The Mechanics and Innervation of the Starfish Tube Foot-Ampulla System

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THE MECHANICS AND INNERVATION OF THE STARFISH TUBE FOOT-AMPULLA SYSTEM

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CONTENTS

The main purpose of the following account is to define the conditions under which the movements of an asteroid tube foot are made possible.

The unco-ordinated movements of a single podium include movements of extension, retraction and bending. Every movement is to be regarded as an end-point in a series of events involving the passage of nervous impulses, the contraction of a part of the musculature and the development of hydrostatic pressure within the system of the tube foot and the ampulla. A more complete analysis of these several factors presupposes a knowledge of the general configuration of the tube foot-ampulla system, the mechanics of movement, and the nerve tracts involved in the passage of impulses to the system.

The general morphology of the tube foot-ampulla system is described, and the mechanics of tube-foot movement are discussed in terms of the morphology of the system and of the physical and physiological characteristics of the component tissues.

Protraction of the foot is made possible by the contraction of the ampulla musculature. The fluid within the ampulla cavity is driven into the podium which elongates by reason of the increased pressure within its cavity. During protraction the longitudinal muscles of the foot relax.

It is considered that a tube foot is unable, of itself, to protract to any significant extent. The reasons are (*a*) that the limited movements of extension which isolated feet are said to undergo are caused by a stretching of the muscles under tensions exerted by the contracted and folded tissues of the ectodermal and connective tissue sheaths; they have no nervous basis and do not contribute to normal protraction, (*b*) that there being no circular muscles in the podium, protraction would imply active relaxation of the longitudinal musculature—a phenomenon for which no valid evidence exists, and (*c*) that the fluid content of the ampulla is of the same order as that which would be required to cause the maximum observed degree of extension of the foot.

The pressure exerted by the fluid entering the tube-foot cavity is translated entirely into an elongation of the foot. An outward bulging of its side walls is prevented by the collagen connective tissue sheath which encircles the muscles and is so designed that it resists lateral pressure while allowing of extension.

Retraction of the podium is effected by the active contraction of its musculature, fluid being driven into the ampulla, the muscles of which relax to allow of the increase in volume of the cavity.

Little, if any, fluid enters or leaves the tube foot-ampulla system during these movements. A valve, the morphology of which is described, is present in the lateral water vessel. It serves to prevent the flow of fluid from the tube foot and ampulla cavity into the radial vessel. A reverse flow of any dimensions is improbable in view of the volume relations of the tube foot-ampulla cavity and the vessels of the water-vascular system. The tube foot-ampulla cavity functions virtually as a closed system.

An account is given of the essential character of the asteroid nerve arc. This latter comprises sense cells and association cells, both of which are ectodermal in position, and motor cells lodged within the mesodermal epithelia of the coelomic cavities. Connexion of motor and association elements is made by neurofibrils which penetrate the connective tissue separating ectoderm from coelomic epithelium.

Descriptions are given of the cytology of the various kinds of neurone to be found within the sensory, association and motor divisions of the nervous system.

The anatomy of the nerve tracts supplying the tube foot and ampulla is discussed. The following summarized account should be read in conjunction with figure 12.

Those nerve tracts which are not wholly intrinsic to the tube foot-ampulla system consist, essentially, of three systems of nervous arcs. Two of these have their motor endings on the ampulla muscles and one on the muscles of the tube foot.

The nervous arcs to the tube foot musculature comprise (1) sense cells of the general epithelium (figure 12, *s.c.*) which transmit excitation to (2) neurofibrils of the association neurones in the subepithelial plexus of the tube foot (*n.ring*, *n.long.*) thence to (3) fibrils of the association plexus of the cord (*fib.ext.*, *fib.int.*). The nerve arc is continued, on the motor side, as (4) motor neurones of the first order (*neur.mot.* 1) situated as 'segmentally' arranged groups in the coelomic epithelium lining the floor of the radial perihaemal canal, and finally to (5) motor neurones of the second order (*neur.mot.* 2 (*t.f.*)) lodged in the coelomic epithelium of the ampulla. The axon fibres of these neurones innervate the foot musculature.

One of the system of nervous arcs which supply the ampulla has a configuration similar to the above in respect to elements (1)–(4), but the motor neurones of the second order (*neur.mot.* 2 (*amp.*)) supply the ampulla musculature. In *Astropecten*, where the ampulla is bilobed, it is the medial lobe which is so innervated.

The second source of innervation of the ampulla involves nerve arcs which are independent of the fibrils of the radial nerve cord. Sense cells of the dorsal sheath and tube-foot ectoderm transmit excitation through the fibrils of the subepithelial plexus directly to motor neurones (*neur.mot.* 1) lying in the lateral part of the ampulla neck. The axons of these neurones innervate the ampulla musculature—in *Astropecten*, the lateral lobe musculature.

The system of nerve tracts so defined provides a basis from which, as further work will show, it is possible to analyse the activities of the tube feet in terms of the motivating capacity of the nervous system.

INTRODUCTION

The tube feet of a starfish, as the primary organs of locomotion, are concerned, to a greater or lesser degree, in almost every action which the animal as a whole is called upon to perform. It is, moreover, the *co-ordinated* movement of the podia which, in walking, in the seizing, holding and manoeuvring of food and in the operation of the 'righting' reaction, to name but a few examples, gives direction, purpose and rhythm to the action. When for any reason co-ordination is incomplete or absent the action either fails entirely in its purpose or is effected with hesitation and lack of precision.

It is not surprising, therefore, that the attention of most observers of starfish behaviour has been focused on the problem of how the movements of the tube feet are co-ordinated. Explanations of the basis of co-ordinated movement have centred round two opposing viewpoints. The one, as put forward by Hamilton (1922), assigns but a minor role to the nervous system in the initiation and maintenance of the co-ordinated movement of the podia. Hamilton believes that the tube feet of a starfish all point the same way during locomotion because, being under similar stresses as a result of a traction effect in a common direction, they orientate similarly. The inadequacy of this attractively simple explanation is at once evident when it is observed that a starfish, inverted over a circular dish just large enough to hold the animal round the periphery of the disk and to leave the arms free, will, if immersed in a larger dish of water, co-ordinate perfectly. The ability of an inverted starfish to orientate its feet in a common direction is proof that traction effects are not primarily responsible for co-ordinated movement. Moreover, since a starfish even in the absence of changing circumstances of peripheral stimulation may effect simultaneous and similar changes in the direction of stepping of its podia, it is clear that orientated movement is subject to a central control. A great body of evidence, from which it will be possible to cite but a few examples, is available in support of the view that it is the central nervous system of the starfish, as represented by the circumoral nerve ring and radial cords, which determines and controls the character and direction of these movements.

In 1881 Romanes & Ewart showed that when the circumoral nerve ring was severed there followed a fundamental change in the pattern of co-ordination, while after section of a radial nerve cord the podia distal to the cut, though co-ordinated among themselves, moved independently of the direction of movement of the remaining feet. Jennings (1907), in his elegant analysis of the behaviour of the starfish *Asterias forreri*, re-emphasized the part played by the central nervous system in the control of the movements of the podia. In furtherance of this view, Mangold (1908, 1921) was able, by cutting the nerve tracts in the neighbourhood of the tube feet so as to isolate them from the various parts of the central and peripheral nervous system in turn, to evaluate in a general way the functions of these various parts in the establishment and maintenance of co-ordination. More recently, Hopkins (1927) has suggested that the circumoral nerve ring includes centres which control the polarity of the feet, for while an isolated arm of a starfish tends always to move in a proximal direction, an arm which is allowed to retain connexion with a portion of the nerve ring will move distally. It is probable also, as Moore's work (1910, 1918) shows, that excitatory and inhibitory phenomena are involved in the co-ordinating process.

This kind of evidence suggests that the central nervous system of the starfish, as represented by the circumoral nerve ring and radial nerve cords, functions not only in the passive role of a central conducting pathway but as a system of nerve centres capable, by their intrinsic nervous discharge, of motivating the tube feet. These nerve centres may, moreover, in consequence of the pattern of distribution of their neurofibrils provide the material basis of the co-ordinating process.

Of the nature and distribution of these nerve centres and tracts, the presence of which may be inferred by experimental analysis, practically nothing is known. The work of Ludwig (1877), Hamann (1883, 1885) and Meyer (1906) is fundamental to our knowledge of the nervous elements within the sensory and association systems of the subepithelial plexus of the ectoderm, while it is to Lange (1876) and Cuénot (1891) that we owe our account of the occurrence of motor cells within the mesodermal epithelia which line the coelomic cavities of the echinoderm. Smith (1937) showed how the sensory and association elements of the subepithelial plexus of the general ectoderm and of the radial nerve cords linked up with the deeper lying motor cells to form the nervous arcs which are the basis of reflex action. It was however realized at the time (p. 168) that the plan of the nervous system so defined was quite inadequate to account for all but the simplest of reactions. There can, indeed, be little doubt that a stage has now been reached in the study of starfish behaviour when a much more adequate knowledge of the nervous anatomy of the animal is required as a prelude to further analysis.

As an initial step in this direction I have included in this paper the results of a study of the character and location of the nerve tracts concerned in the innervation of the asteroid tube foot and its associated ampulla, as revealed by intra-vitam staining methods. It is hoped, in further communications, to present an analysis of the co-ordinated and unco-ordinated activities of the feet in which opportunity will be taken to check and to extend some of the results of this anatomical study.

METHODS

It has been shown (Smith 1937) that much of the gross morphology of the asteroid nervous system may be revealed by the use of polychrome stains such as Mallory's triple stain on sections of Bouin, Heidenhain's 'Susa' or Flemming (F.W.A.) fixed material, but it was pointed out at the same time that a more precise delineation of the tracts would probably be obtained if an intra-vitam staining method, suited to echinoderm material, could be found. This has in fact proved to be the case. The method which has so far given the best results is that which McConnell (1932) used in demonstrating the nerve net of *Hydra* and which was originally developed by Unna (1911, 1916).

It has been my practice to make and use the stain in the following manner. To 20 c.c. of 0·5 % methylene blue (Grübler) made up in distilled water was added 1 drop of concentrated hydrochloric acid (A.R.). After stirring the solution for about a minute, 2 c.c. of 12 % rongalitweiss in distilled water were introduced. On heating this mixture gently until it steams, the methylene blue is reduced to its leucobase; the reaction, once started, is quite rapid, the blue solution changing first to an apple-green colour and then to a colourless fluid in which is suspended a yellow precipitate. After filtering, the solution was set aside overnight in a test-tube plugged with cotton-wool.

On the morning following the preparation of this stock solution, pieces of tissue, such as small lengths of dissected-out nerve cord or whole tube feet, were placed, to the number of five or six to each receptacle, in small dishes each containing 20 c.c. of filtered sea water of salinity of about 35 per mille. To each of these, 0·2 c.c. of methylene-blue leucobase was added, it having been found that the most satisfactory concentration is about 1 %. After a minute or two the solution becomes blue by oxidation of the leucobase. The dishes were then covered so as to exclude dust, and examination of the pieces of tissue made at half-hourly intervals; all specimens were examined in sea water on a slide and under a no. 1 cover-slip. Drawings of the preparations were made on squared paper on a scale appropriate to the size of the image as viewed through a square-ruled eyepiece. One-sixth and one-twelfth objectives were usually employed. Although attempts were made from time to time to fix the blue colour of the nervous elements with ammonium molybdate and picric acid-ammonium molybdate mixtures, the preparations lost so much of their finer detail during the process that it was decided to rely entirely on drawings of freshly stained tissue.

Practically all of the many hundreds of preparations of asteroid nerve cells and fibres made at the laboratory of the Marine Biological Station at Plymouth at various times during the period 1937–9 and subsequently at Cambridge were satisfactory and many were strikingly good. For the benefit of workers who may wish to repeat these results the following observations on the use of the method and on the selection of material may be of value.

The stain should not be used for a period of at least 18 hr. after its preparation. My experience is that its staining properties rapidly diminish in vigour after the sixth day; if observations are to be made over a period of several days, new lots of stock solution should be prepared at intervals of 5–6 days. Staining of the nervous tissue commences after about half an hour's immersion in the fluid, the rate and quality of the staining being little altered within the limits of 13–19° C room temperature. The first elements to stain are those lying nearest the surfaces which were injured during the removal of the tissue from the animal. Badly mutilated tissue will not, however, stain at all.

For some reason not understood, tissue from the starfish *Astropecten irregularis* (Pennant) gives far better results than that of any other asteroid so far examined and, in consequence, this species has been used whenever available. It is not necessary to narcotize *Astropecten* before the removal of tissue, but with other animals, as, for example, *Asterias rubens* L., preliminary narcotization with equal parts of 0·4 M magnesium chloride and sea water should be undertaken; excessive secretion of mucus is thereby prevented and staining takes place the more readily.

