are right in assuming that the electrical properties of these structures are comparable to those of membranes and interspaces elsewhere). The space of several hundred Å, between nerve and muscle membrane, and between the walls of the junctional folds, communicates with the extracellular fluid (without interposition of a cell membrane). This would constitute an undesirable leakage path for electric transmission from nerve to muscle, while it provides a highly desirable low-resistance path for the spread of depolarization from the post-synaptic focus to the rest of the muscle membrane (see Eccles & Jaeger, 1958; Couteaux & Katz, 1957). The whole of this space, including the dense external layer, was at one time classified as a 'membrane complex' (Robertson, 1956*a*, *b*; modified recently, 1959); but it

should be realized that these are the very spaces to which drugs like ACh and curare must have rapid access when applied from the external medium (del Castillo & Katz, 1957*a*, *b*).

As regards the role of the vesicles, very little can be added to what has been said before. There is good evidence that transmission at the nerve–muscle junction involves the release of ACh from the axon terminal, not as independently diffusing molecules, but in discrete multi-molecular packets (Fatt & Katz, 1952; del Castillo & Katz, 1956; Katz, 1958). The size of these quantal units appears to be quite independent of the state of the axon membrane, and to be determined by some other component of the nerve terminal. It has, therefore, been suggested that ACh may be contained in the vesicles and released in an all-or-none manner when the vesicle collides with certain spots of the terminal membrane. Such an event may involve coalescence of the membranes, of vesicle and axon, and diffusion of the vesicular contents into the synaptic space. It is interesting that the vesicles are frequently concentrated near the region of contact with the muscle fibre, and that they appear to 'converge' on special zones of the axon membrane directly opposite the junctional folds. It would seem an admirable arrangement to have the vesicles queuing for such strategically disposed regions of transmitter release, but it would imply that the intracellular distribution of vesicles is brought about by obscure forces of interaction and not just Brownian movement.

If we were not prejudiced by any physiological evidence, we might be tempted to interpret the structural picture in a very different way; for a simpler assumption would be that the vesicles distribute themselves by random motion from their sites of origin. On purely morphological grounds it might therefore be more natural to suppose that the particles are formed by vesiculation at the dense zone of the axon surface, and then spread within the axoplasm by random motion, until they collide, and possibly fuse, with obstacles, e.g. mitochondria, the axon boundary, or a meshwork of filaments. The purpose of this alternative proposition is merely to emphasize that the morphological picture in itself provides no evidence in support of our ideas, though it may quite easily be fitted to them.

Finally, the question may be raised of what the chances are of observing vesicles in the process of 'discharge'. The answer is not at all encouraging: on arrival of an impulse, i.e. at the highest peak of activity, a few hundred units of transmitter are released at one cell junction. The total length of the arborization within one junction is of the order of one mm, so that a single unit-release would occur, on the average, at points 3μ apart. Thus, even if it were possible to fix the specimen at the peak of synaptic activity, the chances of finding a 'discharging vesicle' are very small, and it would be a hopeless task to identify it. On rare occasions one does observe Ω-shaped structures ('opened flasks') on the synaptic surface of the axon; they might represent broken vesicles but could equally well be connected with the formation of new vesicles, or vacuoles at the fibre surface. Phenomena of this kind are seen much more

frequently at the surface of Schwann and endothelial cells. In effect, the statistical properties of the process of transmitter release are such as to make the chances of its detection in the electron-microscope extremely low.

SUMMARY

1. The fine structure of the myoneural junction in fast skeletal muscles of the frog has been examined in the electron microscope.

2. Three cell layers are found at the junction: (i) a nucleated Schwann cell which covers the 'non-synaptic' surface of the nerve terminal and separates it from extraneous connective tissue; (ii) the axon terminal with its accumulation of 'mitochondria' and '500 Å vesicles'; (iii) the muscle fibre with its regular transverse array of synaptic folds.

3. The 'synaptic space' between nerve and muscle membranes contains a dense intermediate layer which follows closely the contours of the muscle surface. Using different methods of fixation and staining (OsO_4, $KMnO_4$), the relative density of this layer is found to vary in a manner quite unlike that of cellular or intracellular membranes.

4. Within the nerve terminal, mitochondria and vesicles are often concentrated in separate regions. Vesicles tend to accumulate in the neighbourhood of discrete zones of the axon surface directly adjacent to post-synaptic folds.

We are indebted to Professor R. Couteaux for valuable discussion and to Mr J. L. Parkinson for his unfailing help. This investigation was supported by an equipment grant made by the Nuffield Foundation.

