THEORIA

A Concept of Intracellular Transmission of Excitation by Means of the Endoplasmic Reticulum

The classical concept of cellular conduction of excitation is that an adequate change in the cell membrane environment causes an alteration of the membrane potential, which initiates a self-propagating wave of action potential and subsequent specific functional events within the cell.

Two of the major problems which arise from this concept are the following:

(1) The precise membrane, or membranes, involved. Classically the only membrane considered was the 'cell surface'.

(2) The mechanism whereby the alterations of the cell surface affect the interior of the cell.

Recent electron microscopy has differentiated the cell surface membranes which separate cytoplasm from extracellular material. It has further shown the presence of intracellular membranes (endomembranes) separating different cytoplasmic phases. Detailed references will be discussed later. From our observations of the disposition of the endomembranes, their relation to the plasma membrane, and their separation of differing cytoplasmic phases, we conclude that the intracellular membranes must possess transmembrane potentials and that they serve for the conduction of excitation as does the plasma membrane. The morphologic evidence is derived from electron micrographs of skeletal muscle fibers, smooth muscle cells, cardiac muscle cells, Purkinje conducting system cells, neurons, neuromuscular junctions, and gland cells, but it is believed that the principles apply to all cells.

The Micromorphologic Background.—In general there are either multiple or single layers of 'cell membranes' separating cells from extracellular spaces or from other cells. They comprise the plasma membranes with or without basal membrane. Within the cell are now observed 'endomembranes', separating intracellular phases. These include: (*a*) membranes of the endoplasmic reticulum, (*b*) membranes of the Golgi apparatus, (*c*) membranes of the mitochondria, and (*d*) nuclear membranes.

The different membrane systems and the several important phases separated by them are exemplified in Figure 1, which represents schematically a muscle fiber with its nerve and myoneural junction.

The sarcolemma of skeletal muscle fibers, cardiac muscle cells, and the cytolemma of smooth muscle cells consists of the outer sheath of denser ground substance (basement membrane), the inner plasma membrane, and the interspace. In addition, embryonic and adult heart muscle cells, and Purkinje conducting system cells show intercalated discs formed by adjacent plasma membranes and their interspace. The same principles apply to the nerves, in which the axonal plasma membrane is either adjacent to a basement membrane, as in vertebrate neuromuscular junctions, or to a plasma membrane, as along the sheath cell of non-myelinated nerves, along interneural synapses, and insect neuromuscular junctions.

The membranes of the endoplasmic reticulum form a continuous system of intracytoplasmic microtubules, vesicles, and clefts of extreme variability. Hence, they separate the continuous internal phase of the reticulum from the continuous cytoplasmic matrix. A second, differentiated phase is separated from the latter by the Golgi membranes, which in some cases may be continuous with the endoplasmic reticulum. The mitochondria lie discontinuously in the matrix, separated from the cytoplasmic phase by their outer membranes, and enclosing membranes that separate two intramitochondrial phases. The matrix further includes the contractile material, neurofilaments, or other specific structures dependent upon the cell type. A special situation is occupied by the nucleus. It is suspended within the fluid phase of the endoplasmic reticulum; the nucleoplasmic membrane separating the nuclear and reticulum phases. Cytoplasmic matrix and nuclear matrix, however, communicate at irregular intervals through small pore canals bounded by adjoined nuclear and reticulum membranes. The pore canal is traversed by only a fine phase border.

In muscle fibers we find an extremely regular transverse and longitudinal arrangement of the endoplasmic reticulum as emphasized in Figure 1. Its tubes are attached to the plasma membrane at the levels of the *Z* and *M* bands. The reticulum tubules surround the *Z* and *M* bands of each sarcomere, and are linked transversely and longitudinally between the fibrils. At the termini of the vertebrate myoneural junctions the reticulum tubules are attached to the digital infoldings of the muscle plasma membrane. In insects the endoplasmic reticulum tubules ramify profusely at the neuromuscular contact fields. The endoplasmic reticulum is continuous with the nuclear membrane, and closely associated with the mitochondria. In nerve cells the endoplasmic reticulum forms a structural component of the Nissl substance and extends longitudinally within the dendrites and axons.

