COMPARATIVE PHYSIOLOGY OF THE NERVOUS CONTROL OF MUSCULAR CONTRACTION

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PREFACE

No characteristic of animal functioning is more distinctive than the power of movement. But the possession of tissues having the capacity for doing mechanical work is of no consequence unless a means exists for harnessing the latent power and releasing it, in a manner calculated to serve the functional needs of the animal. The achievement of this control is the primary function of the nervous system. The control process has so many aspects that several divisions of the science of physiology are included. There are the speed and power of the whole muscle in relation to load and excitation, under different environmental conditions, which set limits to the extent to which complete control is possible; there are the functioning of the motor nerves, the mechanism of transfer of excitation from nerve to muscle, and all those reflex aspects of the functioning of the central nervous system such as were studied by the Sherrington school. We may also add the study of the muscle proprioceptors and the associated servo-mechanisms. Indeed, the whole of the nervous system (including the autonomic nervous system) is associated directly or indirectly with the control of muscle.

There is no reason to doubt that motility and irritability are two distinct physiological processes in every instance in which they are encountered. But the two are coupled together in intimate fashion. It is probable that a coupling mechanism relating muscle-cell irritability to contraction existed before nerve cells were evolved. A similar link is probably present in an elementary state in the muscle cells of the sponges.

The simplest way in which the evolving motor nerve cells could have influenced motility was to affect the irritability of the muscle cells, a direct influence on the contractile elements in the cells probably being much more difficult to attain. Was this the case? If so, what is its mechanism? Do all animals have similar mechanisms? If not, what is the variety of mechanisms encountered and how has it evolved? These are some of the problems which it is the task of the comparative physiologist to explore and with which the present monograph is concerned. Since a myoneural apparatus is present in all phyla except the

Protozoa and the sponges, there is a wide range of animals in which to study the problems.

The task of presenting the comparative data at the present time is rendered difficult by the absence of a detailed account of the comparative anatomy of the myoneural apparatus, and by the fact that 'fundamental' mechanisms which have been discovered in recent years have not yet been incorporated into the general body of physiological knowledge. Since it seems unlikely that the first of these requirements will be satisfied for a considerable time, a summary account is presented here. Also, although the 'fundamental' data have been frequently reviewed already they are too recent to have been incorporated in any text-book of comparative physiology. These data are therefore given here in the hope that a unified presentation will be of help to the student.

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G. H.

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CHAPTER I

The Myoneural Apparatus

INTRODUCTION

AN understanding of physiological mechanisms in the nervous control of muscle must be preceded by knowledge of the anatomy and fine structure of the myoneural apparatus. Only rarely, as in the elucidation of nervous tracts, can the physiologist directly assist the anatomist. The anatomy must always be a little better known than the physiology. But at the present time there are enormous gaps in knowledge in both disciplines. This is particularly the case in regard to all invertebrate phyla, where there has been little interest in the fine morphology since the great era of comparative anatomy in the nineteenth century. Even then the accounts were usually no more than incidental parts of general descriptions. Also the functional approach, which would have led investigators to ask what, from the present viewpoint, are the right questions, had not at that time been developed. The newer interest in the field, which is now developing rapidly, is the result not of fresh anatomical researches, but of the inquiries of experimental investigators. These workers, finding themselves armed with powerful resolving techniques which might be expected to solve many functional problems, can only hope that a new generation of anatomists and histologists will arise to establish the morphological data on which basis alone they may proceed to an understanding of function.

The muscular apparatus of many metazoan invertebrates, instead of being formed of discrete anatomical muscles, frequently consists only of layers of scattered muscle cells embedded in, or attached to, sheets of connective tissue. Muscle cells are encountered even in those Metazoa (the sponges) which possess the lowest level of structural organization. Here they occur in isolated groups where their contraction serves to perform the simple functions of retraction and constriction of oscula, but sponges have no nerve cells, so excitation of these muscle cells must be due entirely to local stresses.