REFERENCES

ANDERSSON-CEDERGREN, E. (1959). Ultrastructure of motor end plate and sarcoplasmic components of mouse skeletal muscle fiber. *J. Ultrastructure Research*, Suppl. **1**, 1–191.

BIRKS, R., KATZ, B. & MILEDI, R. (1960). Physiological and structural changes at the amphibian myoneural junction, in the course of nerve degeneration. *J. Physiol.* **150**, 145–168.

COUTEAUX, R. (1947). Contribution à l'étude de la synapse myoneurale. *Rev. canad. Biol.* **6**, 563–711.

COUTEAUX, R. (1955). Localization of cholinesterases at neuromuscular junctions. *Int. Rev. Cytol.* **4**, 335–375.

COUTEAUX, R. (1958). Morphological and cytochemical observations on the post-synaptic membrane at motor end-plates and ganglionic synapses. *Exp. Cell Res.* Suppl. **5**, 294–322.

COUTEAUX, R. & KATZ, B. (1957). Discussion in Microphysiologie comparée des éléments excitables. *Colloq. int. Cent. nat. Rech. sci., Paris*, **67**, 255–258.

DEL CASTILLO, J. & KATZ, B. (1956). Biophysical aspects of neuromuscular transmission. *Progr. Biophys.* **6**, 121–170.

DEL CASTILLO, J. & KATZ, B. (1957*a*). A study of curare action with an electrical micro-method. *Proc. Roy. Soc.* B, **146**, 339–356.

DEL CASTILLO, J. & KATZ, B. (1957*b*). The identity of 'intrinsic' and 'extrinsic' acetylcholine receptors in the motor end-plate. *Proc. Roy. Soc.* B, **146**, 357–361.

DE ROBERTIS, E. (1958). Submicroscopic morphology and function of the synapse. *Exp. Cell Res.* Suppl. **5**, 347–369.

DE ROBERTIS, E. D. P. & BENNETT, H. S. (1954). Sub-microscopic vesicular component in the synapse. *Fed. Proc.* **13**, 35.

DE ROBERTIS, E. D. P. & BENNETT, H. S. (1955). Some features of the sub-microscopic morphology of synapses in frog and earthworm. *J. biophys. biochem. Cytol.* **1**, 47–58.

ECCLES, J. C. (1957). *The Physiology of Nerve Cells.* Baltimore: The Johns Hopkins Press.

ECCLES, J. C. & JAEGER, J. C. (1958). The relationship between the mode of operation and the dimensions of the junctional regions at synapses and motor end-organs. *Proc. Roy. Soc.* B, **148**, 38–56.

EDWARDS, G. A., RUSKA, H. & DE HARVEN, E. (1958). Electron microscopy of peripheral nerves and neuromuscular junctions in the wasp leg. *J. biophys. biochem. Cytol.* **4**, 107–114.

FATT, P. & KATZ, B. (1952). Spontaneous subthreshold activity at motor nerve endings. *J. Physiol.* **117**, 109–128.

GLAUERT, A. M. & GLAUERT, R. H. (1958). Araldite as an embedding medium for electron microscopy. *J. biophys. biochem. Cytol.* **4**, 191–194.

KATZ, B. (1958). Microphysiology of the neuromuscular junction. *Johns Hopkins Hosp. Bull.* **102**, 275–312.

KÜHNE, W. (1887). Neue Untersuchungen über motorische Nervenendigung. *Z. Biol.* **23**, 1–148.

LUFT, J. H. (1956). Permanganate—a new fixative for electron microscopy. *J. biophys. biochem. Cytol.* **2**, 799–802.

PALADE, G. E. (1952). A study of fixation for electron microscopy. *J. exp. Med.* **95**, 285–298.

PALADE, G. E. (1954). Electron microscope observations of interneuronal and neuromuscular synapses. *Anat. Rec.* **118**, 335–336.

PALAY, S. L. (1954). Electron microscope study of the cytoplasm of neurons. *Anat. Rec.* **118**, 336.

PALAY, S. L. (1956). Synapses in the central nervous system. *J. biophys. biochem. Cytol.* **2**, Suppl. 193–202.

PALAY, S. L. (1958). The morphology of synapses in the central nervous system. *Exp. Cell Res.* Suppl. **5**, 275–293.

REGER, J. F. (1958). The fine structure of neuromuscular synapses of gastrocnemii from mouse and frog. *Anat. Rec.* **130**, 7–24.

ROBERTSON, J. D. (1954). Electron microscope observations on a reptilian myoneural junction. *Anat. Rec.* **118**, 346.