Mitochondria of the muscle fiber are abundant immediately beneath the plasma membrane, close to the *Z* bands, and around the nucleus. They occur in large numbers in both sarcoplasm and axoplasm at the myoneural junctions. In the axon they are observed near the reticulum tubules and the axoplasmic membrane.

Membrane Systems and Potentials.—The electrical phenomena observed in cells are generally attributed to the properties of a hypothetical surface membrane. Electron micrographs reveal the plasma membrane to be the only constant component of all cell surface membranes. It is the barrier between the ion concentrations of the cytoplasmic phase and the ion concentrations of the fluid phase immediately outside. The plasma membrane has a thickness of ca. 100 Å, which is that demanded by calculations from membrane capacitance and dielectric constant. It is of considerable interest that the multiply-connecting endoplasmic reticulum is a further membrane system separating different intracellular phases and ion concentrations. Therefore, not only plasma membranes, but also the endoplasmic reticulum membranes, and other endomembranes must be considered as carrying trans-membrane potentials (see Fig. 1). Alterations of the plasma membrane potential then would induce alterations of the reticulum membrane potential by changes in cytoplasmic matrix ion concentrations at the regions of reticulum-plasma membrane juxtapositions. A potential of activation would be thus transmitted transversely and longitudinally throughout the muscle fiber. If this concept is justified, then the production of spontaneous action potentials in neurons should start in the interior, e.g., nucleus, endoplasmic reticulum (Nissl substance), and mitochondria, or by interaction of these components, to be transmitted secondarily to axon surface membranes and synapses.

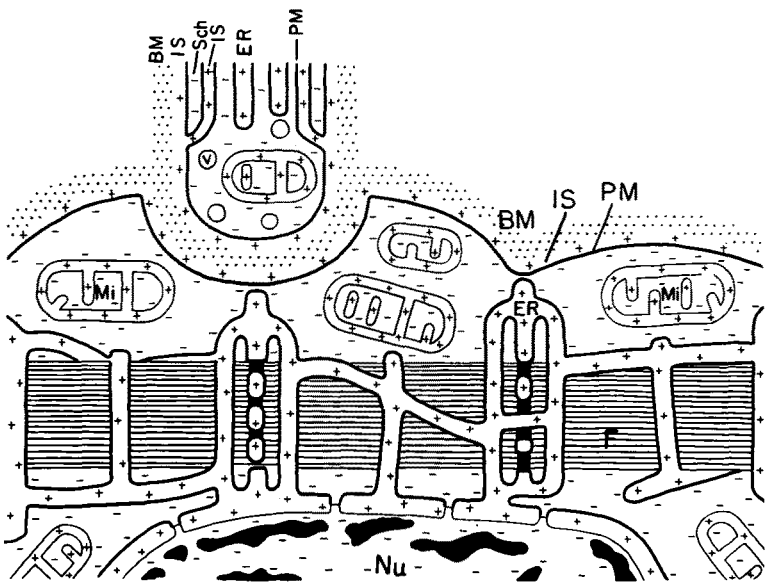

Fig. 1. – Schematic representation of vertebrate muscle fiber with myoneural junction. At upper left, nerve ending with its axonal reticulum (*ER*), vesicles (*V*), and mitochondrion (*Mi*). Axon accompanied on both sides by cytoplasm of Schwann cell (*Sch*) separated from the axon by plasma membrane interspace (*IS*). Neuron basement membrane (*BM*) (stippled) communicates with basement membrane of end plate and sarcolemma. Basement membrane (*BM*) separated from underlying plasma membranes (*PM*) by cytolemma interspace (*IS*). Mitochondria in muscle cell found beneath plasma membrane, near myoneural junction, near *Z* lines and around nucleus (*Nu*). Part of a single myofibril (*F*) is shown, with 2 sarcomeres and 2 central *Z* lines. Endoplasmic reticulum continuous throughout cell; extending transversely from plasma membrane infoldings to nuclear membrane, and longitudinally around the myofibril, with central association at *Z* and secondary at *H* regions. Known plasma membrane resting potential and inferred intracellular distribution of resting potentials indicated by plus and minus signs.