In the coelenterates, which exhibit the lowest level of the tissue grade of construction, the muscle cells are under nervous control. Many of the cells have a double role, since they are functional members of either epidermal or gastrodermal surface layers. The muscular portions of the cells are no more than tails which contain contractile threads. The tails are attached to an intermediate layer of the body-wall (the mesogloea) on which the forces of contraction are primarily exerted. The more advanced coelenterates, both polypoid and medusoid, possess many independent muscle cells in which the non-contractile portion is rudimentary. If the tails of the musculo-epithelial cells were aligned in a random manner only a single, simple mode of response to stimuli would be possible, in the form of local or general shrinking. Even a simple polyp such as *Hydra*, however, can exhibit a range of shapes according to the state of contraction in the muscle cells in different regions of the body-wall. The movements of the different parts of the body can be co-ordinated into the characteristic movement patterns of feeding and locomotion. This is made possible by the fact that the muscular tails of the epidermal and gastrodermal layers of cells are so arranged that there is in each layer a predominant orientation at right angles to that in the other (Gelei, 1925). Thus the inner layer of muscle tails belonging to gastrodermal cells occur at right angles to the oral/aboral axis forming a sheet of circularly arranged fibres which extends throughout the length of the animal. Similarly, the attachments of the epidermal cells in the oral/aboral axis form an outer sheet of longitudinal muscle. Since the animals take the form of a sac, filled with sea or fresh water, contraction of either sheet must cause collapse when the mouth is open; but when this is closed tightly the two sheets of muscle form an antagonistic pair, in which contraction of the one must cause a stretch of the other as pressure is transferred by the contained fluid (cf. Chapman, 1949).

The majority of the muscle fibres are highly attenuated, single-celled elements which, although running on straight courses for considerable distances, branch and make contact with their neighbours sufficiently frequently for the whole to form a thin muscular sheet resembling a network, first clearly demonstrated by O. and R. Hertwig (1879, anemone) and Schäfer (1878, jellyfish). The individual muscle cells of the more advanced forms are deeply embedded in the mesogloea. They may be as long as 1 mm. but are seldom more than 1–2 μ in diameter and often less than 0·5 μ (Pantin, 1952). They are partly organized into tracts which come close to functioning as individual anatomical muscles (Bullock, 1943; Pantin, 1935*a*, *b*, *c*). Such tracts are found in the oral disc of the anemone *Calliactis* as a sphincter, in the mesenteries of *Metridium* as longitudinal retractors, and in the jellyfish *Aurellia* as a circular band.

Striated muscle fibres are encountered fairly frequently, particularly in medusae (Fig. 1 *a*), where marked cross-striations are observed in fibres of the velum and subumbrellar ring (Hertwig & Hertwig, 1879; Krasinska, 1914; Horridge, 1954, with photomicrograph). Even the muscular tails of epidermal cells, which may be regarded as the most primitive type of contractile element (excepting only the myonemes of Protozoa), may be clearly marked with cross-striations. The muscle cells of the ctenophores are usually smooth, although in the lateral muscles of *Eupocamis*, which contract particularly rapidly and vigorously, they are said to be striated (Biedermann, 1896).

Little advance can be made on the coelenterate type of muscular organization as a basic pattern for the achievement of movement until a rigid skeletal apparatus, internal or external, has been evolved. Only then can the muscular sheets be organized into separate anatomical muscles, each with a particular site of attachment, thereby ensuring that the muscular effort is applied in a definite direction. A very limited advance can be achieved by the further development of a basement-membrane system of connective tissue fibres arranged in a lattice. These fibres can be arranged in such a way as to permit extension whilst remaining inelastic and thus serving as a relatively rigid framework for muscles. The animal then becomes more like a piece of rubber tubing than a sac. It possesses a definite shape even when all the muscles are slack. This system has been adopted by annelids (Picken, Pryor & Swann, 1947), nemertines (Cowey, 1952) and nematodes (Picken *et al.* 1947). The way to further progress has been the development of serially repeated transverse septa dividing the body into segments, as in the annelids. The longitudinally aligned muscle fibres can then be attached to the septa and the different segments operated independently. This permits locomotion in a much more efficient manner than is possible with a continuous cylinder of muscle.