Finally, it would be prudent to observe that the methylene blue technique has very real limitations, for it is apparent, when a number of preparations of nerve cord or other tissue are made, that the stain selects only a few of the neurones of any system and leaves others unstained. Moreover, different neurones may be selected in different preparations, even though these latter be of similar parts of the cord and viewed at identical levels (i.e. at the same depth of focus). Only by examination of a large number of preparations can a fairly accurate impression of the systems of neurones, as they exist in the cord, be assured.

In order to check many of the observations made on living tissue, as, for example, in locating aggregates of nerve cells, continued recourse has been made to sectioned material stained with Heidenhain's iron-alum haematoxylin or Mallory's triple stain, but the nerve cells and tracts which are described in this paper have all been demonstrated by vital staining.

THE GENERAL MORPHOLOGY OF THE TUBE FOOT-AMPULLA SYSTEM

The general morphology of the asteroid tube foot and of its ampulla is too well known to require restatement other than in the briefest terms. Nevertheless, if the mode of action of the podium during its extension and retraction is to be appreciated, it is necessary to understand not only the innervation of the system but also its setting within the framework of the animal as well as the mechanical properties of the tissues which compose it.

The ambulacral tube feet of *Astropecten irregularis* (figure 1, *t.f.*) are simple finger-shaped extensions of the hydrocoele without terminal suckers. The wall of the foot is composed of four tissue layers. These are (figures 5 A, B) an outer ectoderm (*ect.*) consisting of ciliated-epithelial, glandular and nervous elements bounded externally by a cuticle (*cut.*), a double connective tissue layer (*sh.ext.*, *sh.int.*), longitudinal muscles (*musc.*) and an inner ciliated epithelium (*coel.epith.*). Within the hydrocoele cavity so contained (figure 1, *cav.t.f.*) is a liquid in which are suspended numerous cells and cell aggregates of varying size and shape.

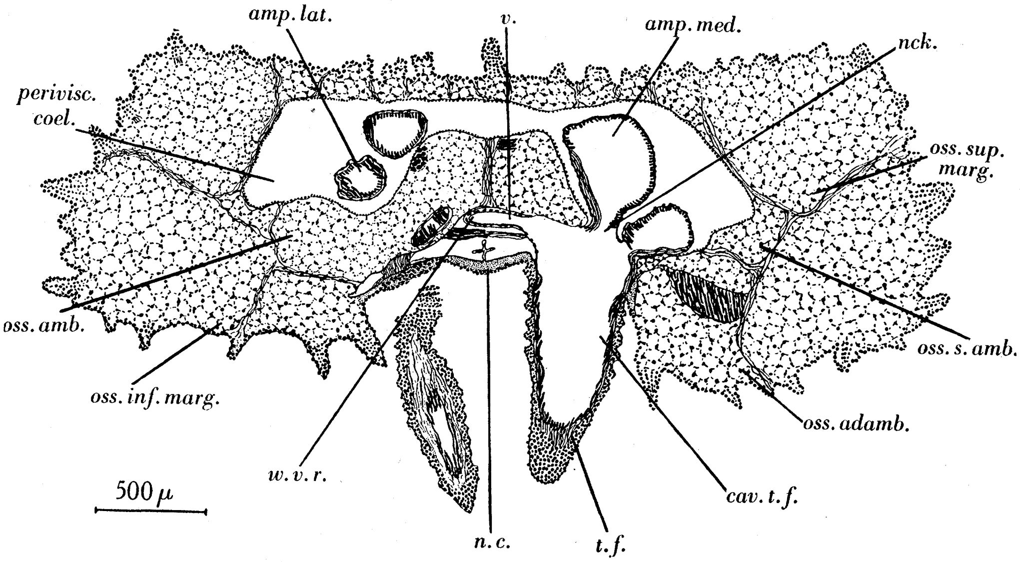

FIGURE 1. Transverse section through the arm of *Astropecten irregularis*. Fixed in Heidenhain's 'Susa' mixture, stained with Heidenhain's iron-alum haematoxylin. *amp.lat.* lateral lobe of the ampulla; *amp.med.* medial lobe of the ampulla; *cav.t.f.* tube-foot cavity; *nck.* neck of the ampulla; *n.c.* radial nerve cord; *oss.adamb.* adambulacral ossicle; *oss.amb.* ambulacral ossicle; *oss.inf.marg.* infra-marginal ossicle; *oss.s.amb.* super-ambulacral ossicle; *oss.sup.marg.* supra-marginal ossicle; *perivisc.coel.* perivisceral coelom; *t.f.* tube foot; *v.* valve; *w.v.r.* radial water vessel.

A narrow canal (figure 1, *nck.*) connects the cavities of the tube foot and ampulla which latter lies wholly within the perivisceral coelom (*perivisc.coel.*) of the arm. The ampulla is almost completely divided into two lobes (*amp.med.*, *amp.lat.*), set across the transverse axis of the arm. The histology of the ampulla wall is similar to that of the podium except that, by virtue of its position within the coelom, its external coat consists of a much-attenuated mesodermal ciliated epithelium.

The narrow neck of the ampulla is lodged within a pore excavated between adjacent ambulacral ossicles, each ossicle being emarginated so as to contribute a demipore. If the tube foot be amputated, the position of the pore is readily seen in oral view of the arm (figure 2, *pore*). The shallow excavation, which in the figure is stippled, marks the area enclosed by the attached base of the foot. It will be seen from the figure that the muscles are for the most part inserted into the distal ossicle of the pair (*musc.insert.*) and that they form a ring broken only at the point of entrance of the lateral water vessel into the tube foot-ampulla cavity. Further it will be noted (figures 1, 3) that the ampulla and its neck are excentrically placed relative to the long axis of the foot; the displacement is lateral in the transverse plane of the arm (figure 1, *nck.*) and proximal along its long axis (figure 3A, *nck.*).

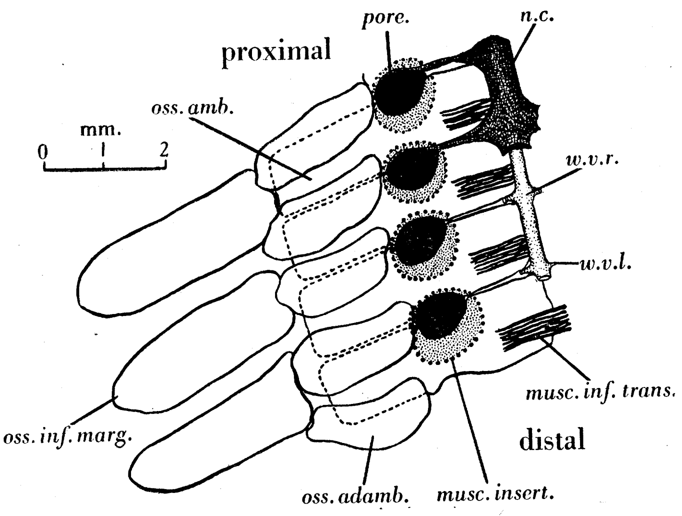

FIGURE 2. Diagram to show the structures visible on one side of a portion of the ambulacrum of *Astropecten irregularis* after removal of the tube feet and adambulacral and infra-marginal spines. Parts of the radial nerve cord and of the radial water vessel have been removed to expose the underlying structures. *musc.inf.trans.* inferior transverse muscle; *musc.insert.* insertion of the tube-foot musculature on the ambulacral ossicle; *n.c.* radial nerve cord; *oss.adamb.* adambulacral ossicle; *oss.amb.* ambulacral ossicle; *oss.inf.marg.* infra-marginal ossicle; *pore.* pore between successive ambulacral ossicles; *w.v.l.* lateral water vessel; *w.v.r.* radial water vessel.

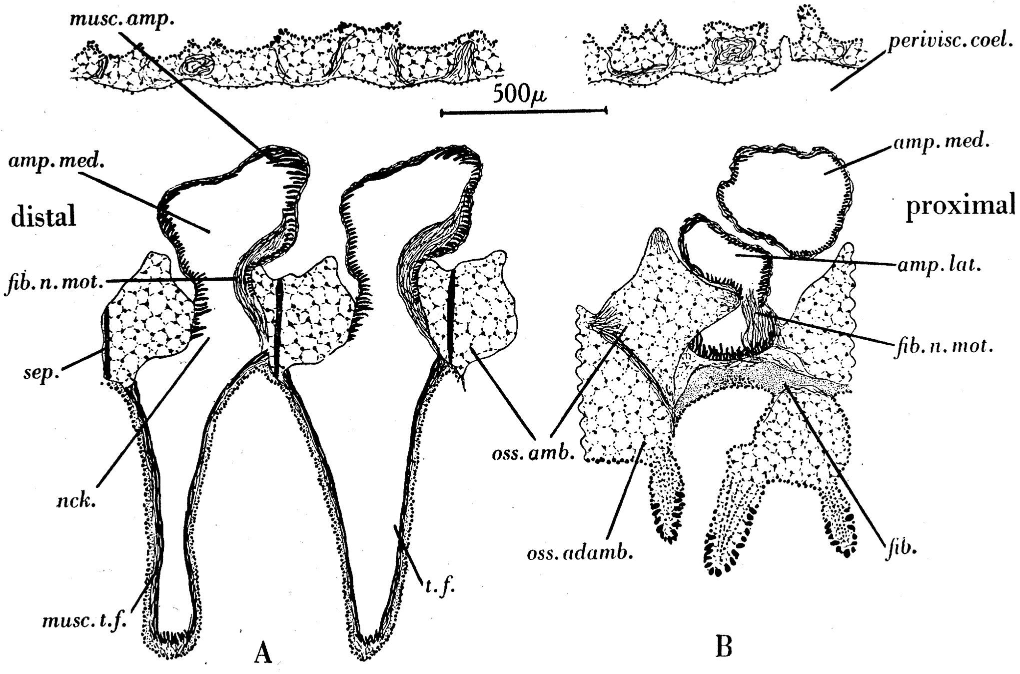

FIGURE 3. Sections from a longitudinal series through the arm of *Astropecten irregularis*. A. Through the medial lobes of two adjacent ampullae. B. Through the lateral lobe and part of the medial lobe of an ampulla. *amp.lat.* lateral lobe of the ampulla; *amp.med.* medial lobe of the ampulla; *fib.* fibrillae of the subepithelial nerve plexus of the body-wall ectoderm; *fib.n.mot.* fibrillae of the motor nerves which supply the ampulla; *musc.amp.* ampulla musculature; *musc.t.f.* tube-foot musculature; *nck.* neck of the ampulla; *oss.adamb.* adambulacral ossicle; *oss.amb.* ambulacral ossicle; *perivisc.coel.* perivisceral coelom; *sep.* line of separation of adjacent ossicles; *t.f.* tube foot.

In *Astropecten* the ampulla and tube-foot musculature are quite distinct. The former (figure 4A) consists of a series of rings, each ring a single smooth muscle fibre. In the ampulla neck (*nck.*) the bands are set horizontally, but in the ampulla itself the rings of muscle are vertical and lie parallel to the long axis of the arm. The individual fibres of the investing connective tissue sheath, on the other hand, run at right angles to the muscles; in this respect they are orientated in a manner similar to the internal fibres of the retention sheath of the podium (*vide infra*, p. 288).

The ampulla of *Asterias rubens* (figure 4B, *amp.*) is a spherical sac. Apart from the fact that the muscles in its neck have a rather different arrangement from the similarly situated ones in *Astropecten* the general disposition of both muscle and connective tissue fibres is much the same in the two forms.

It is not known whether the possession of a simple or of a bilobed ampulla has any functional significance. Taking the class of the Asteroidea as a whole and adopting the classification of Mortensen (1927), it appears that the bilobed type is the more common and that while both forms may occur in any one family only one kind will be found within the limits of a single genus.

The mechanics of tube foot movement

If one of the arms of a starfish be severed and the open end viewed, while still immersed in a dish of sea water, under a binocular microscope of low power, the movements of the tube feet and of their ampullae may be readily examined.

The most obvious feature of these movements is that extension of the podium is always attended by a contraction of its ampulla and that, conversely, the ampulla is distended only when the tube foot suffers contraction. This interdependence of ampulla and podium has, of course, been commented on by a number of authors including Romanes & Ewart (1881), Mangold (1908), Hamilton (1921) and Paine (1929) to name but a few. There is, however, less unanimity of opinion as to the extent of the reciprocation of the two parts of the system. We might, for example, suppose, as Mangold has done, that the extension of the foot is due entirely to pressure developed in the hydrocoele in consequence of the contraction of the ampulla muscles, and that the nervous impulses which evoke extension of the foot do so by excitation of the ampulla musculature. Hamilton, on the other hand, considers such an explanation inadequate. He considers (for reasons which will later be shown to be unacceptable) that the pressures involved are insufficient to cause extension, and he postulates an *active extension* of the foot musculature under the influence of impulses transmitted from the central nervous system. Paine is of a similar opinion except that she regards the impulses as having a local origin (i.e. from the nerve net of the podium), on the grounds that the podia of *Asterias vulgaris* Verrill, even when amputated, are capable of a limited degree of protraction.

