A TEXTBOOK OF

GENERAL PHYSIOLOGY

by

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Dedicated to the memory of
SIR CHARLES LOVATT EVANS, F.R.S.
Emeritus Professor of Physiology in the University of London

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4. *Characteristics of Excitable Tissue*

5. *The Mechanism of Contraction of Muscle*

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Volume one contains the following chapters:

4. *Characteristics of Excitable Tissue*

CHAPTER XV

EXCITABILITY AND PROPAGATION OF THE IMPULSE

In an earlier chapter (p. 564) we have discussed the nature of the potential difference between the inside and outside of certain cells, in particular of nerve and muscle fibres, without, however, attempting to elucidate their functional significance. In the present section we shall be concerned with the problems of excitation and transmission, phenomena that are associated with modifications of these bioelectric potentials. In the complex organism the effects of a stimulus are transmitted by way of specialized cells—*neurones*; transmission is not peculiar to these, however, since a muscle, for example, may exhibit the spread of an excited state in an essentially similar manner to that found in nerve, and even unicellular organisms exhibit responses to local stimulation that are not necessarily confined to the stimulated point. The spread of excitation may thus be a general characteristic of living tissues, attaining, however, a maximal degree of efficiency and speed in the specialized nervous cells. The spread of excitation over a muscle fibre is probably related to the uniform development of contraction, and is thus an important element in its behaviour; nerve and muscle therefore represent specialized tissues in which we may expect to find the properties of excitability and transmission highly developed, so that in this section we may confine attention almost exclusively to them.

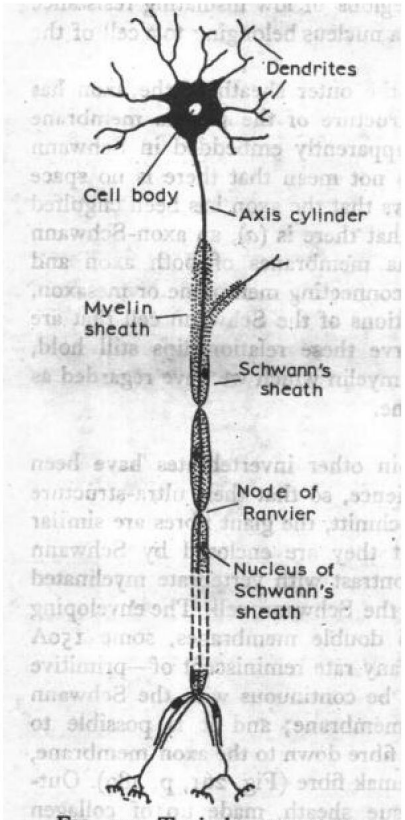

Fig. 519. Typical motor neurone.

The Neurone

A typical vertebrate nerve consists of a bundle of hundreds of fibres, each one of which is an extension of a single nerve cell, or neurone; a motor neurone, Fig. 519, is essentially a cell with a series of processes, one of which, the *axon*, is much longer than the remainder, the *dendrites*. The axon leaves the cell body (soma or perikaryon) as the axon hillock, and eventually makes connection with an effector organ—in this case a number of muscle fibres—or another neurone; the dendrites connect with the axons of other nerve fibres of the

central nervous system.* As we shall see, these junctional regions, the *nerve muscle junction* and the nerve-nerve *synapse*, are specialized regions permitting a close association of the two elements; nevertheless there is reason to believe that the fundamental continuity of the plasma membrane over the protoplasmic surface of the nerve cell is maintained even in these regions, *i.e.*, there is probably no cytoplasmic fusion, so that the neurone is a true unit in transmission. As classically represented, the axon consists, from without inwards, of an outermost sheath or *neurilemma* (*Schwann's sheath*); a *myelin sheath* of mainly lipoid material which acts as an insulating layer, and which may be thick in the typical *myelinated* (or *medullated*) *nerves* of vertebrates or very thin in the *non-myelinated* nerves typically found in invertebrates and in some parts of the vertebrate nervous system. The limiting plasma membrane separates the internal *axoplasm* from the outer layers. The thick myelin sheath of the medullated nerve is interrupted at regular intervals—about 1 mm. in man and 3 mm. in the frog—to give constrictions, or *nodes of Ranvier*, which are therefore to be regarded as localized regions of low insulating resistance (p. 1070). In each internodal region there is a nucleus belonging to a cell of the Schwann sheath or neurilemma.