ROBERTSON, J. D. (1956*a*). Some features of the ultrastructure of reptilian skeletal muscle. *J. biophys. biochem. Cytol.* **2**, 369–380.

ROBERTSON, J. D. (1956*b*). The ultrastructure of a reptilian myoneural junctions. *J. biophys. biochem. Cytol.* **2**, 381–394.

ROBERTSON, J. D. (1959). The ultrastructure of cell membranes and their derivatives. *Biochem. Soc. Symp.* **16**, 3–43.

EXPLANATION OF PLATES

With the exception of Pl. 4*A*, all micrographs were obtained from specimens fixed in OsO_4 and additionally stained with PTA in alcohol. All scale marks represent 1 μ. Plates 1–5 show junctions from frog's sartorius, Pl. 6 from m. adductor longus.

Pl. 1. Longitudinal section through neuromuscular junction. Some details have been traced in Text-fig. 2.

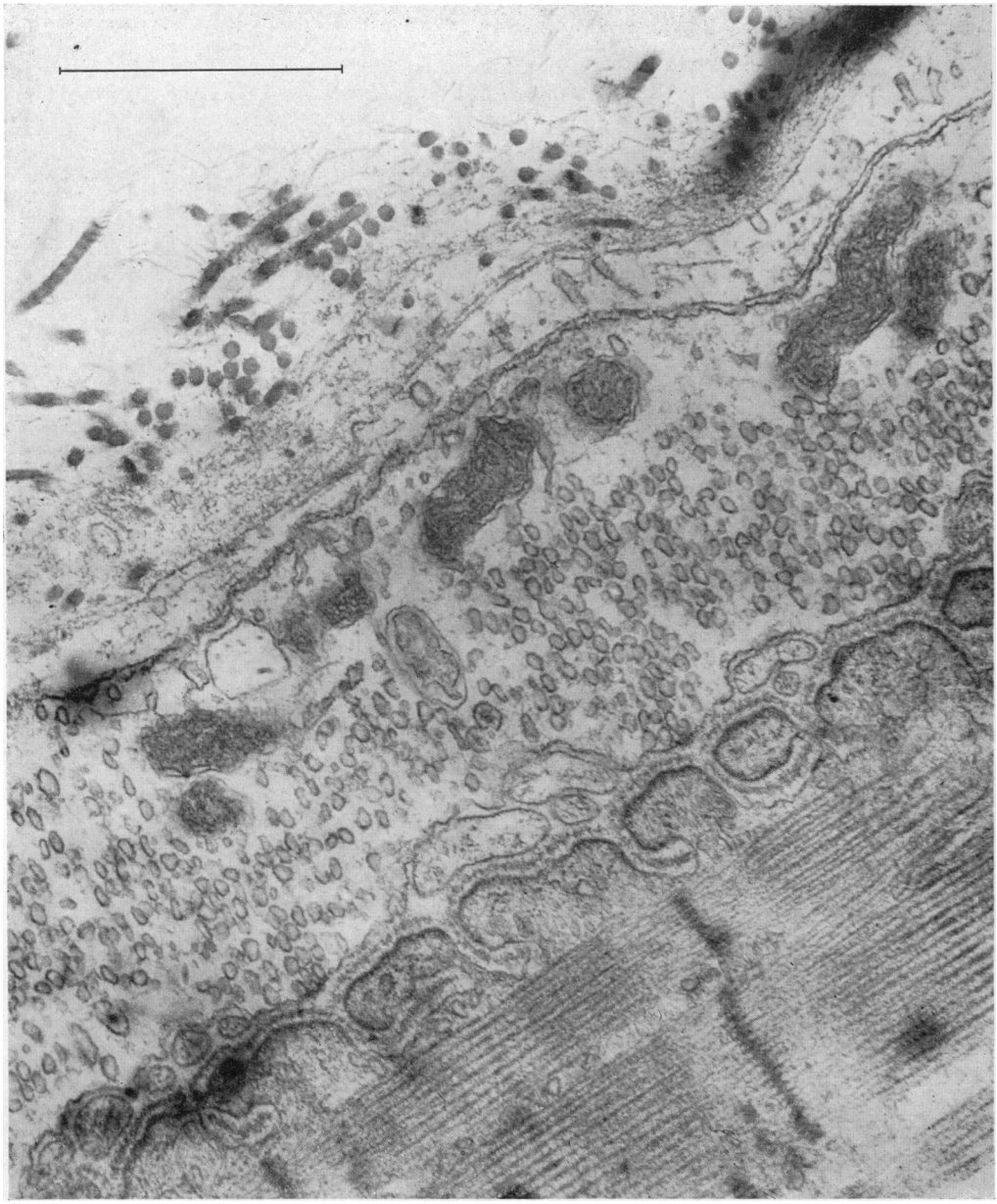
Pl. 2*A*. Longitudinal section passing through nuclear region of Schwann cell (Schw.: Schwann cytoplasm; Schw.n.: Schwann nucleus). *B*. Transverse section. *C*. 'Tangential' section (cf. Text-fig. 1*c*): starting at the top the plane of sectioning passed progressively more deeply into the muscle fibre, so that the axon terminal is seen only in the upper part of the figure, while in the lower part the deeper regions of the junctional folds are still visible.