This conflict of opinion raises a number of questions relating to the mechanics and innervation of the tube foot and ampulla. It involves, among other things, the question as to whether the tube foot and ampulla operate as a closed or as an open system, whether muscle can be actively relaxed, and whether the reciprocally acting system of foot and ampulla presupposes reciprocal innervation with attendant excitatory and inhibitory effects. A detailed examination of the nature and extent of the changes undergone by the tube foot and ampulla during their contraction and expansion provides data relevant to the solution of these questions.

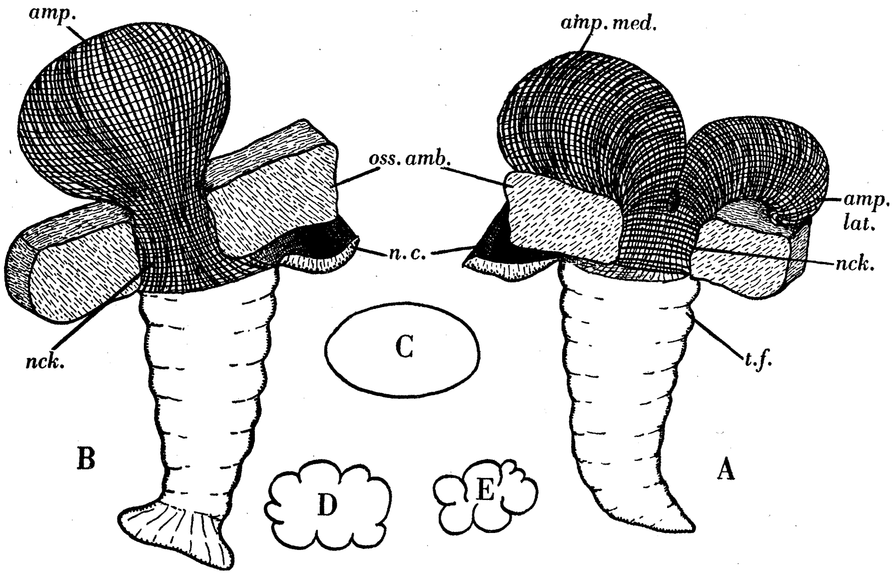

FIGURE 4. Diagrams of the tube foot-ampulla system of A, *Astropecten irregularis*; B, *Asterias rubens*. In the ampulla the heavy lines indicate the direction of the muscle fibres, the lighter lines that of the connective tissue fibres. C, D and E represent, in outline only, the appearance of the ampulla of *Asterias*, as seen from above, during its successive stages of contraction. *amp.* ampulla; *amp.lat.*, *amp.med.* lateral and medial lobes of the ampulla; *n.c.* radial nerve cord; *nck.* ampulla neck; *oss.amb.* ambulacral ossicle; *t.f.* tube foot.

When the tube foot is fully contracted the ampulla appears to be completely distended and is raised above the level of the internal face of the ossicle by the turgor of the neck (figure 4B). The first stage in the contraction of the ampulla consists in the drawing down of the neck (figure 4A), a phase which is accomplished without visible elongation of the podium. Shortly after this, however, it can be seen that the ampulla is being rapidly emptied. At first this is effected without wrinkling of the ampulla wall (figure 4C), but as the volume of the ampulla continues to decrease its wall becomes thrown into folds (figures 4 D, E). When contraction of the ampulla is complete the tube foot is seen to be fully extended and, unless subjected to external forces, is incapable of further extension. The muscles responsible for the contraction of the ampulla appear to be attached along their entire length to the investing sheath of connective tissue. When they contract they first draw the individual fibres of the connective tissue closer together, and so long as this phase is in operation there is no visible deformation of the ampulla wall; the wrinkling occurs when, so to speak, the slack of the connective tissue has been taken up. A similar mechanism of more obvious functional significance is to be found in the foot.

The process of protraction of the podium is marked by one notable feature; the foot maintains its diameter virtually unchanged throughout its movement. The ability of the podium to translate increasing pressure of the fluid contained within its cavity into terms of extension rather than lateral bulging resides in the disposition and properties of the connective tissue sheath which surrounds the musculature. This sheath is seen (figure 5B, *sh.*) to be made up of fibres arranged in two layers, of which those next the muscles (figure 5A, *sh.int.*) are circularly disposed while the outer layer (*sh.ext.*) has its fibres orientated along the long axis of the foot. When dissected out from the surrounding tissue the sheath is found to be non-elastic; it will, for instance, resist any tension that can be exerted by pulling at its free ends with forceps. On staining with Unna's orcein and Van Gieson's acid fuchsin-picric acid mixtures it gives a collagen reaction. Elastin fibres are not present.

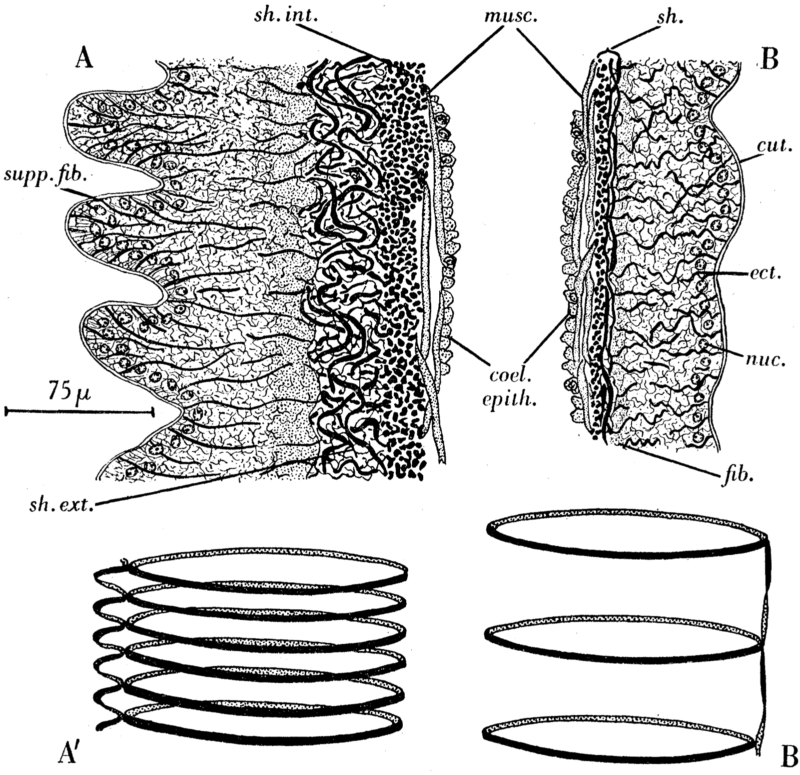

FIGURE 5. A and B are, respectively, longitudinal sections through a part of the tube foot wall in the contracted and expanded state. A′ and B′ represent, in diagrammatic form, the arrangement of the longitudinal (outer) and circular (inner) fibres of the connective tissue sheath in the contracted and expanded podium. *coel.epith.* coelomic epithelium; *cut.* cuticle; *ect.* ectoderm; *fib.* fibrillae of the subepithelial plexus; *musc.* muscle fibre; *nuc.* nucleus; *sh.* connective tissue sheath; *sh.ext.* longitudinal fibres of the connective tissue sheath; *sh.int.* circular fibres of the connective tissue sheath; *sup.fib.* supporting fibres of the ectoderm.

Figures 5 A and B show the arrangement of the fibres in the contracted and extended foot. Each collagen fibre of the inner layer may be regarded as a non-extensible band embracing the musculature. If the foot be contracted, the fibres are, of necessity, piled into layers (figure 5A, *sh.int.*). On extension of the foot, however, they are drawn apart (figure 5B, *sh.int.*), but whether the sheath be contracted or extended the external diameter of the foot is not greatly altered, though the cavity of the contracted foot may suffer constriction by internal bulging of the musculature. The arrangement of the fibres under these two extreme conditions is shown schematically in figures 5 A′ and B′. The rings represent some of the circular fibres of the inner layer, while the single marginal fibre is one of the many which comprise the outer layer of the sheath.

The collagen sheath not only ensures that the podium shall attain its maximum length under the pressure of the hydrocoele fluid but it also prevents an outward bulging of the muscles (figures 5 A, B, *musc.*) when they contract; in this latter respect it recalls the retention bands of vertebrate muscle (Le Gros Clark 1939).

It can be shown that in *Asterias rubens* (where the shape of the ampulla allows its volume to be ascertained within a reasonable margin of error) the volume of the cavity of the expanded ampulla is of the same order as the maximum possible increase in volume of the foot cavity consequent on the extension of the podium.* This would indicate that the elongation of the tube foot is essentially, as Mangold (1908) suggested, a passive process; its muscles relax under loading forces initiated by the contraction of the ampulla musculature and transmitted through the medium of the contained fluid, the extent of the elongation being limited by the quantity of fluid which the ampulla can discharge into it. Hamilton's (1921) objection that the forces exerted by the ampulla are insufficient to account for the full protraction of the podium would appear to be based on a misconception of the action system involved; a contraction of the ampulla musculature adequate to expel fluid from the ampulla will, if the system is a closed one and the muscles of the foot are free to relax, be bound to elongate the foot to an extent provided for by the increased amount of fluid emptied into it. There is, however, one condition which requires to be satisfied if this interpretation of the mechanics of the system is to be acceptable. This condition is that the tube foot-ampulla system is self-contained in regard to its fluid content and that fluid cannot be drawn, in significant quantities, from other sources during the ordinary processes of protraction and retraction.

If a tube foot together with its ampulla be removed from a starfish, the isolated system does not lose its fluid. Perhaps, under these conditions, the intrinsic musculature of the lateral water vessel contracts and effectively seals off the system. But what of the system *in situ*? It may well be, of course, that the muscles of the water vessel contract as in the isolated system, but there seems to be no way of observing this directly. There is, however, a further method of obstructing fluid flow, namely, by the insertion of a valve into the lateral water vessel. Lange (1876) figured and described a valve in this position in *Asterias rubens*, but his observation seems frequently to have been overlooked by subsequent authors, and in giving a more detailed figure of this valve (figure 6) than Lange provided I desire chiefly to re-emphasize the correctness of his description.

The valve (figures 1, 6, *v.*) is a tubular extension of the lateral water vessel (*w.v.l.*) into the cavity of the tube foot (*cav.t.f.*) terminating at its orifice in a thickened lip which can be introverted by contraction of a muscle (*musc.v.*) so as to obstruct or close the orifice.

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* The following measurements are of a tube foot and ampulla of *A. rubens*:

Protracted podium: Length 8·0 mm.
Av. internal diameter 0·62 mm.
Volume $\pi \times 0{\cdot}77$ mm.^{3}.
Retracted podium: Length 1·25 mm.
Av. internal diameter 0·36 mm.
Volume $\pi \times 0{\cdot}04$ mm.

Difference in volume of the protracted and retracted podium $\pi \times 0{\cdot}73$ mm.^{3}.

Cavity of ampulla of height 1·6 mm., transverse diameter 1·8 mm. and longitudinal diameter 1·5 mm., is within the limits of $\pi \times 0{\cdot}6$ mm.^{3} and $\pi \times 0{\cdot}9$ mm.^{3}.

It can be readily appreciated from the figure that while the valve will permit of flow of fluid from the water vessel to the tube foot-ampulla cavity there is no possibility of a return flow. It is, moreover, very unlikely that fluid enters the system in significant quantities as a further consideration will show. The volume of the fluid in the stone canal, circumoral and radial water vessels of a medium-sized starfish, as calculated from measurements made from sectional material, does not exceed 1–2 % of the total capacity of all the tube foot-ampulla systems. A tube foot cannot be recharged with fluid from such an inadequate supply system in quantities sufficient to promote significant movement. When it is further observed that the tube foot of an ophiuroid, which lacks an ampulla, is capable of only very limited extension, while the echinoid podium with its great capacity for extension has a relatively larger ampulla than has the asteroid, it becomes increasingly probable that, for all practical purposes, the tube foot and ampulla of the starfish together function as a closed mechanical system, and that any fluid which may be drawn from the radial vessel serves merely to replace fluid lost by diffusion.

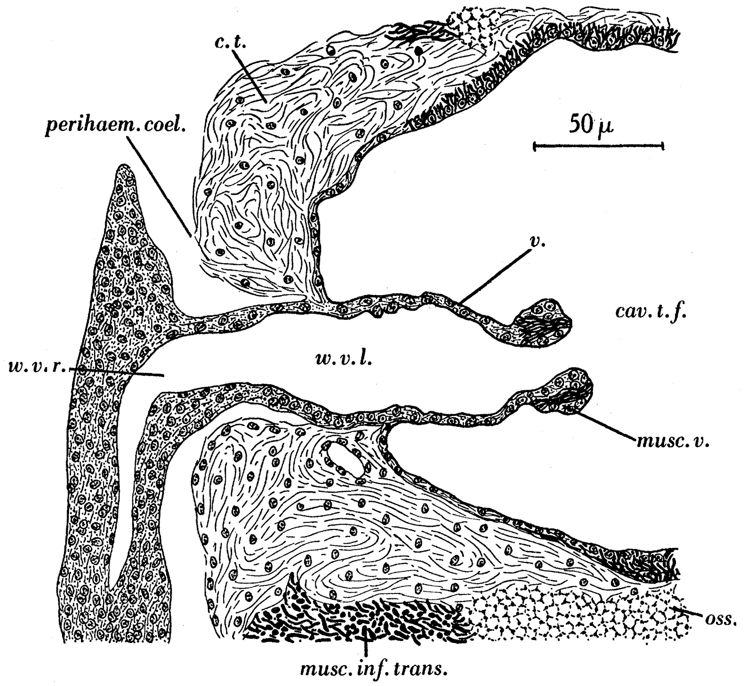

Figure 6. Part of a horizontal section through the arm of *Asterias rubens* to show the valve of the lateral water vessel. *c.t.* connective tisssue; *musc.inf.trans.* inferior transverse muscle; *musc.v.* muscle of the valve; *perihaem.coel.* radial perihaemal canal; *v.* valve; *w.v.l.* lateral water vessel; *w.v.r.* radial water vessel.