Ultra-Structure. The ultra-structure of the outer sheath of the axon has been discussed earlier in relation to the structure of the plasma membrane (p. 477). We may recall that the axon is apparently embedded in Schwann cytoplasm but, as Gasser showed, this does not mean that there is no space between the two cells. Fig. 261 (p. 480) shows that the axon has been engulfed by an invagination of the Schwann cell so that there is (*a*), an axon-Schwann double membrane consisting of the plasma membranes of both axon and Schwann cell, and (*b*), the so-called surface-connecting membrane or mesaxon, representing the space between the two portions of the Schwann cell that are in close apposition. In the myelinated nerve these relationships still hold, but are obscured by the numerous layers of myelin which we have regarded as essentially spirally wound Schwann membrane.

Invertebrate Fibres

The giant fibres of the squid and certain other invertebrates have been studied extensively because of their convenience, so that their ultra-structure is of some interest. According to Geren & Schmitt, the giant fibres are similar to vertebrate non-myelinated fibres in that they are enclosed by Schwann cells, but the nuclei indent the axon, by contrast with vertebrate myelinated nerve where the nuclei lie in the surface of the Schwann cell. The enveloping Schwann cytoplasm contains some 3 to 6 double membranes, some 150Å thick, which would appear to be—or are at any rate reminiscent of—primitive myelin sheath. These membranes appear to be continuous with the Schwann surface membrane and the axon plasma membrane; and it is possible to follow spaces leading from the surface of the fibre down to the axon membrane, similar to the mesaxons described in the Remak fibre (Fig. 261, p. 480). Outside the Schwann layer is a connective-tissue sheath, made up of collagen fibrils well orientated in the axial direction.

The giant axon is surrounded by several Schwann cells some 0·1–0·2μ thick near their ends and some 0·8–0·9μ near the nucleus; these are in a single row around the axon, to form a layer that is crossed by tortuous intercellular channels.

* The distinction between axon and dendrite is made on both morphological and functional grounds; it is sufficient for our purpose to note that the neurone is so disposed that impulses pass normally into the dendrites through the cell body and thence along the axon. The long processes, constituting the sensory nerves of the spinal ganglia (p. 1127), are often described as axons, though functionally they behave as dendrites.

External to the Schwann cells is a thick basement membrane (0·2μ thick), and beyond this are alternate layers of connective-tissue cells and fibrils forming the endoneurium (Villegas & Villegas, 1968). Sections through the Schwann sheath indicate the presence of some three to six membranes apparently running parallel to the axon (Geren & Schmitt) and analogous with the myelin layer of myelinated nerve. Careful examination of these in the electron-microscope has shown that