Pl. 3. Transverse sections. In *A*, the Schwann nucleus is seen in the top right part; a 'finger' of Schwann cytoplasm protrudes, from the right, into the synaptic region. Magnifications of *A* and *B* are identical.

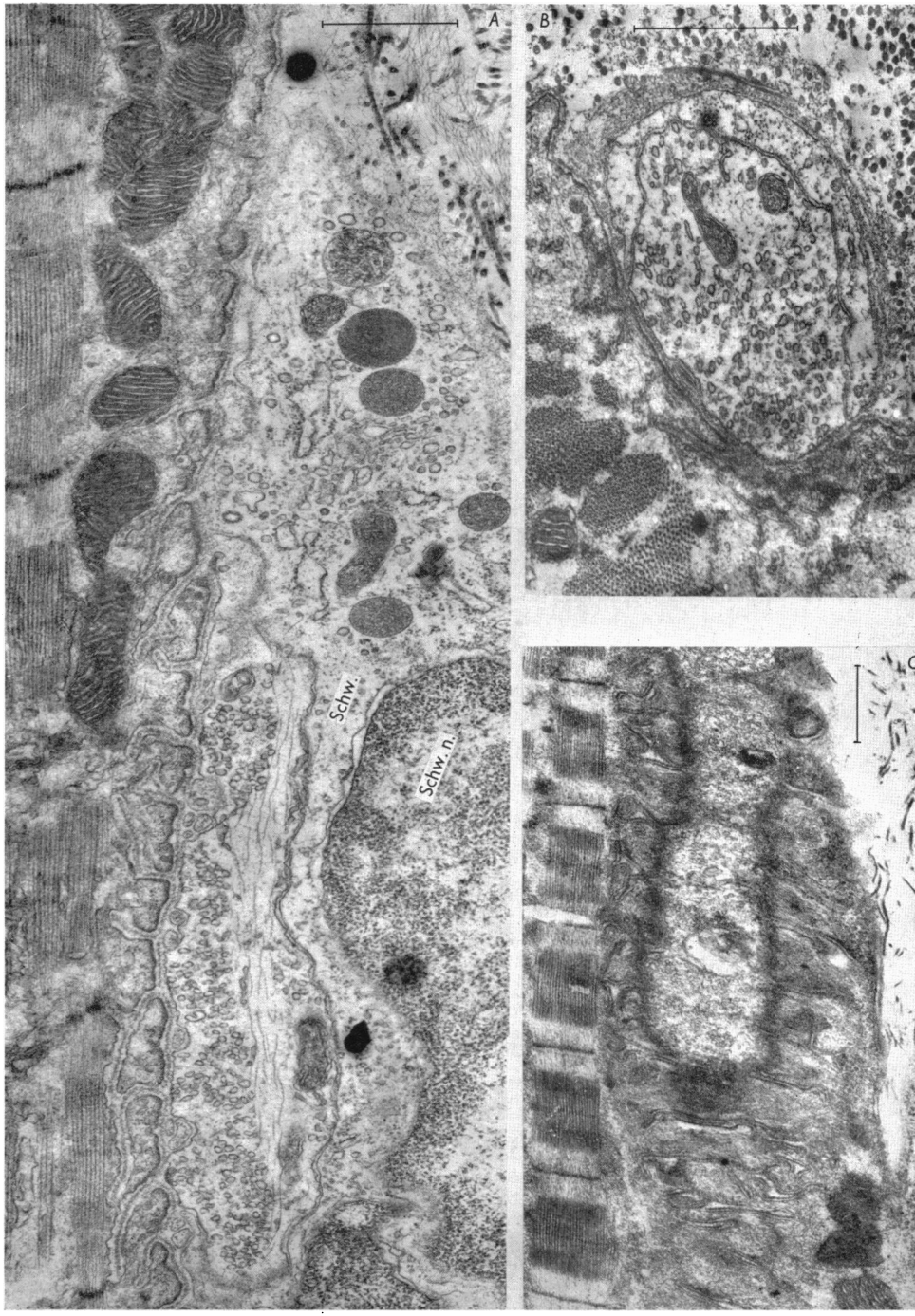
Pl. 4*A*. $KMnO_4$-treated muscle. Longitudinal section, showing Schwann layer, vesiculated nerve ending and post-junctional folds. *B*. OsO_4 + PTA. Transverse section passing 'tangentially' through a junctional fold (dark area under the nerve ending).