There is, however, one feature in the behaviour of the tube foot which does not appear, at first sight, to be explicable on this mechanical basis. Paine (1929) records that excised podia of *Asterias vulgaris*, though they usually remain in a contracted state until they die, may, after a lapse of time, 'elongate very slowly by an almost imperceptible movement'. Podia removed from *Asterias rubens* or *Astropecten irregularis* do not, in my experience, show these movements and, unfortunately, Paine gives no indication of the extent of the elongation of the amputated feet of *Asterias vulgaris*. It is evident that hydrostatic pressure, generated by muscular contraction, can play no part in producing these limited movements, and Paine explains them in terms of impulses which, originating within the nerve net of the foot, cause relaxation of the foot musculature. It is a matter of some importance, when we come to examine the innervation of the tube foot, to determine how these movements are initiated. A later argument may be anticipated by observing that, though it may readily be shown that a tube foot may be made to *contract* its musculature by the application of an appropriate stimulus to its surface, there is no known nervous basis whereby an *active elongation* of the muscles of an isolated podium can be explained. If, on the other hand, this elongation is due solely to mechanical forces, then the problem is somewhat simplified.

The muscles of a tube foot are unstriated. Bozler (1936), in discussing the properties of smooth muscle, shows that, in contradistinction to striped muscle, it exhibits many of the properties of a viscous fluid. Under the application of a constant load, the muscle, after an initial sudden extension, continues to lengthen slowly and at a constant rate; elongation can, moreover, occur under very small loads. On removal of the external force, the muscle does not return, as would an elastic body, to its former resting length, but remains at its new length while the tension which had developed during its elongation rapidly decreases. Let us refer to these properties in considering the autonomous movements of the excised podium.

The foot undergoes full contraction under the stimulation resulting from its amputation. After a while, as Paine reports, elongation occurs and the question we have to ask is this: are there any forces coming into play which would tend to cause mechanical extension of the foot?

In the contracted podium the ectoderm and the connective tissue fibres of the outer sheath are thrown into folds, and it would be natural to suppose that, in tending to straighten, they would exert a slight tension on the contained tissues, including the muscles. As smooth muscle reacts readily to small loads the foot might continue to extend until an unfolding of the tissues produced a diminution of the loading force. It is probable, therefore, that mechanical agencies do exist which would make it unnecessary to postulate, as Hamilton (1921) and Paine (1929) have done, that nervous excitation initiates the extension of the excised podium.

In summarizing the mechanical aspect of tube-foot movement it may be said that active contraction of the tube foot occurs as a result of the excitation and contraction of the foot musculature. Active extension of the podium results from the transmission of excitation to the ampulla, the muscles of which contract; the degree of extension in the closed system is limited by the capacity of the ampulla, while the form that the podium takes during its extension is defined by the properties of its connective tissue sheath.

The nature of the nerve supply and the reactions of the podium to excitation transmitted along tracts capable of definition are compatible with such a system. It should at the same time be emphasized that in so referring to the 'transmission of excitation' (or, as in other parts of this paper, to the 'passage of impulses' down a nerve fibre), no attempt is made to suggest that the events which intervene between the application of the stimulus and the elicitation of the response have in any way been defined, nor that transmission of excitation is to be explained solely in terms of the passage of impulses.

THE INNERVATION OF THE TUBE FOOT AND AMPULLA

(a) *The generalized anatomy of the asteroid reflex arc*

In a recent paper (Smith 1937) the opinion was expressed that, in view of our present knowledge of the distribution of the nervous elements within the body of an asteroid, no justification remained for the retention of the terminology whereby the nervous system was divided into ectoneural, hyponeural and apical parts. Cuénot (1891), in applying these terms, was embodying within them sound and important observations; in particular, he noted that while much of the asteroid nervous system (in effect its ectoneural and hyponeural components) was to be found within the ectoderm, other parts resided in the mesodermal epithelia which line the various coelomic cavities. There can be no doubt whatever of the accuracy of this description as applied to the general lay-out of the system, but the subdivision of the coelomic system into hyponeural (Lange's nerves) and apical parts is inadequate and arbitrary for reasons which have been stated at length (Smith 1937). In this connexion, it may be recalled that the apical system of Cuénot is part of a more extensive series of nerves, which were together designated the lateral motor system. Furthermore, it will be shown in this paper that the greater part of Cuénot's hyponeural system is not involved in the innervation of the inferior transverse muscles of the arm as Lange (1876) and Cuénot (1891) had supposed, but is concerned with the transmission of nervous impulses to the muscles of the tube foot and ampulla.

The disposition and function of the asteroid nerve elements as given by Smith (1937) may best be summarized if one describes, in general terms, the course along which excitation is transmitted when passing from a receptor in the skin to a deep-lying effector such as a muscle. If the skin of a starfish be stimulated, impulses arise within the centripetal fibres (figure 7, *centrip.fib.s.c.*) of the sense cells (*s.c.*) which are present in all parts of the ectoderm in very great numbers. Excitation is transmitted via the cell body of the sense cell to the centrifugal fibre (*centrif.fib.s.c.*). This latter makes synaptic connexion with one of the fibrils of another set of nerve elements, the association ganglion cells or internuncial neurones (*neur.assoc.*, *neur.assoc.'*), the fibrillae of the sensory and association cells together making up the intricate network of the subepithelial fibrillar plexus. The sense cells and ganglion cells constitute the ectoneural system of Ludwig (1899), but, in view of the marked morphological and functional differences which will be found to exist between the two parts of the system, the terms 'sensory' and 'association' will be used for its subdivisions.

Between the association neurones and the motor cells of the coelom wall there is always a boundary of mesodermal connective tissue (*b.z.*). This layer may be very thick and contain calcareous ossicles (*oss.*), but in places it is thinned out to a few microns only. It is through these weak boundaries that connexion between association and motor neurones is made. It would appear that the penetrating fibril may be association in character (*neur.assoc.'*) or that it may be motor (*neur.mot.* 1), so that the synapse may sometimes be on the coelomic side of the boundary, at others within the subepithelial plexus. It would be better, however, at this stage, not to stress the point unduly, as further work on the nature and position of the synapses between internuncial and motor neurones is required in confirmation.

The motor elements (*neur.mot.* 1, *neur.mot.* 2) are not distributed in a haphazard fashion in the epithelial linings of the coelomic sinuses but are developed in the neighbourhood of the muscles (*musc.*) which they are to innervate, though this is not obvious in the particular instance of some of the motor neurones (*neur.mot.* 1) which are involved in the nerve supply of the tube-foot and ampulla musculature. There appears, however, to be one established criterion of motor nerve development, namely, that the subepithelial plexus of the association system, where it lies near the origin of a motor nerve, shows considerable expansion (*fib.exp.*); these local fibrillar expansions always mark the presence of deeper seated motor nerves. This is most marked in the instance of the radial and circumoral nerve cords.

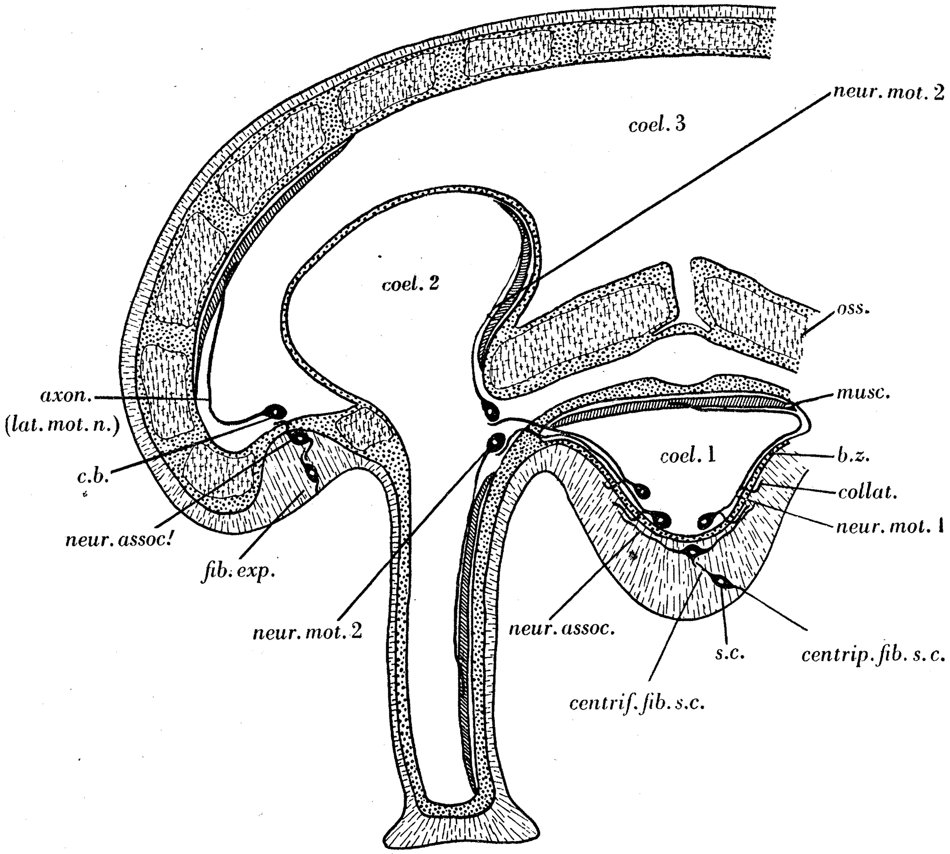

FIGURE 7. Diagram to show the general anatomy of the nerve tracts within the starfish arm and the pattern of innervation of the muscles lying within the various subdivisions of the coelom. *axon*, axon of motor neurone; *b.z.* boundary zone of connective tissue between the subepithelial nerve plexus of the ectoderm and the coelomic epithelium; *c.b.* cell body of motor neurone; *centrif.fib.s.c.* centrifugal fibre of sense cell; *centrip.fib.s.c.* centripetal fibre of sense cell; *coel.* 1, 2, 3, perihaemal, water-vascular and perivisceral divisions of the coelom; *collat.* collateral fibre of an axon; *fib.exp.* fibrillar expansion of the subepithelial plexus opposite the point of origin of a motor nerve; *lat.mot.n.* lateral motor nerve; *musc.* muscle; *neur.assoc.*, *neur.assoc.'* association (internuncial) neurone; *neur.mot.* 1, *neur.mot.* 2, motor neurones of the first and second order; *oss.* ossicle; *s.c.* sense cell.

As to the detailed character of the motor elements the following comments can be tentatively offered. It is probable, though by no means certain, that their cell bodies (*c.b.*) are always terminal and that each has a long centrifugal fibre (*axon*) with or without collateral branches (*collat.*). The axon may run direct to a muscle, but if the muscle to be innervated is lodged in a part of the coelom which is morphologically separated from the cavity in which the motor element has its origin, then a further motor neurone must intervene before the muscle can be innervated (*neur.mot.* 1 and *neur.mot.* 2 of *coel.* 1 and 2). This feature is especially well displayed in the innervation of tube foot and ampulla from the radial nerve cord.

With this general anatomical plan of the asteroid nerve arc in mind we will consider in more detail the series of nerve elements concerned in bringing about reactions of the tube foot-ampulla system to stimuli applied to various parts of the starfish body.

(*b*) *The sensory system*

Receptor cells are found in all parts of the ectoderm of starfishes but are especially abundant in the tips of the tube feet. Both Meyer (1906) and Smith (1937) have reported on the form and distribution of the primary sense cells as they appear in fixed preparations. The following description, based entirely on the appearance of the cells after intra-vitam staining with methylene blue, confirms, in essentials, the accounts already given.

The receptor cells of the starfishes *Asterias* and *Astropecten* appear to conform to a single morphological type. When fully stained, each sensory cell is seen to consist of a bipolar spindle-shaped body (figure 8A, *s.c.*) of length 5–10μ and maximum diameter 1–1·5μ, drawn out at either end into a fibre. It may be epithelial in position, but more frequently it lies below the epithelium. The free end of the centripetal fibre (*centrip.fib.s.c.*) is unbranched and lies flush with the surface of the ectoderm, between the individual cells of which the fibre is intruded. In no instances do the centripetal fibres penetrate the thin cuticular coating of the ectoderm (*cut.*) which does not, apparently, interfere with the reception of tactile and chemical stimuli. The length of the fibre is necessarily dependent on the position of the cell body and is therefore greatest where the epithelium is thickest. In the tip of the tube foot, for example, the centripetal fibre may acquire a length of 200μ.