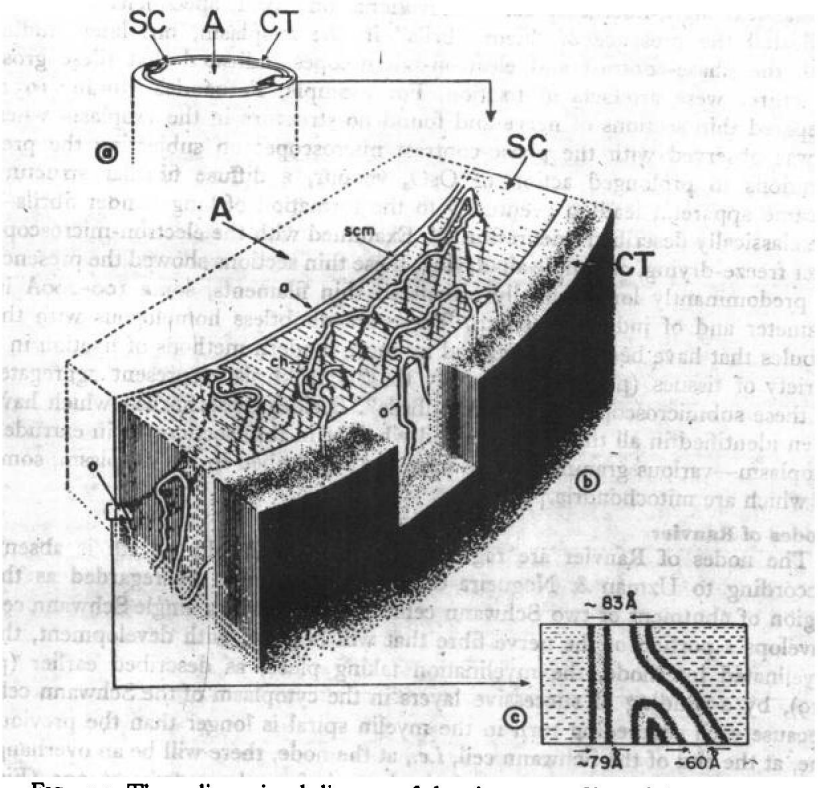

FIG. 520. Three-dimensional diagram of the giant nerve fibre of the squid. (a) A segment of the nerve fibre showing the axon (A) covered by the Schwann cell (SC). The latter is covered by the connective tissue (CT). (b) Enlarged portion of the fibre in which channels (ch) are shown as slits crossing the Schwann cell from outer surface to axonal surface. Note the openings (o) of the channels (ch). It may be seen that some of the channels, which in ultra-thin sections appear ending in a blind alley, are found to be continuous at different levels, as has been observed in serial electron micrographs. (c) Highly enlarged view of one of the channel openings (o) in which the continuity of the channel walls with the Schwann cell membrane (scm) is demonstrated. The fine structure of the axolemma (a) is shown. No difference can be appreciated between this structure (a), the Schwann cell membrane (scm), and the channel wall structure (ch). (Villegas & Villegas. *J. gen. Physiol.*)

they consist of double-edged osmiophilic layers, whilst the less dense zones between the edges are channels connecting the outside of the Schwann cell to the space surrounding the axon as illustrated by Fig. 520. Studies on the permeability to water by Villegas & Villegas gave a $\mathscr{P}$ value of $1{\cdot}42.10^{-4}$ cm./sec. and from this they deduced that the fraction of the total area of the axon available for diffusion was some 0·23 per cent., and this would correspond with the

area of the channels connecting the connective tissue to the space between axolemma and Schwann cell membrane.*

The arthropod axon is surrounded by a lemnoblast and is described by Edwards, Ruska & de Harven as *tunicated*, since it is loosely mantled by several cytoplasm-enclosing membranes of the lemnoblast.

Neurofibrils

Classical light-microscopical observations on fixed specimens of nerve indicated the presence of "neurofibrils" in the axoplasm, but later studies with the phase-contrast and electron-microscopes indicated that these gross structures were artefacts of fixation. For example, Fernández-Morán (1952) prepared thin sections of nerve and found no structure in the axoplasm when it was observed with the phase-contrast microscope; on subjecting the preparations to prolonged action of OsO_4 vapour, a diffuse fibrillar structure became apparent, leading eventually to the formation of long slender fibrils—the classically described "neurofibrils". Examined with the electron-microscope after freeze-drying, or simple air-drying, these thin sections showed the presence of predominantly longitudinally orientated thin filaments, some 100–200A in diameter and of indefinite length. These are doubtless homologous with the tubules that have been demonstrated by more modern methods of fixation in a variety of tissues (p. 44). Presumably the "neurofibrils" represent aggregates of these submicroscopic "neuroprotofibrils". Besides these fibrils—which have been identified in all the axons studied whether in thin sections or in extruded axoplasm—various granular bodies have been described in the axoplasm, some of which are mitochondria.†

Nodes of Ranvier

The nodes of Ranvier are regions in which the myelin sheath is absent. According to Uzman & Nogueira-Graf, the node must be regarded as the region of abutment of two Schwann cells. On this basis, a single Schwann cell envelops a portion of the nerve fibre that will become, with development, the myelinated internode, the myelination taking place, as described earlier (p. 479), by a winding of successive layers in the cytoplasm of the Schwann cell. Because each succeeding turn in the myelin spiral is longer than the previous one, at the end of the Schwann cell, *i.e.*, at the node, there will be an overhang, the innermost lamella ending perhaps $\frac{1}{2}$–1μ before the outermost one (Fig. 521*a*). At the node, where the Schwann cells end, the axon is thus covered only by Schwann cytoplasm. According to Robertson, the Schwann cytoplasm in

* The measurement was one of the diffusion of tritiated water and passage was presumably largely determined by the migration along the tortuous channels shown in Fig. 520; responses to osmotic gradients, on the other hand, must have been determined by the axolemma; a filtration permeability coefficient of $7{\cdot}8.10^{-10}$ ml./cm^2. sec. cm. H_2O pressure was derived from the osmotic measurements. By applying Solomon's theoretical treatment (p. 416), an equivalent pore radius of 4·25A was deduced for the axolemma (Villegas & Barnola, 1961; Villegas, Caputo & Villegas, 1962) which may thus be regarded as the main seat of diffusional resistance to transport across the axon. In a preliminary note, Villegas & Villegas (1962) have pointed to the possibility of a third component, in addition to axolemma and Schwann sheath; this is the layer of endoneurium cells external to the basement membrane of the Schwann cells.