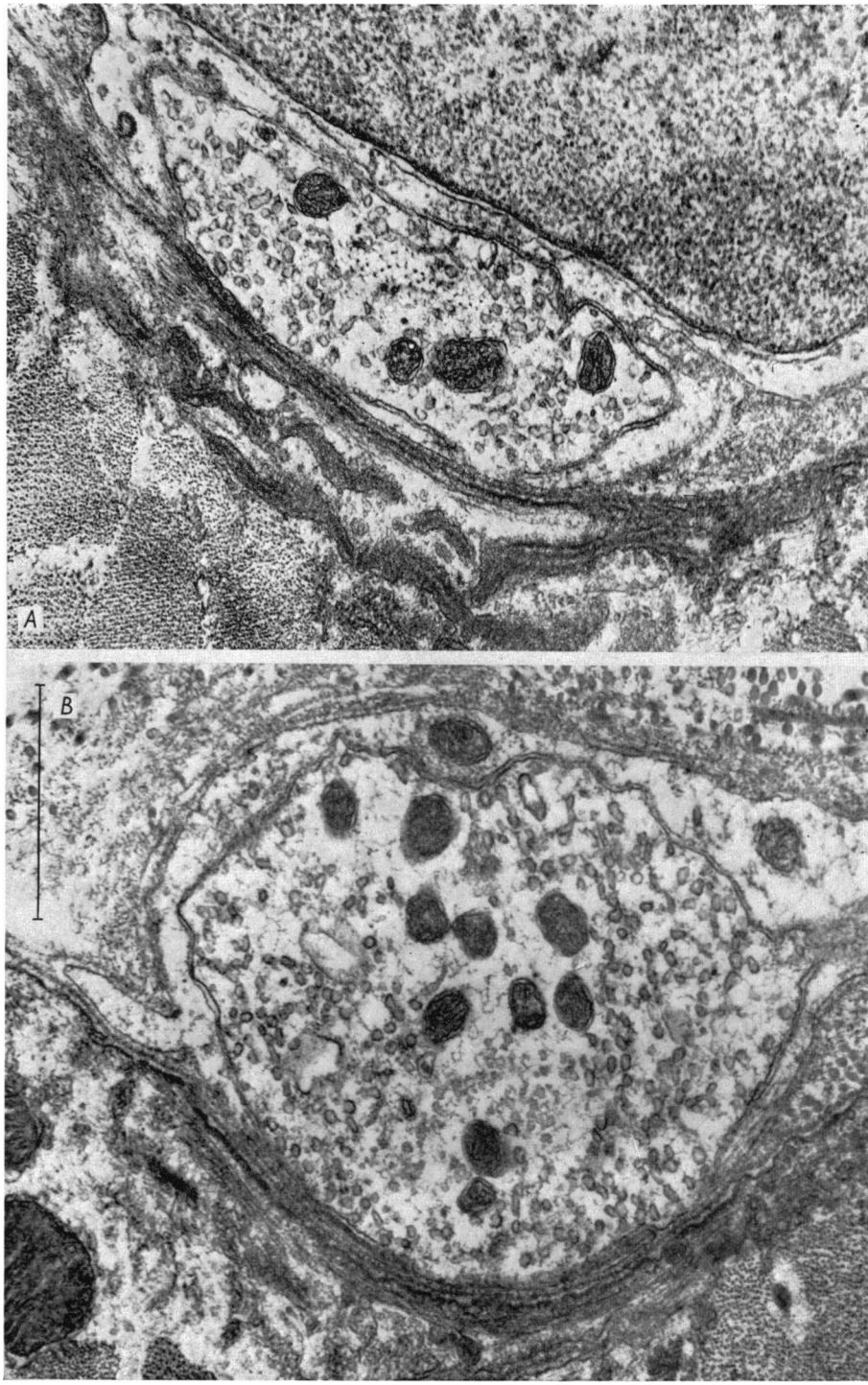
Pl. 5. Longitudinal sections. Special points: accumulation of presynaptic vesicles opposite to junctional folds in *A* and *B*; in *B*, Schwann cell with nucleus (S.n.) and a 'finger' (s.f.) protruding from the right; c.t. connective tissue space.