A centrifugal fibre (figure 8A, *centrif.fib.s.c.*) runs from the cell body into the deeper lying tissues. It is usually unbranched but may exhibit a distal dichotomy. The alinement of the centrifugal fibre within the tracts of the subepithelial plexus will be described in connexion with the association cells (p. 296).

On staining with methylene blue, the cell body takes up the stain first, the colour developing in the fibrils somewhat later. There appears to be some difference in the finer structure of the centripetal as compared with the centrifugal fibre, for it is noticeable that, while the former never becomes beaded even after prolonged immersion in the stain, the centrifugal fibre, after an initial period during which there is no sign of beading, later develops beads along its length. It shares this latter property with all the remaining fibrillae of the nervous system whether they belong to association or to motor elements. The beaded condition is, however, pathological, for it is never exhibited by the freshly stained fibril and, moreover, it precedes a final condition (figure 13C) in which the entire fibril breaks up into large globules—the products of fusion of individual beads.

It is interesting to note that in well-fixed F.W.A. material subsequently stained with Delafield's haematoxylin no varicosity of the fibrils is visible, and, although Meyer (1906) has reported that the centrifugal fibres of the sensory cells of *Asterias rubens* have a beaded appearance, this must be considered, on the evidence of vitally stained material, to be an abnormal condition.

While the dispersion of the sense cells throughout the greater part of the ectoderm is more or less at random, in the tip of the podium they are aggregated into distinct groups.

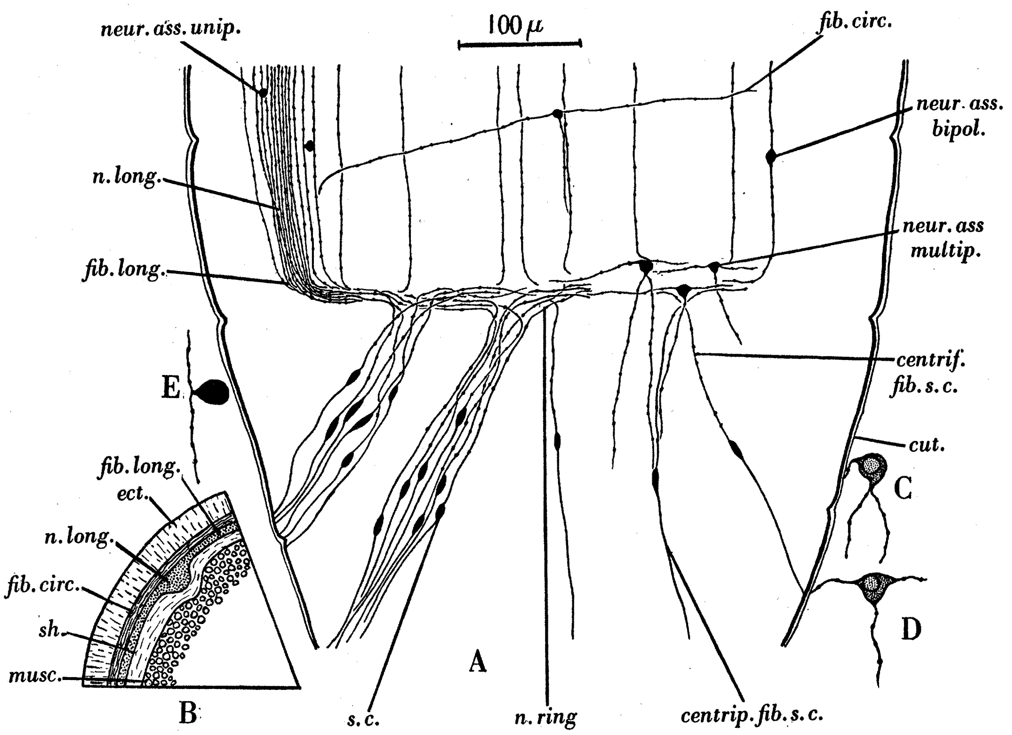

Figure 8. A. A composite figure based on a large number of intra-vitam methylene-blue stained preparations showing the sensory and association (but not the motor) neurones of the distal end of the tube foot of *Astropecten irregularis*. B. Diagram of the proximo-medial sector of a tube foot as seen in transverse section; it shows the relations of the circular and longitudinal neurofibrils of the association system and the position of the longitudinal nerve. C, D. Tripolar ganglion cells of the nerve ring. E. '*T*' neurone of the longitudinal nerve. *centrif.fib.s.c.* centrifugal fibre of sense cell; *centrip.fib.s.c.* centripetal fibre of sense cell; *cut.* cuticle; *ect.* ectoderm; *fib.circ.* circularly disposed fibrils of the subepithelial plexus; *fib.long.* longitudinally disposed fibrils of the subepithelial plexus; *musc.* muscle; *n.long.* longitudinal nerve; *n.ring*, nerve ring; *neur.ass.bipol.* bipolar association neurone; *neur.ass.multipol.* multipolar association neurone; *neur.ass.unipol.* unipolar association ('*T*') neurone; *s.c.* sense cell; *sh.* connective tissue sheath.

This arrangement, however, has no neurological significance. It is brought about by the intervention of interdigitating wedges of connective tissue, the disposition of which is dictated by mechanical considerations (Smith 1937, p. 135).

In *Marthasterias glacialis* (L.) the number of sensory endings per square millimetre of surface of ectoderm is never less than 4000 (Smith 1937). Counts of cell bodies in fixed and in vitally stained preparations show that in the tip of the *Astropecten* podium the number exceeds 8000 for the same area.

(*c*) *The association elements*

Although isolated starfish podia will react to direct stimulation by retracting or by bending, the full range of their activities can only be developed if nervous connexion with the radial nerve cord is maintained (Smith 1945). The part of the nervous system responsible for the transmission of excitation from the sensory elements of the foot to the nerve cord is the chain of association (internuncial) neurones which extend through the subepithelial plexus of the podium into the much thickened plexus of the radial nerve cord. It will be convenient, for the purposes of description, to enumerate and describe these elements in the order in which they are involved as the transmitters of excitation from the receptors of the foot to the motor neurones which, by innervating the muscles of the foot and ampulla, complete the nervous arc.

The free distal ends of most of the centrifugal fibres of the sense cells pursue a circular course around a part of the periphery of the podium where they contribute in great measure to the circular fibrillar zone (figure 8 A, B, *fib.circ.*) which directly underlies the bases of the epithelial cells. In consequence of the great number of sensory cells in the tip of the foot, the circular fibrillar zone here undergoes a local enlargement into the so-called nerve ring (figure 8A, *n.ring*), but it is important to realize that this is only part of a more general plexus of circularly disposed fibrils.

Methylene-blue staining reveals large numbers of association cells among the fibrillae. These cells (figure 8A, *neur.ass.multip.*, figure 8 C, D and figure 9 *a*, *c*, *d*, *f*, *g*, *j*) are usually of the multipolar variety and have a diameter of 10–20μ; tripolar neurones are by far the most common. These latter (figures 8 C, D, 9*a*) have one or two fibrils which receive impulses from sense cells, the centrifugal fibres of which are too short to reach the nerve ring; the remaining fibril or fibrils contribute to the plexus of the ring. The fibrils of the nerve ring make connexion with longitudinally running fibrils which together form a well-defined inner longitudinal plexus (figure 8 A, B, *fib.long.*). Around the greater part of the periphery of the podium this zone is present as a very thin sheath bounded externally by the circularly disposed fibrils (*fib.circ.*), but, in the proximal and medial quadrant of the foot (i.e. by reference to the line of the ambulacral groove), fibrillae are present in numbers sufficiently great to form a conducting path which, being visible in sections, has been called the longitudinal nerve (figure 8 A, B, *n.long.*). Within this 'nerve' the constituent fibrils appear to run the length of the tube foot without interruption, finally to enter the radial cord.

In contradistinction to the ganglion cells of the circular zone of fibres those of the inner, longitudinal plexus are usually bipolar (figure 8A, *neur.ass.bipol.*, figure 9 *b*, *h*) or unipolar (figure 8A, *neur.ass.unipol.*, figure 9*e*) in character, though multipolar cells also occur. They have a length of about 15–25μ and a diameter of 6–8μ. Unipolar neurones are of the '*T*' type. Their cell bodies take up methylene blue more readily than do the multipolar neurones, so that at a time when the latter are still somewhat diffusely stained the '*T*' neurones are dark blue in colour.

It would be well to emphasize at this point that little attention has been paid during the course of this work to the detailed cytology of the nerve cells. In figures 8, 12 and 14, where neurones are figured, the whole cell body is shown as a uniformly stained dark blue mass. It is convenient when tracing the course of neurofibrils to allow the staining to attain to this final phase, though the picture which the neurone then presents is a travesty of its real self. I find it quite impossible to portray in a black and white figure the transient delicacy of outline and the details of cytoplasmic structure of the neurone in the initial stages of its staining. Some of the representative types of neurone found within the various levels of the association plexus of the radial nerve cord and tube foot, and to which reference is made at various places in the text, are shown in figure 9. I have preferred, in the legend to the figure, to describe the cell inclusions in objective terms rather than to attempt to relate them to the normal constituents of the neurone, such as the nucleus, vacuoles, Golgi bodies and Nissl granules, though this correlation should not eventually present any great difficulty.

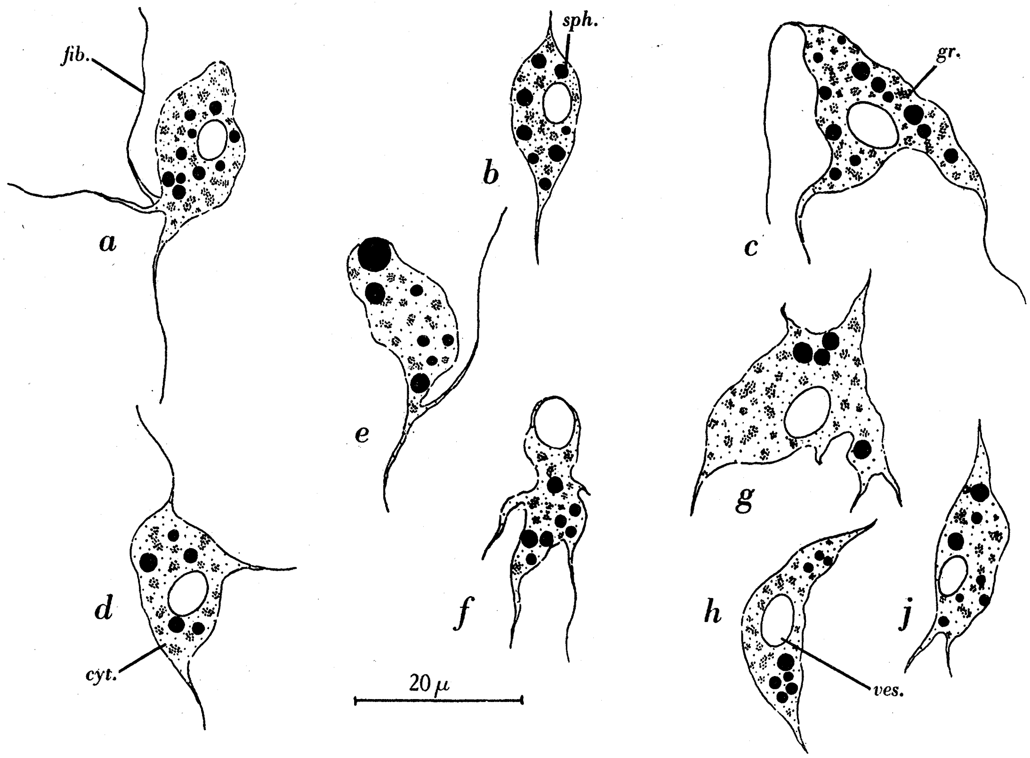

FIGURE 9. Drawings of representative types of association neurones from the subepithelial plexus of the tube foot and radial nerve cord of *Astropecten irregularis* and *Asterias rubens* to show their appearance in the early stages of methylene blue intra-vitam staining. *cyt.* unstained ground cytoplasm; *fib.* neurofibril; *gr.* brown-pigmented granules; *sph.* intensely staining spherical body; *ves.* unstained vesicular body.

To return, however, to the description of the general configuration of the nerve cells and fibrils of the association plexus. It would seem that the whole pattern of distribution of sensory and association cells is related to the transmission of excitation from the receptors of the foot to the nerve centres of the radial cord. Impulses travelling from the centrifugal fibres of the primary sense cells are delivered across a synapse (or what, on physiological grounds, one supposes to be a synapse) to the transversely orientated fibrillae of the outer, circular plexus, thence to the longitudinal fibrillae of the inner zone whence they are transmitted to the nerve cord. On morphological grounds one would further suppose that the longitudinal nerve is the principal conducting tract from the foot to the cord.