† Maxfield (1953) has isolated, by fractional centrifugation, a protein from extruded squid axoplasm which he calls "axon filaments"; a dried solution appeared under the electron-microscope as fibrils of indefinite length, 90–160A in diameter. These filaments could be reversibly broken down to smaller particles by alkaline K-phosphate, in which case dried specimens showed no fibrils that could be resolved. Elfvin (1961) has described thin (100A) and thick (300A) filaments in unmyelinated nerve. More recently Peters & Vaughan (1967) have described the microtubules and filaments in the developing nerve—at early stages the microtubules of 233–260A diameter dominate the picture; but later the number of neurofibrils, with 90–100A diameter, increases; these, in cross section, also appear as tubules. The suggestion is made that the filaments come from microtubules.

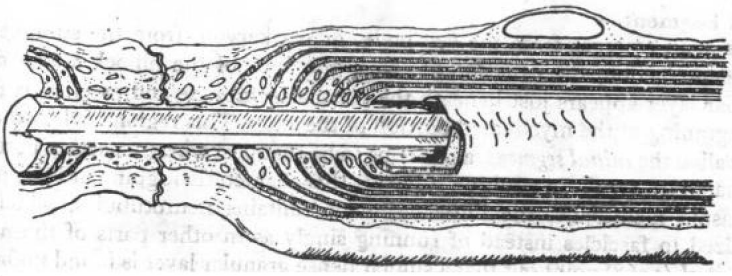

FIG. 521*a*. Diagrammatic representation of cutaway view of early node of Ranvier illustrating relationship between myelin lamellar endings, Schwann cell cytoplasm, and axon. The myelin lamellæ are cross-hatched; cut surfaces of the Schwann cell cytoplasm are stippled and include outlines of formed cytoplasmic organelles. (Uzman & Nogueira-Graf. *J. biophys. biochem. Cytol.*)

the node breaks up into a number of processes, so that, in effect, the axon membrane is naked in this region.

Schmidt-Lantermann Clefts

The myelin sheath in each Schwann segment is interrupted by several oblique partitions, the *incisions* or *clefts of Schmidt-Lantermann*, easily recognizable in polarized light, and first identified in the electron-microscope by Rozsa, Morgan, Szent-Gyorgyi & Wyckoff. According to Robertson, these

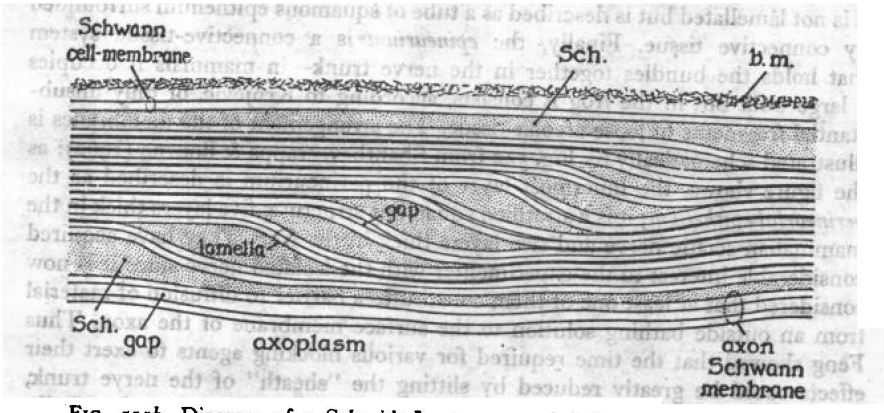

FIG. 521*b*. Diagram of a Schmidt-Lantermann cleft in a segment of myelin in longitudinal section. The outer Schwann cell membrane is shown above as two lines, making a unit about 75A across. Two such units are seen below, making the axon-Schwann membrane. Each myelin lamella is composed of two such units in contact, and the heavy dense lines are produced where adjacent lamellæ are in contact. In the cleft the lamellæ may separate to give a gap. The basement membrane (b.m.) is indicated above, but not below. (Robertson. *J. biophys. biochem. Cytol.*)

clefts arise by a staggered separation of the myelin lamellæ, as indicated in Fig. 521*b*, so that, in effect, the gaps are not empty but traversed by successive elements in the concentrically wound myelin lamellæ. If the myelin lamellæ are formed in Schwann cytoplasm, it may be supposed that these spaces are filled with this cytoplasm, and a consideration of the three-dimensional aspect of the clefts will show that they constitute a helical layer of Schwann cytoplasm, leading eventually to the surface of the axon.