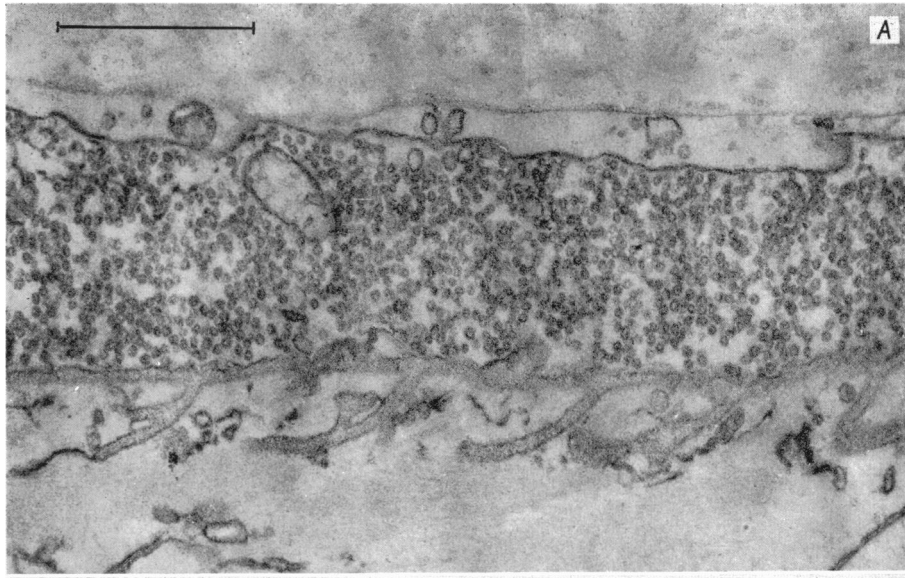
Pl. 6. Longitudinal sections. M. add. longus. Note: variety of membrane-bound compartments and vesicular aggregates in the axon terminal.