Within the radial cord itself there is a comparable, yet in many respects a different, arrangement of fibrils in the plexus. In the first place the plexus is very thick (120μ as compared with 20μ in the tube foot), as, indeed, might be expected, since it is made up not only of the fibrillae of its own intrinsic sensory and association systems but also of the fibrils which it receives from the longitudinal tracts of the tube feet as well as those from the general ectoderm. The centrifugal sensory fibres and the fibrils of multipolar (usually tripolar) association neurones, similar in all essential respects to those of the tube foot, together contribute to the outer or superficial zone of fibrillae (figure 10 A, B (α)) in which the fibrils run in all directions but have an overriding tendency to longitudinal orientation (figure 10 A (α)). Many of these fibrils run to a deeper level in the cord (figure 10 A, B (β)), where junction is made with longitudinal fibrillae arranged in linear series (figure 10 A (β), *fib.long.*). These latter are arranged in bundles which have been shown (Smith 1937) to be separated one from the other by intervening palisades of non-nervous supporting fibres which, since they are not susceptible to intra-vitam methylene-blue staining, have been omitted from the figure.

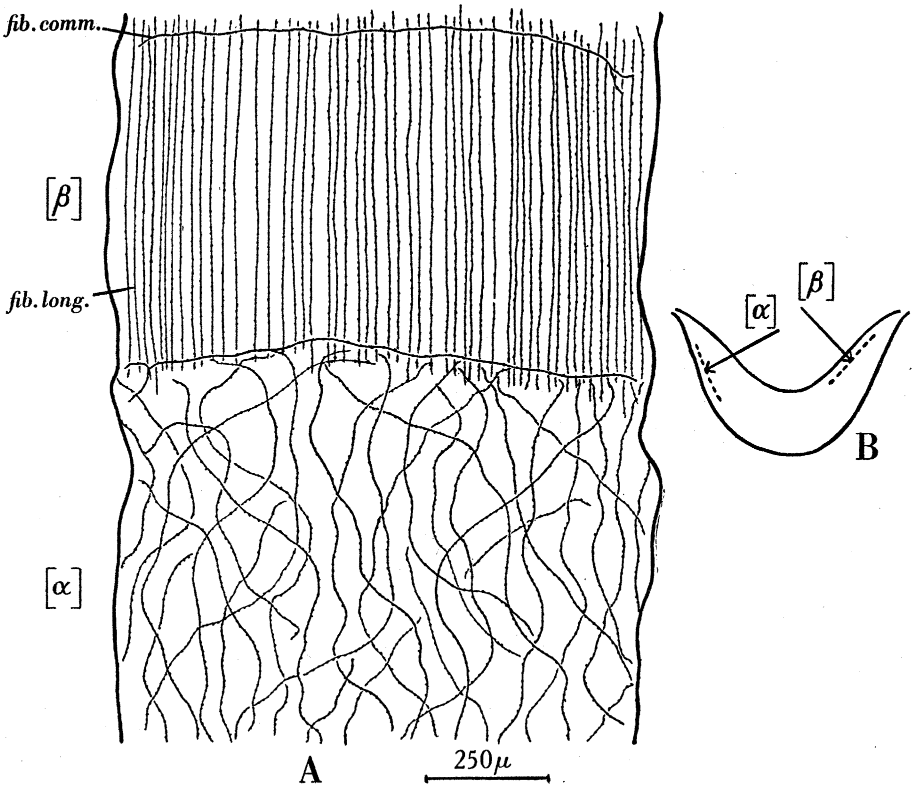

FIGURE 10. A. Diagram to show the orientation of the neurofibrils within the superficial (α) and the deeper (β) layers of the subepithelial plexus of the radial nerve cord. The levels of (α) and (β) are shown in B, an outline drawing of the cord as seen in transverse section.

As with the corresponding elements of the tube foot, the longitudinal fibres may take their origin from unipolar, bipolar or multipolar bodies. It is not possible to trace individual fibres along their entire length, for it is rare for more than a small part of a fibre to be stained at any one time. There is some reason for believing, however, that the deeper-lying fibrils of the radial cord are very long, certainly extending to several centimetres, and it is possible that some of them run an uninterrupted course along the whole length of the cord and so enter the circumoral nerve ring. One might infer from the constitution of this deep fibrillar zone of the radial nerve cord that its fibrillae provide pathways for the rapid conduction of impulses and serve thereby to co-ordinate the responses of the organs of the various arms always provided, of course that it is through these fibrils that connexion with the motor neurones is ultimately made. Before consideration is given to this latter point, however, reference must be made to a further system of association elements. These are the transverse or commissural fibrils (figure 10A, *fib.comm.*), the cell bodies of which have not been seen. Either or both ends of the commissural fibrils may be branched. There can be little doubt that these transverse fibrils ensure co-ordinated response of the tube feet and muscles of the two sides of the arm, a co-ordination which is a marked feature of the behaviour of all starfishes.

In summarizing the implications of the anatomical configuration of the sensory and association systems it may be stated, in conclusion to this section, that, provided the physiological characteristics of the neurones permit it and the stimuli be adequate, the final destination of the impulses delivered to the internuncial neurones of the asteroid nervous system will, in so far as they remain confined to the association system, be the deeper-lying longitudinal fibrillae of the radial nerve cord and circumoral nerve ring.

It now remains for the description of the innervation of the podium to be extended to the systems of motor neurones through which nervous connexion with the muscles of the tube foot and ampulla is finally made.

(*c*) *The motor system*

(1) *The coelomic sinuses of the arm*

The motor neurones of the starfish are lodged in the coelomic epithelium. The coelom is not a simple cavity but is subdivided into compartments separated one from the other by walls of connective tissue faced with epithelial cells. Since motor nerves are potentially capable of transgressing the boundaries of a single sinus it is necessary, in order to trace their course, to define the limits of the separate cavities and to note their morphological relations one to the other.

The primary division of the coelom of echinoderms is into perihaemal, water-vascular and perivisceral portions. Other, secondary, cavities may arise during the course of development, but these may be excluded from this account as they do not enter into the constitution of the tube foot-ampulla system. The coelomic sinuses relevant to the systems to be described are shown, diagrammatically, in figure 11.

The radial perihaemal canal (figure 11, *perihaem.coel.*), which is part of the perihaemal system of cavities is partitioned by a septum (*sept.*) containing strands of 'haemal' tissue. Flooring the cavity is a layer of connective tissue (*b.z.*) below which again lie the longitudinal fibrillae of the association system of the cord. The roof of the sinus (*rf.*) consists of connective tissue within the stroma of which is imbedded the calcareous stereom of the ambulacral ossicles (*oss.amb.*). In the figure, the ossicle of the left-hand side of the arm has been removed; on the right-hand side a small piece of the ossicle and the roof of the perihaemal canal have been cut out in order to expose the perihaemal canal the more completely.

As the diagram shows, the perihaemal sinus of the arm suffers a series of constrictions along its length at points corresponding to the transverse diameter of each tube foot (*t.f.*). Alternating with these constrictions and corresponding to the intervals between successive podia, the perihaemal sinus opens out laterally (*perihaem.coel.lat.*) and partially encircles the foot. A fuller description of the nervous elements associated with the perihaemal canal may be anticipated by stating that while cell bodies (*c.b.*) are found in groups, each of which is limited to the region of one of the constrictions, their axons (*axon*) push out towards the bases of the podia along the lateral extensions of the radial canal. The cell bodies and axons, which in the figure are shown in white, have, in consequence, a 'segmental' arrangement along the floor of the sinus.

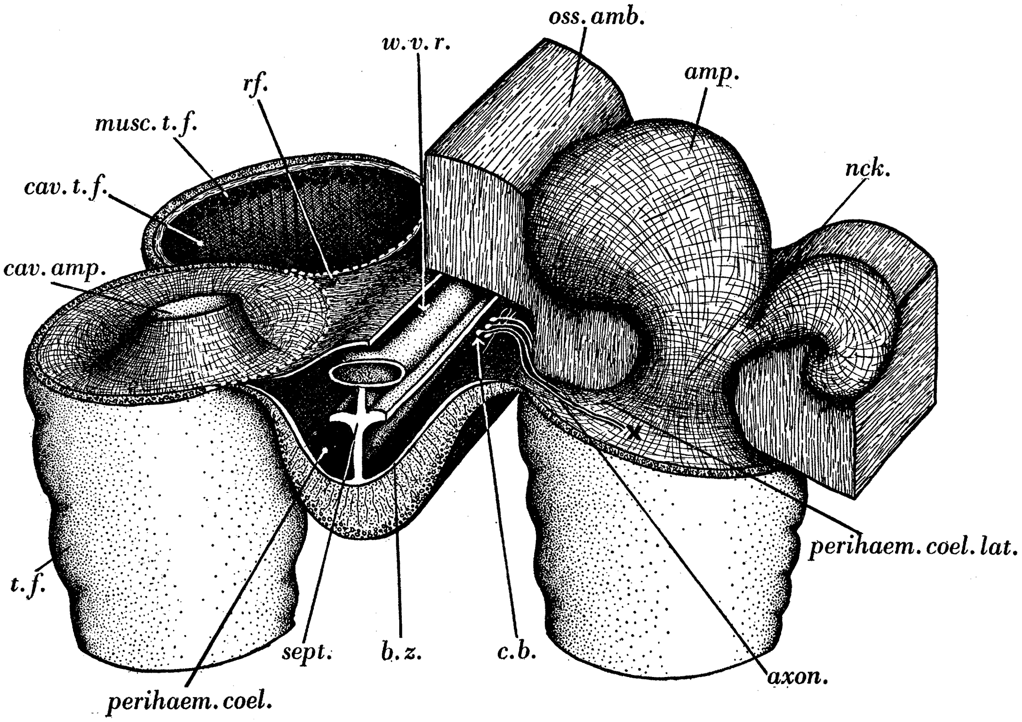

FIGURE 11. Diagrammatic stereogram (not to scale) showing the anatomical relations of the tube foot and ampulla of *Astropecten irregularis* with the neighbouring structures and sinuses of the arm. On the right-hand side of the figure the proximal ambulacral ossicle of the pair contributing to the tube-foot pore, a small part of the distal ossicle, and the underlying roof of the radial perihaemal canal have been removed. On the left the ossicles have been completely removed as have the ampullae. In one instance the ampulla neck is also lacking and the cavity of the foot is exposed. *amp.* ampulla; *axon*, axon of motor neurone in the wall of the perihaemal sinus; *b.z.* boundary zone of connective tissue; *c.b.* cell body of motor neurone; *cav.amp.* cavity of ampulla; *cav.t.f.* tube-foot cavity; *musc.t.f.* tube-foot muscle; *nck.* ampulla neck; *oss.amb.* ambulacral ossicle; *perihaem.coel.* radial perihaemal canal; *perihaem.coel.lat.*, lateral extension of the radial perihaemal canal; *rf.* roof of the perihaemal sinus; *sept.* septum; *t.f.* tube foot; *w.v.r.* radial water vessel; *X* is the point where the axons of the motor neurones of the perihaemal sinus perforate the connective tissue to enter the cavities of the tube foot and ampulla.

The water-vascular cavity, in so far as it concerns the arm of the starfish, consists of a radial water vessel (*w.v.r.*), 'segmental' lateral canals (not shown in figure 11 but see figure 2, *w.v.l.*) and the cavities of the ampulla (figure 11, *cav.amp.*) and of the tube feet (*cav.t.f.*). The walls separating the water-vascular and perihaemal cavities are of connective tissue with inner and outer epithelial linings. On the right of the figure an ampulla is shown intact, and it is apparent that connexion could be made between the two sinuses by perforation of the floor of the lateral sinus (*perihaem.coel.lat.*). It is, in fact, by this means that the axon fibres of the motor neurones leave the perihaemal system and enter the confluent cavities of the tube foot and ampulla (figures 11, 12, *X*).

(2) *The motor neurones of the perihaemal sinus*

In order to study the neurones of the perihaemal sinus, the radial nerve cord has been carefully dissected away from the underlying structures of the arm, cut into pieces of convenient length ($\frac{1}{2}$–1 in.) and immersed in a 1 % solution of rongalitweiss-reduced methylene blue in sea water. Examination under a one-sixth or one-twelfth objective shows that, after a period of about 1 hr., a number of cell bodies and neurofibrillae begin to take the stain.

The cord is best examined with its internal surface (i.e. the floor of the perihaemal canal) uppermost on the slide. If this be done, the superficial fibrillar zone (figure 12, *fib.ext.*) will be visible at the lowest level of focus. On raising the objective, first the deeper lying fibrillae (*fib.int.*) of the plexus and then the interlacing fibres of the connective tissue lamina come into view. These latter, which are unstained, constitute the boundary zone (*b.z.*) separating the subepithelial plexus from the epithelium of the perihaemal sinus; above them, and within this epithelium, lie the groups of motor nerve cells (*neur.mot.* 1) with their darkly staining cell bodies. Many years ago Lange (1876) described cells in this position. He reported that they had a segmental arrangement along the cord, each group corresponding in position to one of the inferior transverse muscles of the arm (figure 2, *inf.trans.musc.*), and he supposed that the function of the neurones which he had figured was to innervate these muscles. Cuénot's (1891) work supported Lange's conclusions and the existence of a series of motor nerves lying above the nerve cord has been accepted by all subsequent authorities.