Initial Segment

The axon emerges from the cell body, or perikaryon, from the summit of a conical projection, the *axon hillock*. Near the apex of the hillock a thin dense granular layer appears just beneath the plasma membrane and extends as far as the beginning of the myelinated portion of the axon. This region of the axon has been called the *initial segment*, and seems to be that part where an action potential is initiated (p. 1191). In addition to having this characteristic granular layer under its plasma membrane, the initial segment contains neurotubules, which are organized in fascicles instead of running singly as in other parts of the nerve. Palay *et al.* (1968) point out that a similar dense granular layer is found under the plasma membrane at the node of Ranvier. They hazard the suggestion that the fasciculation of the neurotubules subserves a contractile function, perhaps providing the motive force for the protoplasmic streaming from parikaryon to axon.

Outer Membranes. The individual fibres of a nerve trunk are bound together by three connective-tissue systems, namely the *endoneurium*, *perineurium*, and *epineurium*. Essentially, the endoneurium constitutes a covering for the individual fibres, and it may be described as a connective-tissue tube made up of two layers, the *inner endoneurium*, or sheath of Plenk & Laidlaw, made up of circularly orientated argyrophil fibrils, and an outer endoneurium, or sheath of Key & Retzius.* The *perineurium* is a connective-tissue sheath surrounding a bundle of nerve fibres which, in mammals, is a laminated capsule, each lamella being covered with endothelial cells. In the frog, on the other hand, it is not lamellated but is described as a tube of squamous epithelium surrounded by connective tissue. Finally, the *epineurium* is a connective-tissue system that holds the bundles together in the nerve trunk—in mammals it occupies a large bulk but in the frog it consists, according to Krnjević, of only unsubstantial fragments of loose areolar tissue. The arrangement of the membranes is illustrated schematically by Fig. 522 from Shanthaveerappa & Bourne (1962): as the figure shows, the innermost layer of the perineurium is described as the *perineurial epithelium*; it is a stratified squamous structure, five layers thick in the mammalian sciatic nerve and two layers thick in the frog. They have acquired considerable interest to the experimenter with the isolated nerve since it is now considered that at least one of them constitutes a barrier to diffusion of material from an outside bathing solution to the surface membrane of the axon. Thus Feng showed that the time required for various blocking agents to exert their effects could be greatly reduced by slitting the "sheath" of the nerve trunk, or removing it. As a result of further studies, for example those of Crescitelli, of Huxley & Stämpfli, and most recently of Krnjević, there can be little doubt that the "connective-tissue sheath" of nerve does indeed constitute a barrier to diffusion which may reduce the effective diffusion coefficient of K^+ by a factor of 30 or more, whilst with lipid-soluble substances, such as urethane, little or no "barrier action" is observed. The only point in dispute seems to be the anatomical identification of the barrier; most investigators have described this as the epineurium, and have characterized the "desheathed nerve" as essentially a nerve without epineurium, but with perineurium and endoneurium intact.

* The neurilemma, or Schwann membrane of classical light-microscopy, may be described as the innermost portion of the tubular wall that surrounds the nerve fibre, *i.e.*, the innermost portion of the endoneurium. In the electron-microscope it is a fine granular membrane, only a few hundred Ångstrom units thick, reinforced by a network of submicroscopic fibrils. The fibrils have an axial repeat of 600–660A and are apparently collagen; the longitudinal fibrils interweave with circular fibrils to form a network which constitutes the *inner endoneurium* of Plenk & Laidlaw.