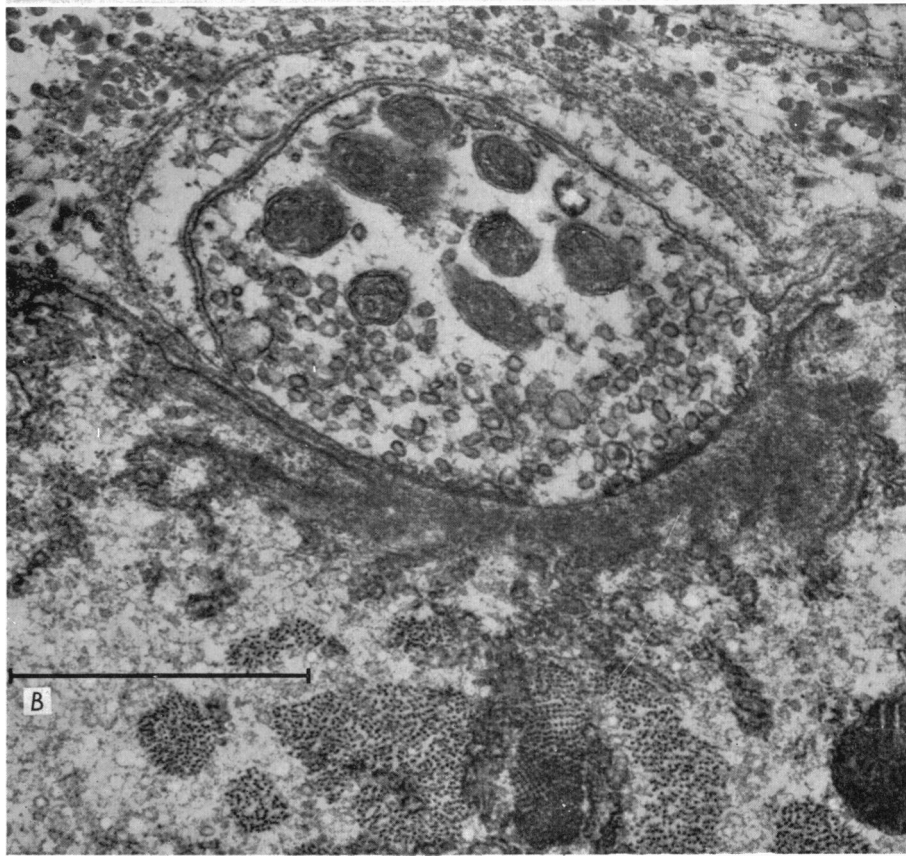


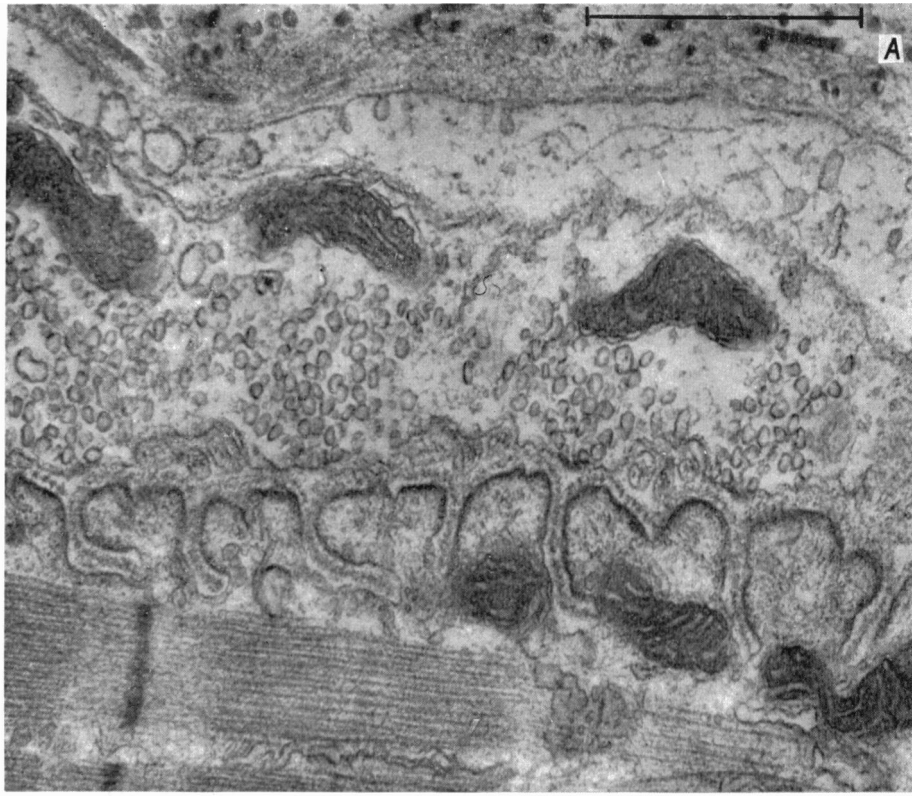
(*Facing p.* 144)

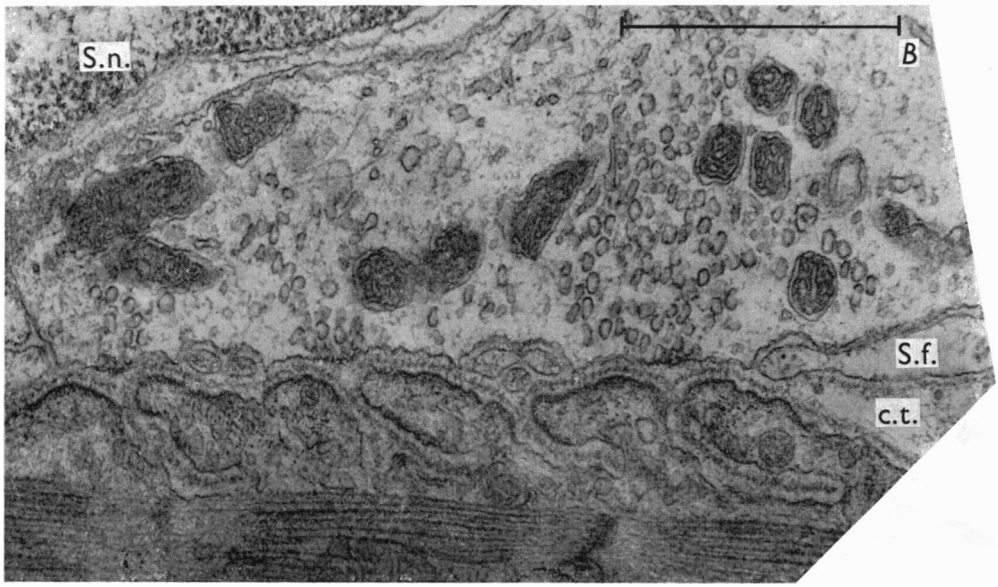

A
B
C
Schw.
Schw. n.