The nerve cells which can be demonstrated by the use of methylene blue may be the neurones which Lange found, but, if they are, his conception of their function must be extended, for while it is probable that they do supply the inferior transverse muscles they are primarily concerned in the innervation of the tube foot and of its ampulla.

Each group of neurones comprises upwards of twenty separate elements. It will be seen (figure 12B, *neur.mot.* 1) that the groups are arranged opposite the tube feet and that they have a transverse extension over the middle half of each side of the nerve cord.

In the initial stages of staining (figure 13A) only the cell bodies (*c.b.*) and the more closely adjacent parts of the axon are visible. The pear-shaped neurone has its blunter end directed towards the mid-line of the ambulacrum, while its tapered end is drawn out into a single fibril—the axon. The cell body contains a central nucleus (*nuc.*) which, like the ground substance of the surrounding cytoplasm, is but lightly stained. Within the latter, however, are a number of intracellular structures (*sph.*) with a high avidity for the stain. These deeply staining spheres are uniformly distributed throughout the neurone except that there is a clear area of cytoplasm (*cyt.*) near the point of origin of the axon.

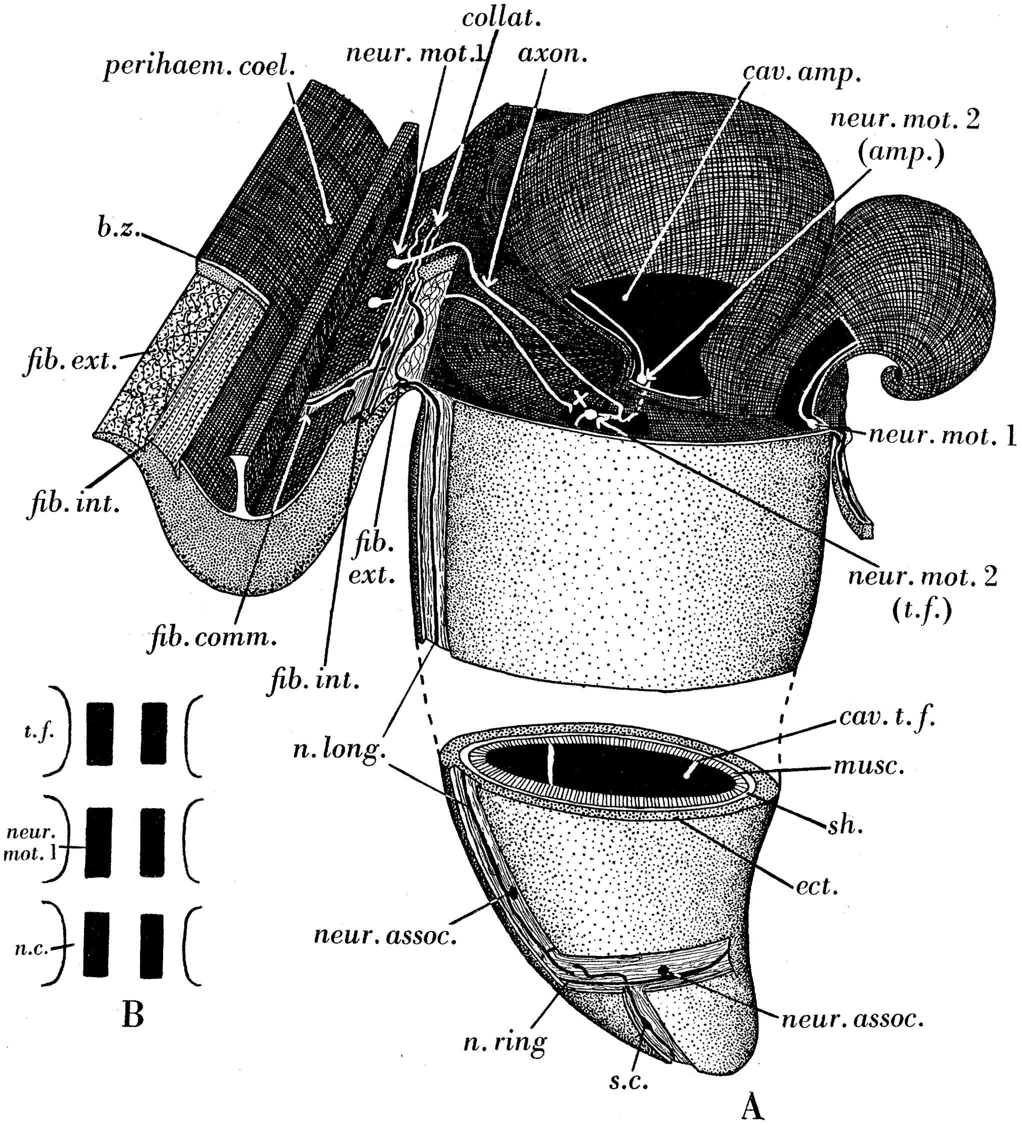

FIGURE 12. A. Diagrammatic stereogram (not to scale) of a tube foot and ampulla of *Astropecten irregularis* showing their nerve supply. Sensory and association nerves are figured in black, motor nerves in white. Where necessary, tissue has been removed to show the level of the various neurofibrillar zones. B. Diagram showing the areas occupied by the motor neurones of the radial perihaemal canal on the internal face of the nerve cord. *axon*, axon of motor neurone; *b.z.* boundary zone of connective tissue; *cav.amp.* ampulla cavity; *cav.t.f.* tube foot cavity; *collat.* collateral fibre of motor axon; *ect.* ectoderm; *fib.comm.* commissural fibre; *fib.ext.* fibrils of the superficial plexus of the radial nerve cord; *fib.int.* fibrils of the deep plexus of the radial nerve cord; *musc.* muscle; *n.c.* radial nerve cord; *n.long.* longitudinal nerve of the tube foot; *n.ring*, nerve ring of the tube foot; *neur.assoc.* association (internuncial) neurone; *neur.mot.* 1, *neur.mot.* 2 (*amp.*), *neur.mot.* 2 (*t.f.*), motor neurones of the first and second order, the latter supplying respectively the ampulla and tube foot; *perihaem.coel.* radial perihaemal canal; *s.c.* sense cell; *sh.* connective tissue sheath; *t.f.* tube foot; *X* is the point where the axons of the motor neurones of the perihaemal canal enter the tube foot and ampulla cavity.

The second phase of staining is illustrated by figures 13B and 14. The cell body is of an intense blue colour throughout, without distinction of nucleus and cytoplasm. Collateral branches of the axon (*collat.*, *collat.′*) are now made visible for the first time. They arise from the axon at intervals along its length and they may be from two to ten in number. Each of the collaterals makes an almost perfect right angle with the main fibre and, as it extends away from the axon, sinks to a lower level. By this means the collateral fibre is enabled not only to penetrate the rather loosely woven mass of the connective tissue fibres of the boundary zone, but is brought into alinement with the longitudinally arranged fibrillae of the association system. Though it is not possible to determine the precise nature of these junctions it seems safe to infer that the role of the collateral fibrils is to effect synaptic junction between the internuncial and motor systems of neurones.

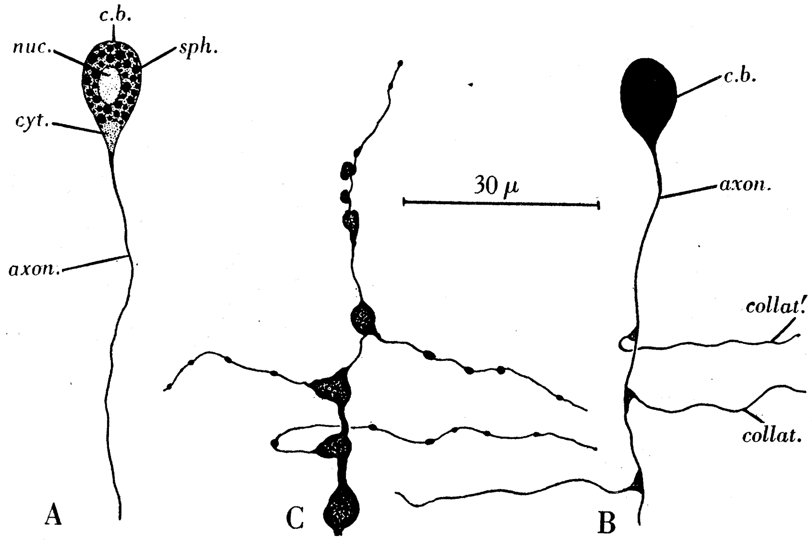

FIGURE 13. Stages in the intra-vitam methylene-blue staining of the motor neurones of the radial perihaemal canal of *Astropecten irregularis*. A, B and C. Neurones after 1, 2 and 5 hr. staining, respectively. *c.b.* cell body of neurone; *collat.*, *collat.′* collateral fibre of the axon; *cyt.* clear area of cytoplasm; *nuc.* nucleus; *sph.* intensely staining spherical body.

In most instances (figures 13B, 14, *collat.*) the collateral fibre extends away from the axon on the side from which it took its origin, but on occasions (*collat.′*) it may turn through 180° almost immediately after leaving the axon to run the greater part of its course in a direction opposite to that of its initial growth.

In the final stages of the staining process (figure 13C) the cell body, more often than not, loses all traces of colour. As the axon is now beaded and has, in addition, larger globules of deeply staining material condensed in the junctions of the axon with its collateral branches, it is probable that retrogressive changes within the neurone have resulted in a flowing out of the deeply staining intracellular material. Shortly after this condition has been reached (after about 5 hr. of staining), the whole preparation takes on a diffuse blue colour and the nervous elements are no longer visible as differentiated objects.

Figure 14 shows a complete group of motor neurones with cell bodies (*c.b.*), axon fibres (*axon*) and collateral branches (*collat.*, *collat.′*) all clearly visible. The axon, as it approaches the margin of the sinus, may branch but this is not usual. In either case it ceases to take a course transverse to the axis of the arm but turns to run longitudinally along the margin of the canal, either towards the base or the tip of the arm. On reaching the interval between two podia it bends again to run outwards along the lateral canal of the perihaemal sinus and, arriving at the base of the ampulla neck (figures 11, 12, *X*), penetrates the canal floor to enter the hydrocoel. It is here that it makes connexion with further neurones (figure 12, *neur.mot.* 2 (*amp.*) and (*t.f.*)) which are the subject of description in the section which follows.

In concluding this section it may added that the motor neurones within the radial perihaemal sinus of *Asterias rubens* closely resemble those of *Astropecten irregularis* in form and arrangement. The main differences are that in *Asterias rubens* there is a supernumerary criss-cross plexus of fibrils in the area of the motor neurones and axon fibres, and that the neurones of a 'segmental' group are more numerous than in *Astropecten*. These differences do not, however, affect the general form of the motor nerve supply to the foot.

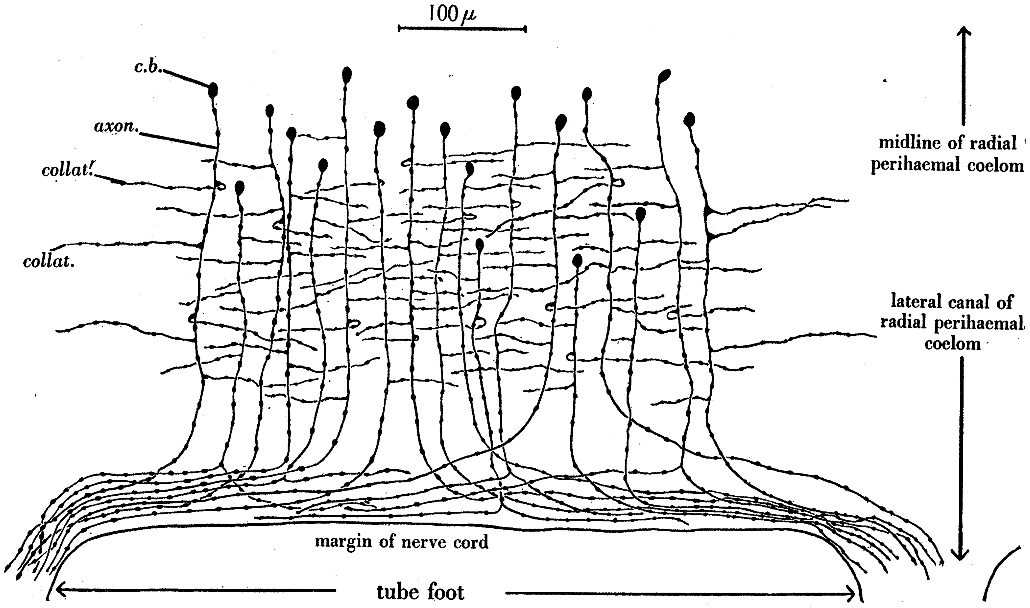

FIGURE 14. One of the 'segmental' groups of motor neurones of the radial perihaemal canal of *Astropecten irregularis*. *c.b.* cell body; *collat.*, *collat.'* collateral fibre of the axon.