The muscle cells of coelenterates are innervated from nerve cells which form part of a plexus or network of bi-, tri- and multipolar cells in which only a few definite tracts can be distinguished. Considerable attention has been focused on the structure of the net, as being the most elementary type of nervous system, and on the nature of the connexions between the nervous elements of this network. Schäfer (1878) described the nerve net in the medusa *Aurellia aurita* and regarded the connexion between nerve cells as occurring without fusion of the cytoplasm at the junctions. The nature of the network in actinians was extensively studied by O. & R. Hertwig (1879) and shown to be essentially similar to that of the medusa. Their work was confirmed by Groselj (1909) and that of Schäfer by Bozler (1927*a*, *b*), the latter discovering the multipolar cells. Discontinuity between the nerve cells was emphasized by Woollard & Harpman (1939), and by Batham & Pantin (1951). In *Hydra*, however, Hadzi (1909) and McConnell (1933) believed that there is cytoplasmic continuity between the nerve cells. Cytoplasmic continuity has not been claimed for the neuromuscular junctions.

Small knobs have been described at the points of contact between nerve elements and muscle fibres. Schäfer (1878) described them as follows: 'In some instances the nerve ending takes the form of a fusiform dilatation which may contain a small nucleus; in other cases the dilatation is more marked, and may even form a triangular, flattened expansion within which a number of nuclei can be detected. These expansions of the end of the nerve appear to represent a primitive form of the motorial end-plate.' A somewhat similar ending on a muscle fibre of *Beroë* is described in Schneider (1902). Hertwig (1880) figured these junctions (Fig. 2*a*). There may be a small swelling with a nucleus at the point of contact, and more than one termination from different fibres may occur on the same muscle fibre.

Pantin (1952) has presented a photomicrograph of a nervous structure in *Metridium* which he calls a motor end-plate on the retractor muscle field. This is a large structure with a claw and numerous fine nervous processes, and should supply a large number of muscle cells—if in fact it is a motor ending. Bozler (1927*a*) found simple endings on *Rhizostoma* muscle fibres, describing for some fibres a condition in which there were several endings on a muscle fibre arising from a single nerve fibre. This kind of innervation will be termed *multiterminal innervation*. There were other muscle fibres in Bozler's preparations on which several endings derived from different nerve fibres were present (see also Fig. 2*a*). This second condition will be termed *polyneuronal innervation*. Both conditions are seen most clearly in the arthropods (see below, p. 16).

In the mesenteries of *Metridium* (Pantin, 1952), the nerve net consists primarily of bipolar cells with long axons running on a straight course parallel to the oral/aboral axis for up to 1 mm. Together they form a through-conducting tract which offers less synaptic delay than is the case in the general nerve net, so that conduction reaches its highest rate in this tract. The powerful retractor muscle sheets of the mesenteries receive a motor nerve supply directly from the through-conducting tract. The innervation of the greater part of the musculature occurs directly from local branches of the general nerve network. Sensory cells make direct synaptic contact with the adjacent region of the network.

PLATYHELMINTHS

The platyhelminths possess bilateral symmetry and have evolved a nervous system with a primitive brain, but the muscular apparatus has made little significant advance on the two-cylinder organization, beyond the utilization of transverse bands and the elaboration of longitudinal tracts. Musculo-epithelial cells have, however, been entirely replaced. The muscle cells are all uninucleate, spindle-shaped, non-striated elements; rapid movements are not found in the phylum. A few discrete, anatomical muscles have been developed, but only in connexion with the pharynx and with the copulatory apparatus.

In association with the body-wall the nerve net is still present as a conspicuous feature (Monti, 1897; Sabussow, 1905; Hyman, 1951). The muscle fibres receive branches directly from this subepidermal nerve net; but it can be shown experimentally that a directing and co-ordinating influence comes straight from the brain via nerve trunks which are present as thick strands in the network (Moore, 1924). Whether this motor influence takes effect directly on the muscle cells or via the nerve net is problematical. Monti gives a figure of a motor-nerve terminal on one of the smooth muscle cells of *Dendrocoelum*. It is a nucleated, triangular expansion similar to those described in coelenterates (Fig. 2*b*).

NEMERTINES, ROTIFERS AND MINOR PHYLA

The histology of the muscle fibres of these groups has been little studied. The muscle cells of nemertines are of the same type as those found in platyhelminths, and apart from the basement-membrane system of the former, referred to above, there is no great difference in structural organization. By contrast, the muscles of the microscopic rotifers are in the form of a complete set of anatomical, discrete units. This development is probably a consequence of their having evolved a chitinous cuticle. The animals are so small that an anatomical muscle may be composed of a single fibre, a situation which is encountered elsewhere only in the larval Arthropoda and a few small insects. Most of the muscle fibres of rotifers are probably unstriated, but in some of the published figures, for instance, those of Hudson & Gosse (1886), there is a suggestion of striation.