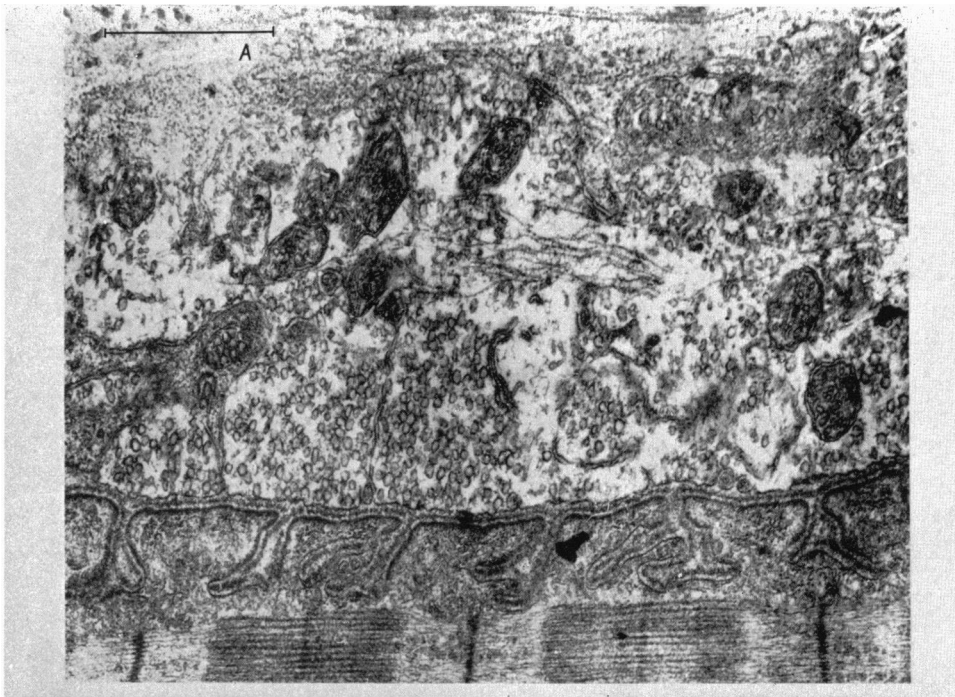

A
B

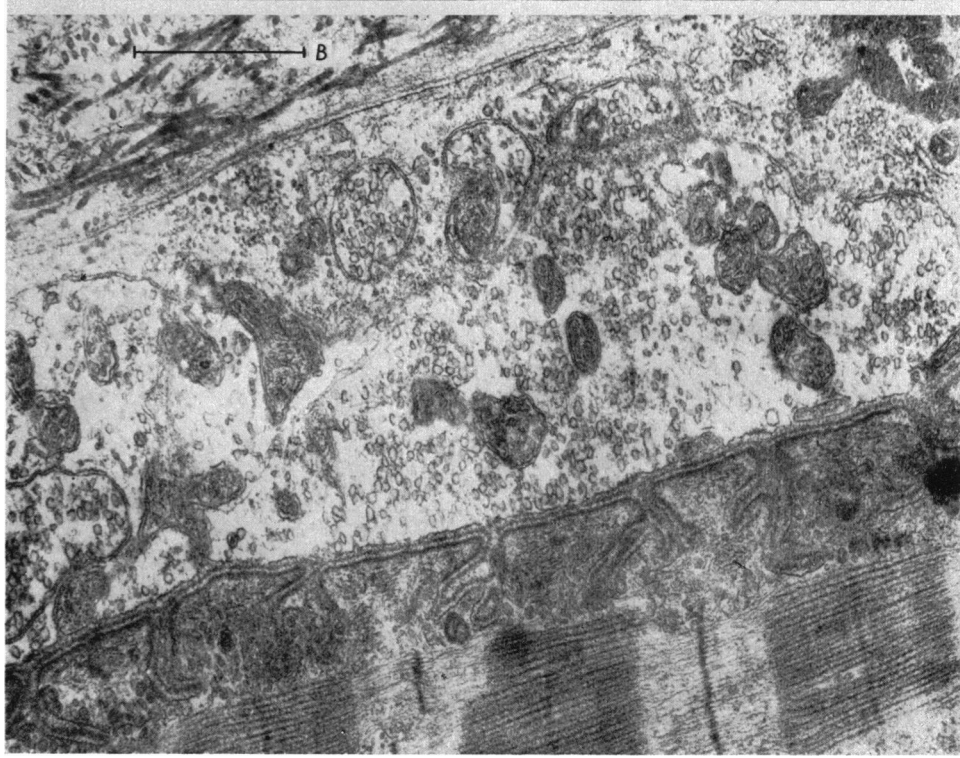

A


B


A


S.n.
B
S.f.
c.t.


A


B