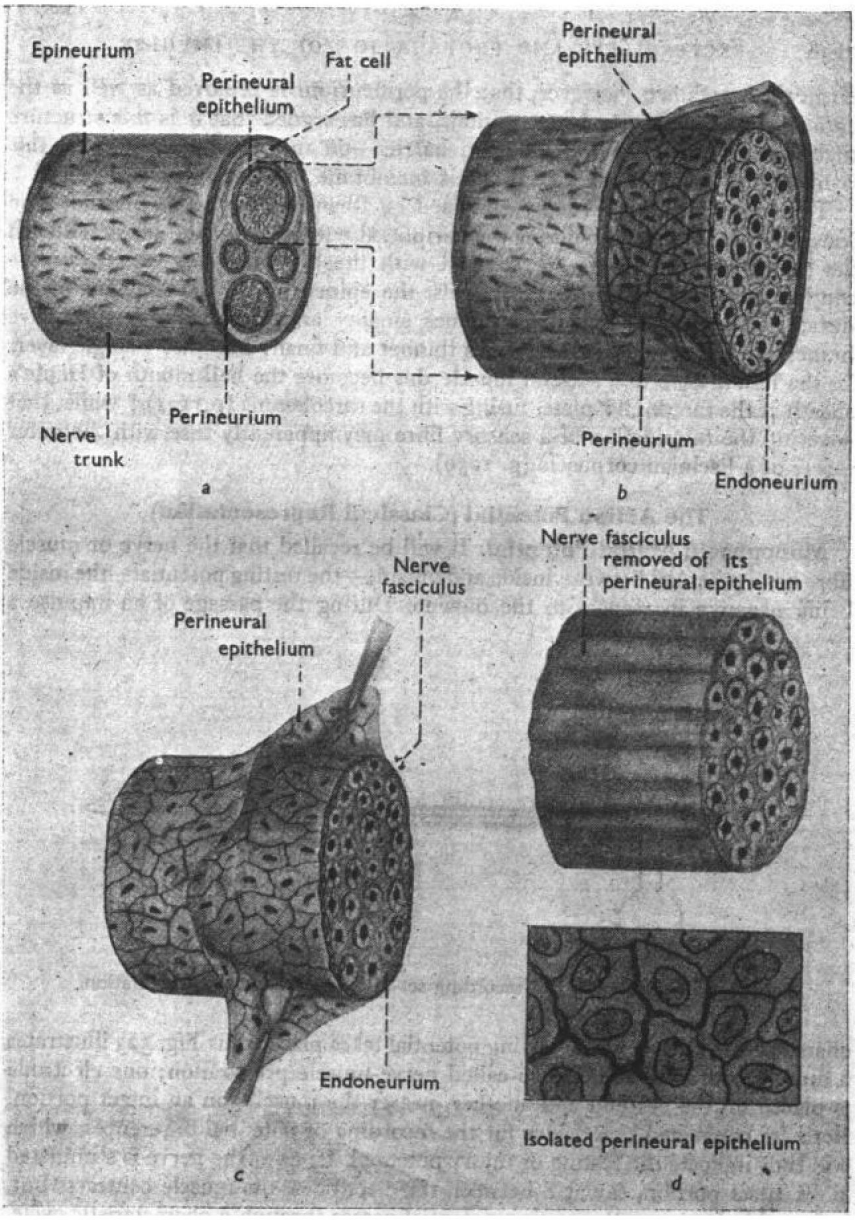

FIG. 522. The outer membranes of peripheral nerve; the figures demonstrate the various stages of isolation of this "perineural epithelium" under a binocular dissection microscope.

(*a*) The nerve trunk as a whole, with many fasciculi along with their connective tissue components, the epi-, peri-, and endo- neurium. The perineural epithelium is shown in the diagram surrounding each nerve fasciculus, lying under the perineurium.

(*b*) One nerve fasciculus is removed along with the perineurium, perineural epithelium and endoneurium. Part of the perineurium is removed to show the multiple layered perineural epithelium, lying immediately under the perineurium and on the entire surface of the nerve fasciculus.

(*c*) The nerve fasciculus removed from its entire perineural connective tissue layer, leaving the perineural epithelium covering the nerve fasciculus. The multiple layered nature of the perineural epithelium is indicated.

(*d*) Nerve fasciculus removed of its perineurium and perineural epithelium, leaving the nerve fibres of the fasciculus and the endoneurium intact. The isolated perineural epithelium can be seen lying flat on the glass slide. A capillary network in this perineural epithelium is also shown. (Shanthaveerappa & Bourne. *J. Anat.*)

Krnjević has shown, however, that the perineurium is removed as well as the epineurium in desheathed preparations, and has argued that it is this structure that is responsible for the diffusion barrier—on morphological grounds this is likely since it contains a continuous membrane of closely apposed cells.*

This view has been strongly endorsed by Shanthaveerappa & Bourne, who consider that the perineurium is the peripheral equivalent of the pia-arachnoid; the emerging nerve roots are covered with these membranes which become continuous with the perineurium, whilst the epineurium is the analogue of the dura. As the nerve fasciculus becomes smaller and smaller, with successive branching, the perineurium becomes thinner and finally becomes a single layer. In the motor fibre to a skeletal muscle this becomes the bell mouth of Henle's sheath at the motor end-plate, fusing with the sarcolemma (p.1131),† whilst that covering the termination of a sensory fibre may apparently fuse with the outer layers of a Pacinian corpuscle (p. 1259).