(3) *The motor neurones of the tube foot-ampulla cavity*

The motor neurones of the tube foot-ampulla cavity (figure 12, *neur.mot.* 2 (*amp.*) and (*t.f.*)) are lodged in the neck of the ampulla. It is from these second-order neurones that axon fibres extend either into the ampulla or tube foot, there to terminate on the muscles and so to complete the rather complex anatomical arc which originated with the sensory cells of the foot and general ectoderm.

The cell bodies of the motor neurones are arranged in two main groups one of which (A and B, enclosed within the broken line on the left of figure 15) lies in the proximal and medial quadrant of the ampulla neck while the other (figure 15C) is situated in its lateral part. In order to see the constituent cells and fibres of these motor centres at all the neck of the ampulla must be exposed by removal of at least one of the encircling ambulacral ossicles. The appearance of the stained preparation, when this has been done, is shown diagrammatically in figure 15.

On staining with methylene blue certain features of the innervation of the tube foot and ampulla musculature are at once made evident but, unfortunately, the least satisfactory part of the preparation is that which concerns the ampulla neck (figure 15, *nck.*), the reason

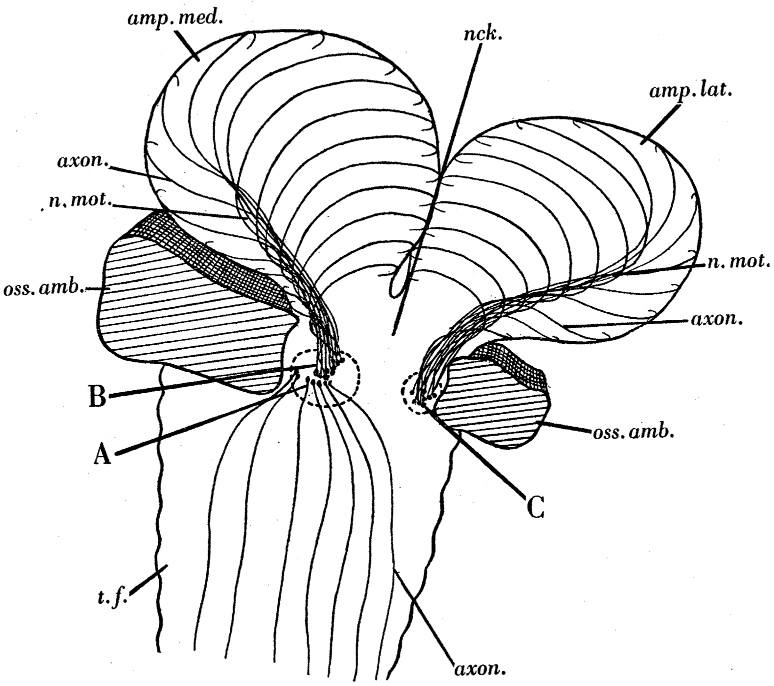

FIGURE 15. Diagram of a tube foot and ampulla of *Astropecten irregularis* showing the cell bodies and axon fibres of the motor neurones of the second order which are associated with the system. One of the two ambulacral ossicles contributing to the tube foot pore has been removed so as to expose the ampulla neck in which are lodged the cell bodies of the neurones. *amp.lat.* lateral lobe of the ampulla; *amp.med.* medial lobe of the ampulla; *n.mot.* motor nerve of the ampulla; *nck.* ampulla neck; *oss.amb.* ambulacral ossicle; *t.f.* tube foot. A. Cell bodies of neurones which innervate the tube foot musculature. B, C. Cell bodies of neurones which innervate the ampulla musculature. A and B are involved in nerve arcs which pass through the nerve cord. C receive impulses from the dorsal sheath ectoderm and have no direct connexion with the central nervous system.

being that it is very difficult to remove the covering ossicle without tearing the closely adherent tissues. In consequence, the cell bodies of the neurones within the neck always receive some damage and will not stain readily with vital dyes. While, therefore, it is possible to report the presence of neurones in this region it is not possible to give any details of their form or arrangement.

The tube foot musculature receives its nerve supply from a single group of motor neurones of the second order (figure 12, *neur.mot.* 2 (*t.f.*); figure 15, A). They are part of a nerve arc which also involves, as has been seen, sense cells, internuncial neurones and motor neurones of the first order. The axons of these motor neurones of the second order run into the cavity of the podium to terminate on and innervate the muscle fibres. The quality of staining of these axon fibres has not been sufficiently good to warrant the positive assertion that each motor fibril innervates one muscle fibre and one only, though there is certainly no evidence to the contrary.

Unlike the podium, the ampulla has a double nerve supply. One set of fibrils arises from cell bodies (figure 12, *neur.mot.* 2 (*amp.*); figure 15, B) which are grouped in the neighbourhood of the neurones of the foot. Their axons leave the cell bodies in numbers sufficiently great to constitute a 'motor nerve' (figure 15, *n.mot.*), clearly visible in fixed material stained with Mallory's triple stain. Its constituent fibres run in the coelomic epithelium along the proximo-medial border of the cavity of the medial lobe (*amp.med.*) of the ampulla and, as each of the muscle fibres of the ampulla is reached in turn, one of the neurofibrils (*axon*) leaves the motor nerve to pursue a complete circle around the ampulla in company with the muscle which it innervates. An adequately stained preparation will, in consequence, show a series of hoops each of which is a blue neurofibril overlying an unstained muscle fibre.

All the neurones of the groups A and B are involved, primarily, in nervous arcs which include also the motor neurones of the perihaemal sinus and the association cells of the nerve cord. It must be supposed, therefore, on anatomical grounds, that contraction of the musculature of the tube foot, in some cases at least, involves the passage of impulses through the centralized nervous system of the radial cord. The exceptions to this rule would be the contractions brought about through the medium of local nervous arcs within the foot itself, the existence of which can readily be inferred from the movements which amputated feet are able to effect. It is probable that all the normal contractions of the medial lobe of the *Astropecten* ampulla involve the passage of impulses from the radial cord, since the internal position of the ampulla would not make it accessible to local stimulation and any local nervous arcs (if present) could hardly be operative.

When, however, one examines the second source of innervation of the ampulla, namely, in respect to its lateral lobe, a remarkably different type of nervous arc is found. The motor neurones of group C (figure 15) send out axon fibres to the lateral lobe in numbers and disposition comparable to those which supply the medial lobe but, in this instance, they have no connexion with the neurones of the radial nerve and perihaemal sinus. Their cell bodies (figure 12, *neur.mot.* 1) are motor neurones of the first order, and they lie in the proximo-lateral quadrant of the ampulla neck at a point where the subepithelial plexus of the adjacent ectoderm, lateral to the tube foot, is greatly expanded. The implication of this association of plexus and motor nerve is that the latter is in direct receipt of excitation transmitted through the nerve tracts of the subepithelial plexus of the podium and of the ectoderm lateral to the foot; the nerve tracts of the radial cord do not enter into the constitution of these particular nervous arcs.

With the description of the nervous arcs which supply the foot completed, this account may be concluded by considering, very briefly, the extent to which the features in the nervous anatomy of the radial nerve cord and the tube foot-ampulla system, here presented, may be well-founded and where, for various reasons, the description may not be entirely adequate.

CONCLUSION

The purpose of the foregoing account has been to describe, in essentially anatomical terms, the mechanics and innervation of the tube foot and ampulla of the starfishes *Asterias rubens* and *Astropecten irregularis*. In respect to the latter aspect of this study it may justifiably be claimed that the salient characters of the nervous arcs which supply the tube foot-ampulla system have, for the first time, been demonstrated.

Observations on the activities of starfish podia under varying conditions of peripheral stimulation and of innervation (Smith 1945) lend support to the view that the nerve tracts which have been revealed by intra-vitam staining have, indeed, a distribution and constitution of the kind described. Reference to this paper will show that these observations confirm, in particular, the existence of two features in the innervation of the system which would hardly have been suspected without recourse to methods of direct anatomical observation. They are (i) the double motor supply to the ampulla (figure 15, neurones B and C) and (ii) the occurrence, on the motor side of the nervous arcs which supply the foot-ampulla system via the tracts of the radial nerve cord, of two systems of neurones (figures 7 and 12, *neur.mot.* 1; *neur.mot.* 2).

While, however, an analysis of the movements of the feet appears to corroborate the more general features of the nervous anatomy of the system, it clearly indicates, at the same time, that the anatomical picture, here presented, is inadequate in at least two major respects.

First, the circumoral nerve ring, of which the present paper gives no account, is the site of aggregations of neurones which exercise a dominant role in co-ordinating the activities not only of the tube feet but also of other muscular systems within the body of a starfish. Any account of the nerve supply of the podia must, in consequence, be incomplete so long as the nervous anatomy of the circumoral ring remains unexplored.

Secondly, it is evident from experimental studies (Smith 1945) that the orientated movements of the podia during locomotion and in other co-ordinated activities require that some of the second order neurones of system A (of figure 15) shall make connexion with the motor neurones of the first order, and shall innervate the musculature of the foot, according to a definite pattern. It has, so far, not been possible to demonstrate any definite configuration of these neurones by the use of vital dyes.

The chief merit of this account, incomplete though it undoubtedly is in these and other particulars, will, I think, be shown to be in its revelation of the fundamental relations of the nervous structures involved in the supply of the podium, a knowledge of which is an essential prerequisite to the experimental analysis of the conditions under which the various activities of the podia can be displayed. It may be anticipated that such an analysis will, in turn, greatly facilitate the further examination of the anatomy of the starfish nervous system.

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LIST OF ABBREVIATIONS

amp.	ampulla.
amp.lat.	lateral lobe of ampulla.
amp.med.	medial lobe of ampulla.
axon	axon of motor neurone.
b.z.	boundary zone of connective tissue.
c.b.	cell body of motor neurone.
c.t.	connective tissue.
cav.amp.	ampulla cavity.
cav.t.f.	tube foot cavity.
centrif.fib.s.c.	centrifugal fibre of sense cell.
centrip.fib.s.c.	centripetal fibre of sense cell.
coel. 1, 2, 3	divisions of the coelom.
coel. epith.	coelomic epithelium.
collat., *collat.'*	collateral fibre of the axon.
cut.	cuticle.
cyt.	cytoplasm.

ect.	ectoderm.
ect.lat.	lateral ectoderm (dorsal sheath).
fib.	neurofibril of the sub-epithelial plexus.
fib.circ.	circularly disposed neurofibrils.
fib.comm.	commissural fibril.
fib.exp.	expansion of neurofibrillar zone of the subepithelial plexus.
fib.ext.	superficial fibrillae of the radial nerve cord.
fib.int.	deep fibrillae of the radial nerve cord.
fib.long.	longitudinally disposed neurofibrils.
fib.n.mot.	fibrillae of motor nerve.
gr.	granule in cytoplasm of neurone.
lat.mot.n.	lateral motor nerve.
musc.	muscle fibre.
musc.amp.	ampulla muscle.
musc.inf.trans.	inferior transverse muscle.
musc.insert.	insertion of tube foot muscle into ossicle.
musc.t.f.	tube foot muscle.
musc.v.	muscle in valve of lateral water vessel.
n.c.	radial nerve cord.
nck.	ampulla neck.
n.long.	longitudinal nerve.
n.ring	nerve ring of the tube foot.
neur.assoc., *neur.assoc.′*	association (internuncial) neurone.
neur.ass.multip.	multipolar association neurone.
neur.ass.unipol.	unipolar association ('*T*') neurone.
neur.mot. 1	motor neurone of the first order.
neur.mot. 2	motor neurone of the second order.
neur.mot. 2 (*amp.*)	second order motor neurone of the ampulla.
neur.mot. 2 (*t.f.*)	second order motor neurone of the tube foot.
nuc.	nucleus.
oss.	ossicle.
oss.adamb.	adambulacral ossicle.
oss.amb.	ambulacral ossicle.
oss.inf.marg.	infra-marginal ossicle.
oss.s.amb.	super-ambulacral ossicle.
oss.sup.marg.	supra-marginal ossicle.
perihaem.coel.	radial perihaemal canal.
perihaem.coel.lat.	lateral perihaemal canal.
perivisc.coel.	perivisceral coelom.
pore	tube foot pore.
rf.	roof of perihaemal sinus.
s.c.	sense cell.
sep.	line of separation of ossicles.
sept.	septum.

sph.	deeply staining spherical body in the neurone.
sh.	connective tissue sheath.
sh.ext.	external layer of connective tissue sheath.
sh.int.	internal layer of connective tissue sheath.
supp.fib.	supporting fibre.
t.f.	tube foot.
v.	valve.
ves.	unstained vesicular body in the neurone.
w.v.l.	lateral water vessel.
w.v.r.	radial water vessel.