Known and Inferred Responses to Potential Alterations. The imbalance initiated by the local currents from the end plate would have the following consequences upon the entire system in the muscle fiber:

(1) Propagation of the action potential along the plasma membrane, accompanied by ion displacements.
(2) Alteration of the ion concentration surrounding the endoplasmic reticulum at its points of peripheral attachment.
(3) Propagation of a potential of activation along the membranes of the endoplasmic reticulum.
(4) Ionic imbalance around the sarcomere at the *I* (*Z*) and *H* (*M*) regions, where the reticulum is intimately associated with the fibrils.
(5) Alteration of mitochondrial trans-membrane potentials and consequent ionic imbalance in the inner mitochondrial phases.
(6) Alterations in the nuclear membrane proper, nucleoplasm, and pore regions.
(7) Energy release by inversion of membrane potentials.
(8) Restoration of ion balance and potentials.

It should be emphasized that these events are cyclic in nature and interact. For example, the mitochondria receive stimuli from the advancing cytoplasmic imbalance and are influenced by 3 carbon products from fibril activity. In turn they produce the energy for the restorative processes. As mentioned above, the events should be the reverse in neurons. Action potentials are initiated by metabolic processes in the interior and secondarily transmitted to the cell surface.

It is important to recognize that under the conditions considered nothing 'flows' or is 'transported', but that only a series of reversible local disturbances succeed each other. By the structural relationships now known a much more intimate influence of action potentials occurs upon the cellular interior than previously could be considered. Distances for ion movements are brought within the range of a few millimicrons across membranes, and the areas of influence narrowed to ½ micron or less.

Recognition of the Endoplasmic Reticulum and Theories Regarding its Function.—As a necessary background for the consideration of the function of the endoplasmic reticulum in intracellular conduction, we may note some pertinent electron microscope observations and interpretations. The presence of a discreet network of intracellular tubules and vesicles in a wide variety of cells was first shown by PORTER *et al.*[1]. For more detailed information the reader is referred to recent reviews[2], and to specific papers on muscle[3], and nerve[4].

Attention was directed early to the relation of the endoplasmic reticulum (ergastoplasm) with cytoplasmic basophilia[5], and to its possible role in muscle adenylic acid metabolism[6]. Its part in nucleocytoplasmic exchange has been considered more recently, particularly in relation to exchange of RNA[7]. The proposal has been

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made that exchange of inorganic ions and small molecules occurs across reticulum membranes by way of the perinuclear cisterna and that large molecules pass through the pores of the nuclear envelope[8]. Probably this mechanism represents an important circulation of gene-specific products from the nucleus to the cytoplasm and the resupply with breakdown products to be utilized once more by the nucleus[9]. By this mechanism the cytoplasm not only is regulated by, but also regulates nuclear activity. A functional parallel exists between plasma membrane (control of environment-cell exchange) and reticulum membrane (control of nucleus-cytoplasm exchange). It is important to note that the nucleo-cytoplasmic, as well as the cell surface exchange, can now be considered to be under the influence of nerve potentials.

The endoplasmic reticulum forms part of the Nissl substance[10] which loses characteristic staining properties upon exhaustion of the neuron. In like manner the endoplasmic reticulum disappears in certain liver cell injuries and during regeneration reappears first around the nucleus and immediately beneath the plasma membrane[11]. It is notable that nerve cells and cells under considerable nervous control, such as gland and muscle cells, are rich in ergastoplasm and endoplasmic reticulum respectively. A special functional situation obtains in neurosecretory cells in which nervous and secretory activity coexist, and secretory products are very likely elaborated at the expense of Nissl substance[12].

That an intracellular network exists in striated muscle, and that such a network may bear an important relation to the excitation of the contractile material was postulated in the last century[13]. On the basis of electron micrographs, BENNETT and PORTER[14] first suggested the possible mechanics of involvement of the reticulum during contraction. It was thought that the reticulum might itself move, or might participate in the movement of material from sarcoplasm into fibril. As a second possibility, it has been pointed out that its membranes may flow by formation in one region and breakdown at a distance; thus serving as carriers of particles and ions[15]. Two investigating groups have arrived at what we consider the most logical interpretation for the problem of conduction, i.e., that the endoplasmic reticulum possesses a trans-membrane potential and serves in impulse transmission by membrane depolarization[16].