The Action Potential (Classical Representation)

Monophasic Action Potential. It will be recalled that the nerve or muscle fibre has a potential between inside and outside—the resting potential—the inside being negative in respect to the outside. During the passage of an impulse a

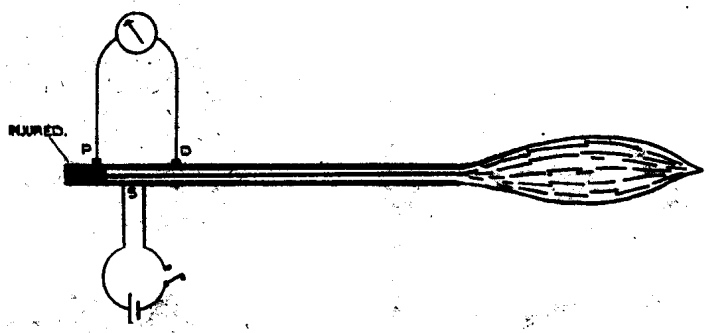

FIG. 523. Stimulation and recording set-up for nerve-muscle preparation.

characteristic change in the resting potential takes place; thus Fig. 523 illustrates a muscle with its nerve, the so-called nerve-muscle preparation; one electrode is placed on the cut end and another, nearer the muscle, on an intact portion. Both are connected to a device for the recording of potential differences, which will thus indicate the resting or injury potential. If, now, the nerve is stimulated at an intact portion, say at S between the electrodes, the muscle contracts but, before this happens, the resting potential passes through a characteristic cycle, falling rapidly to zero and returning to its original value, the whole cycle lasting perhaps only a few thousandths of a second (msec.). If the difference of potential between P and D is plotted against time, we will obtain a curve similar to that in Fig. 524 (*a*), the potential of the electrode D, in respect to P, being first 30 mV positive, falling to zero, and then rising to its initial value. It is more usual, however, in making records, to balance out the resting potential

* Shanes has drawn attention to a 40 per cent. increase in volume that occurs on soaking a desheathed frog's nerve in Ringer's solution; it would seem to be a gain in extracellular fluid; in the toad nerve no such swelling occurred.

† According to Shanthaveerappa & Bourne, capillaries pass through the perineurium to supply the individual fibres of the fasciculus; it would thus be interesting to determine whether there is a "blood-axon barrier"; if the capillaries are similar fundamentally to those of the central nervous system this would be the case (p. 728).

with a potentiometer, so that, before stimulation, the recorded potential between the electrodes is zero. Stimulation results in a change of potential such that D now becomes negative in respect to P, and the action-potential record, obtained by plotting the potential of D against time, is turned upside down as in Fig. 524 (*b*), potentials above the abscissa being called *negative*. The detailed nature of the action potential and its full interpretation will be dealt with later; for the moment we may note that the record is called a *negative spike*, and that the transmission of the effect of stimulation seems to be associated with a transient fall in the resting potential to zero.* This fall could happen

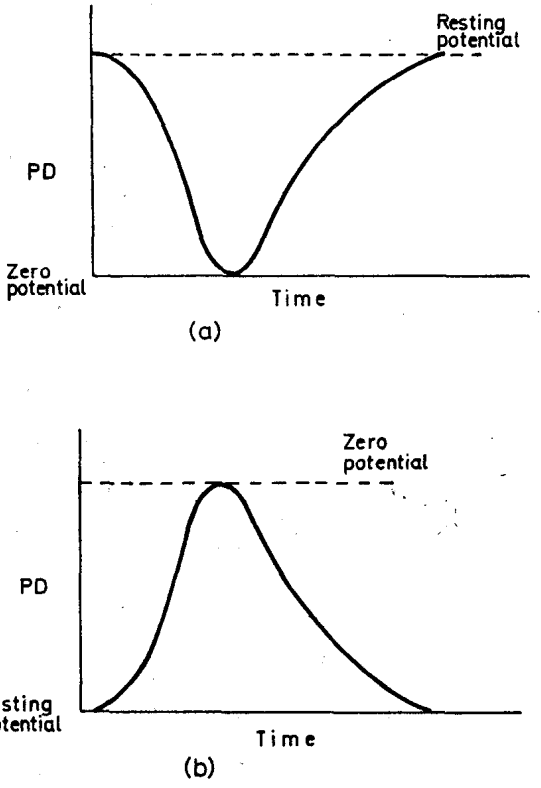

FIG. 524. Monophasic action potential, classically regarded as a falling to zero of the resting potential.