Anatomical muscles are encountered also in the Chaetognatha (*Sagitta*), and these contain striated muscle fibres (Prenant, 1903; Burfield, 1927). In general those minor phyla which have an external skeleton (e.g. Polyzoa) also possess anatomical muscles, whereas the worm-like animals (e.g. Phoronida) retain the twin-cylinder muscle sheets.

NEMATODES

Very curious muscle cells are encountered in nematodes (Fig. 1*e*). Owing to their unique method of development, in which cell division ceases after a small fixed number of cleavages, many of the cells are large, particularly in the larger species (Chitwood & Chitwood, 1937). The longitudinal muscle cells of *Ascaris megalocephala* are so large that they can easily be dissected out. They form a cylinder beneath the ectoderm, a layer one cell thick, in which the muscle cells are closely packed together. Each cell has an outer, fibrillar portion as seen in cross-section, which is either flattened (the platymarian condition), or

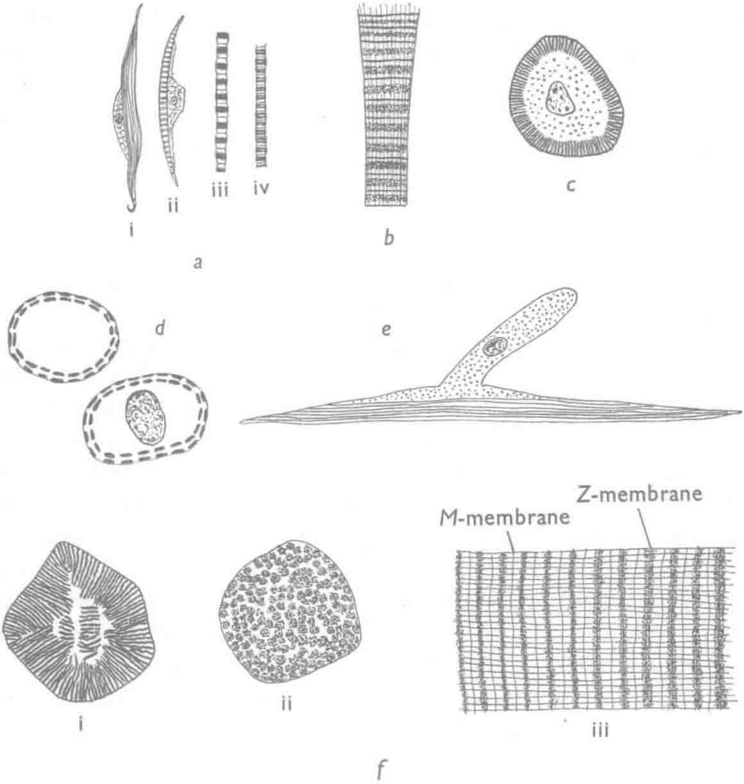

Fig. 1. The structure of some muscle fibres of invertebrates. (*a*) Coelenterate (medusa): (i), (iii) Smooth and striated muscle fibres from *Pelagica*. (ii), (iv) Two striated fibres from *Neoturris*. (After Krasinska, 1914.) (*b*) Annelid (polychaete). Striated muscle fibre from 'gizzard' of *Syllis*. (After Haswell, 1889.) (*c*) Annelid (leech). Transverse section through unstriated muscle fibre of *Hirudinea*. Note 'cortex' of lamellar fibrils and medulla of ordinary non-fibrillar sarcoplasm containing nucleus. (After Bhatia, 1941.) (*d*) Mollusc (cephalopod). Transverse section through striated muscle fibres of arm of octopus showing double layer of peripheral fibrils, central non-fibrillar sarcoplasm with nucleus. (After Plenk, 1933.) (*e*) Nematode. Giant muscle cell of *Ascaris*. The portion containing the nucleus lies in the plane at right angles to the long axis of the cell. (*f*) Insect. Transverse sections through muscle fibres (i) of wing of *Periplaneta*, (ii) of leg of locust. Note radial sarcostyles in (i), round sarcostyles with subfibrils in (ii). (iii) Part of whole muscle fibre of *Cyclochila* showing closely packed sarcostyles, both *Z*- and *M*-membranes. (After Tiegs, 1955.)