The membranes of the endoplasmic reticulum separate phases with different protein and ion concentrations. Following fixation, the inner phase of the endoplasmic reticulum appears less opaque than the cytoplasmic matrix; indicating a lower protein concentration in this phase. The semipermeability of the membrane has been postulated[17] for the ergastoplasm of pancreas, and the suggestion made that the tubules could behave like osmometers. Experimentally it has been shown that the reticulum tubules swell osmotically in muscle degeneration following ischemia[18], and that the intrareticulum and perinuclear spaces are augmented in water resorbing intestinal epithelium[19]. These facts necessarily imply water movements, alterations of ion concentrations, a transmembrane potential, and lead to the possibility of impulse conduction.

Indeed, the endoplasmic reticulum has been considered to play a role in ion exchange, water balance, nuclear-cytoplasmic exchange, degree of shortening of the sarcomere, and conduction of excitation. The multiplicity of function derives from the morphologic arrangement and selective permeability of the reticulum membranes.

Further Evidences for the Role of Endomembranes in the Conduction of Excitation.—DAVSON and DANIELLI[20] calculated from membrane capacitance and dielectric constant a necessary thickness of the order of 100 Å for the irritable membrane (see KATZ[21] for discussion of physical properties of cell membranes). Electron micrographs in fact show a plasma membrane thickness of ca. 100 Å. The same value obtains for the membranes of the endoplasmic reticulum. It has been postulated further that parts of the plasma membrane may migrate into the cell to participate in the formation of the endoplasmic reticulum[22]. A rough calculation of the surface ratio between plasma membrane and reticulum membrane, in fibrillar muscle fibers, indicates that the contact surface between reticulum and cytoplasmic matrix is at least 5 times greater than that between plasma membrane and matrix. The volume included by the reticulum membranes per surface unit compares with the space between plasma membrane and basement membrane, or between two adjacent plasma membranes in the case of the intercalated discs in heart muscle[23]. Within the membranes of the endoplasmic reticulum or between the plasma membranes of the intercalated disc, there should be little loss of ions due to the fact that these systems are closed. In the case of the sarcolemma, the basement membrane must serve as a restricting ion barrier. Otherwise more ions would be lost from the interspace to the interstitium. The lack of this restricting barrier in the intercalated disc means facilitation of longitudinal cell to cell conduction.

There is no evidence of propagated muscle potentials in insects and crustaceans[24], but probably a summing of local non-propagated potentials, suggesting that the muscle response is a series of local shortenings. Morphologic evidences of multiple nerve endings and longitudinal nerve-muscle contact in insects[25] and lobster[26]

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support the electrical data. Similar multiple nerve endings, 'small fiber system', have been shown in the frog for certain tonically reacting muscles[27] which show local, non-propagated shortening in the vicinity of the small motor nerve ending. It has been further found that the fine nerve twigs terminate opposite *Z* bands in insect muscle[28], suggesting that conduction could occur along the 'Grundmembran'. It must be noted, however, that the 'Grundmembran' concept meant the separation of two equal phases of cytoplasmic matrix and not the separation of two ionically unequal phases as achieved by the endoplasmic reticulum.

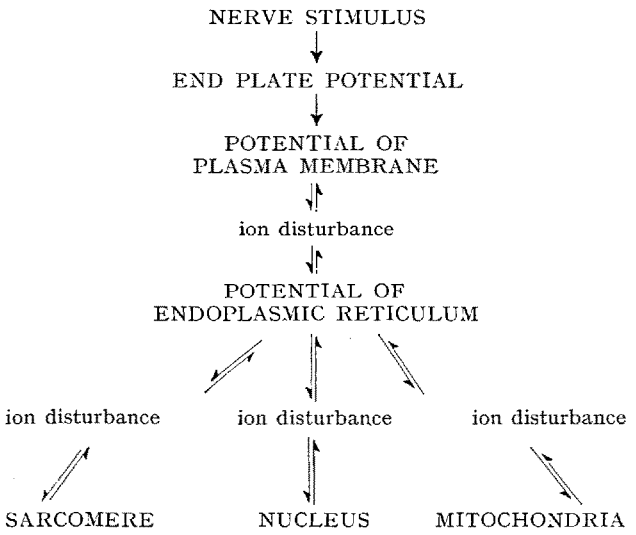

Fig. 2. – Diagram of probable sequence of events from nerve action potential through to muscle cell nucleus.