if the effect of the propagated disturbance were to abolish the potential difference between inside and outside of the cell at the point D, *i.e.*, if the electrical condition of the point P were unaffected. That this is the truth of the matter is shown by the fact that the time at which the spike occurs depends on the distance of the point of stimulation from D, and not on its distance from P. Thus the membrane is said to be depolarized by the passage of the propagated disturbance.

An essentially similar type of action potential is obtained by stimulation of the plant cell, *Nitella*, but in this case the process is very much slower, lasting some 15 seconds.

* As we shall see, the action potential consists of something more than a *fall* in the resting potential; the sign is actually reversed.

Diphasic Action Potential. If the conducted effect of the stimulus—the propagated disturbance—consists essentially of the abolition of the resting potential across the wall of the cell, then, since this disturbance travels at a measurable rate, we may expect to find characteristic changes in the difference of potential between two electrodes on intact nerve as the disturbance passes first under one electrode and then under the other. We may expect the changes illustrated in Fig. 525. At (*a*) the disturbance has not reached either electrode: both of these are on intact nerve so that their potential difference is zero. At

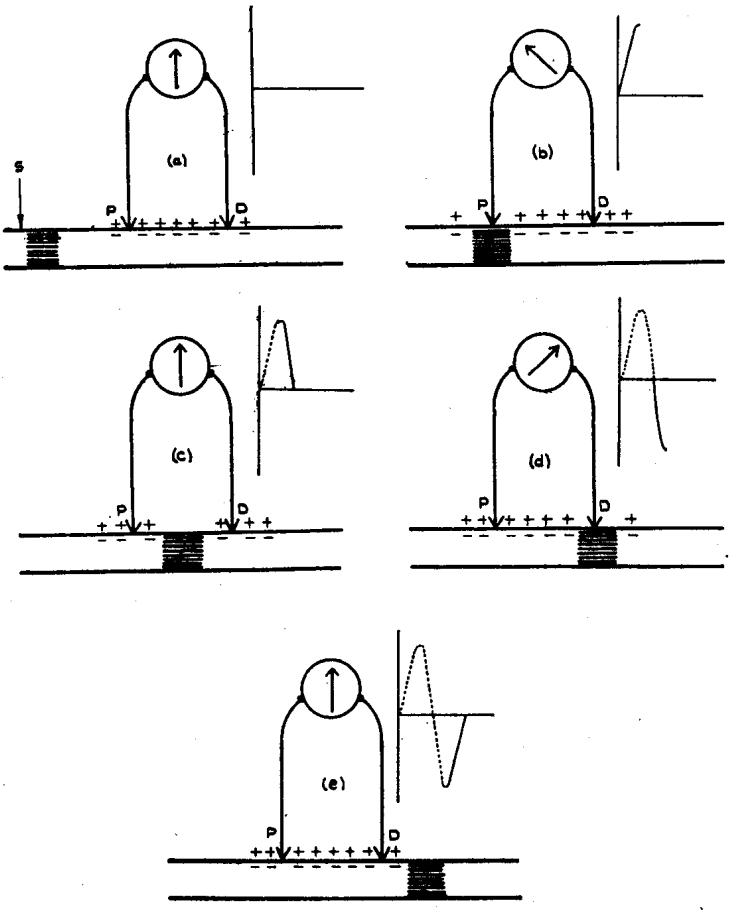

FIG. 525. Schematic illustration of the diphasic action potential.

(*b*) the disturbance has reached the proximal electrode, P, to abolish its resting potential; consequently P becomes negative in respect to the distal electrode, D, and positive current flows through the recording instrument from D to P. At (*c*) the disturbance is between the electrodes, and the resting potential at P has been re-established; there is thus no difference of potential between the electrodes —the *isoelectric phase*. At (*d*) the disturbance has reached the distal electrode, abolishing its resting potential. D thus becomes negative in respect to P and current now flows in the external circuit, *i.e.*, through the recording system, in the reverse direction. Finally, at (*e*) the disturbance has passed on and the