U-shaped (the coelomyarian condition). The rest of the cell is composed of granular cytoplasm (sarcoplasm). A median extension of the cell containing the single nucleus runs in the plane of transverse section of the body. According to Roskin (1925), the strongly staining fibrils can be dissected out of the cell. The only other muscular tissue of the nematodes consists of numerous small, discrete, anatomical muscles, associated with spicules, copulatory apparatus, etc. There are no circular or transverse muscles. The accessory muscles are composed of cells which are small, fusiform and unstriated.

The mode of innervation of the extraordinary muscle cells of the nematodes is not known. Nerve branches have been seen to be associated with the nucleated, central extension of the large muscle cells, but no details have been given. Roskin (1925) described some of these muscle cells as showing a series of regularly spaced local contractions following stimulation, an observation which suggests the presence of multiterminal innervation. The small total number of cells in the animals enables each nerve cell to be labelled and identified (Chitwood & Chitwood, 1937). There can be no doubt that motor innervation comes directly from some of the central nerve cells, there being no trace of a peripheral network. Thus in some respects the myoneural apparatus of the nematodes is one of the most advanced to be found in a lower invertebrate; but the absence of segmentation, appendages and skeleton and the habit of parasitism which has been adopted by most members has meant the retention of only the simplest kinds of movements. The group is consequently not very interesting from the present point of view, although the process of neuromuscular transmission from the nerves to the giant muscle cells may be an unusual one.

ANNELIDS

The special advance made by the annelids, as mentioned earlier, has been the evolution of metameric segmentation, with the consequent serial repetition of nerve centres, primitive appendages and septa. The muscle fibres of annelids usually occur grouped together in neat bundles with a relatively small amount of associated connective tissue. They are often flattened along one axis forming a ribbon, with the single nucleus peripherally placed. An elaborate system of oblique and dorso-ventral muscle sheets crosses the body. The appendages and jaws are operated by sets of discrete, anatomical muscles. Although still largely in the primitive, twin-cylinder condition, the muscular apparatus of the annelid body-wall thus possesses a considerable degree of organization.

Haswell (1889) figured markedly striated muscle fibres which he obtained from the gizzard of various species of the polychaete genus *Syllis*. Some of the bands are very wide and a few have only one, centrally placed cross-striation. According to Dahlgren & Kepner (1908), the appearance of striations in these annelid fibres may not be genuine, but caused by the presence of fibrils which connect the cells laterally. Some of the observations recording striations in annelid fibres are undoubtedly spurious. The 'striated muscle band' of *Nephthys* supposed to be enclosed in a 'delicate sarcolemma' (Emery, 1887) is in fact no more than a band of tough connective tissue overlying the nervous system (original observations). Prenant (1903), however, confirmed Haswell's observations on *Syllis* and later (1929) also in other genera, so the real existence of striated fibres in some annelids must be regarded as probable. In large polychaetes the muscle fibres are quite large, up to 30–50 μ in diameter and a few millimetres in length; they should be amenable to direct, modern, physiological analysis.

Those annelids which are capable of rapid longitudinal contractions such as the Sabellidae and Lumbricidae have longitudinal muscles which show a characteristic and beautifully orderly appearance in transverse section. The muscles are divided in the long axis by thin septa. To each of these septa the fine, leaf-like muscle fibres are attached, close together, one above the other, forming a block with a 'fir-tree' appearance in transverse section. Each fibre has a single nucleus, usually placed peripherally, but sometimes within the cytoplasm. In some species there is a gelatinous connective tissue between the leaves (muscle cells) of the block, and the muscle nuclei appear in this. The whole structure of the block then approaches that of a syncytium, a condition which may actually be realized in some instances (cf. figure of muscle block from *Ophiodromus vittatus* in Prenant, 1929). The condition of these near-syncytial muscle blocks illustrates a possible way in which evolution of the syncytial skeletal muscle fibres of arthropods and vertebrates may have occurred.