Not only the nerve-muscle relationship but also the reticulum-contractile material relationship becomes important. Phasic muscle fibers possess a well organized longitudinal and transverse endoplasmic reticulum and well defined fibrils (Fig. 1), whereas tonic fibers are characterized by non-fibrillar contractile material[29] and sparse reticulum with regular peripheral attachments but less systematic longitudinal distribution[30].

Application of small electric impulses to restricted regions of a single muscle fiber by means of a micropipette causes a response when applied to the *I* region but the same stimulus is ineffective against the *A* band. The resulting local contraction travels inwardly, indicating that the *Z* band (reticulum!) is involved in excitation and conduction[31]. Thus, it is evident that a periodic variation in thresholds and excitability exists along the muscle fiber, in correspondence with the periodic attachment of the endoplasmic reticulum to the plasma membrane at the *Z* band levels. High excitability and low threshold reside at *I* and the inverse at the *A* regions. This in turn suggests that a segmental decrement occurs in the surface action potential of muscle fibers, perhaps similar to the internodal decrement in nerves[32]. The sarcomere, together with its endoplasmic reticulum and surrounding cytoplasmic matrix, now takes the place of the muscle fiber as the functional element for potentials, tension, contraction and anaerobic metabolism. Morphologically, the sarcomere has been defined as the *Z* to *Z* distance of the contractile material. Since the contraction is symmetrical, involving the migration of the two proximal halves of the *A* region towards the *Z*, the true motor unit should be the *M* to *M* distance, with the *Z* as the center[33].

As far as can be determined from electrophoretic and other measurements[34], the cell surfaces, cytoplasmic inclusions and nuclei are positive relative to the cytoplasmic matrix, and the nuclear envelope positive to the nuclear matrix. From these determinations and the known relations of the endoplasmic reticulum, we can conceive of the charges being distributed as shown in Figure 1. It is inferred that the permeability characteristics of the endoplasmic reticulum are equal to those of the plasma membrane. The phase inside the endoplasmic reticulum differs from that outside the plasma membrane. However, both the plasma membrane and the endoplasmic reticulum membranes are exposed to the cytoplasm as a common phase. Hence, they must permit similar exchange of ions, e.g., Na and K, must possess similar mechanisms for maintenance of ionic gradients and impulse transmission, and have a common cytoplasmic source of energy for restoration. It has been shown that metabolic inhibitors have an effect on skeletal muscle different from that on nerve [35]. However, it is quite possible that several types of enzymic 'pumps' might exist according to the cytoplasmic phases and membranes of the tissues involved. The Donnan equilibrium, which applies only between homogeneous phases, does not hold for muscle and nerve cells. It has been suggested that this is due to permeability barriers or to the heterogeneity of the cell interior[36]. Such permeability barriers and heterogeneity of cell interior can now be seen as the endomembranes and the additional intracytoplasmic phases separated by them.

Taking the muscle cell as a model for the demonstration of the role of endomembranes in conduction of excitation, one can derive a general scheme of the probable sequence of potential and ionic disturbances from nerve stimulus through to muscle nucleus as presented in Figure 2.

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Zusammenfassung

Die morphologische Beobachtung von getrennten cytoplasmatischen Phasen in geschlossenen Membranen führt zur Annahme von Membranpotentialen innerhalb der Zelle. Schreibt man den Membranen des endoplasmatischen Reticulums die Eigenschaften der Plasmamembran zu, so leiten sie die Erregung ins Zellinnere und dienen als Potentialspeicher.

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