ON THE PHYSIOLOGY OF THE LANTERN RETRACTOR MUSCLE OF PARECHINUS ANGULOSUS.

by

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A C K N O W L E D G E M E N T S.

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My thanks are also due to the staff and my fellow research students in the Department of Zoology for their assistance and counsel while this work was in progress.

Resumé

The lantern retractor muscles of regular echinoids act in almost isometric conditions and a study of their control was considered to be of interest. The retractor muscles cannot be indirectly stimulated from the radial nerves, but proved to be very photosensitive. Their responses to light were therefore studied. The muscles show a complex pattern of contraction in response to illumination, which includes both excitatory and inhibitory components. The possible genesis of these patterns is discussed and examined in the light of the responses of the muscles to direct current stimulation and to drugs. It is concluded that many of the features of the response are undoubtedly neurogenic in origin, but that myogenic activity may possibly also be involved.

C O N T E N T S.

Introduction.

Our detailed knowledge of the myoneural physiology of the echinoderms is effectively limited to the specialized muscles moving the spines of the echinoids and the retractor systems of the holothurians.

Romanes (1885) and von Uexküll (1896) both studied the spine responses of echinoids, but a reasonably clear picture of the myoneural connections involved was only obtained when Kinosita (1940) investigated the responses of Anthocidaris crassispina. More recently Millott (1954, 1956) and Millott and Yoshida (1959, 1960 a,b,c.) have investigated the responses of the spines of Diadema antillarum and demonstrated pathways of conduction through the radial nerve.

The retractor muscle of the holothurian Cucumaria sykion Lampert has been studied in detail by Pople and Ewer (1954, 1955, 1958). This investigation included studies on the spontaneous activities of the muscles as well as circumoral conduction and control. C. sykion has no rigid skeleton apart from the ossicles round the buccal cavity to which the retractor muscles are attached. When the tentacles of the animal are pulled in the retractor muscles are grossly shortened. They act therefore essentially isotonically in contrast to the muscles moving the jaws of the echinoid lantern which act over shorter distances and hence behave in an essentially isometric manner.

It was thought that a comparison of the isotonic system found in the holothurian with the isometric system in the echinoids would be instructive. At the same time an investigation of the control and coordination of the various parts of Aristotle's Lantern could be undertaken. These aims could not be achieved for it proved impossible to stimulate the muscles of the lantern indirectly through the radial nerve. Kinosita (1940) also met this difficulty as he could not stimulate the spine muscles of Anthocidaris crassispina through the radial nerve. He overcame this problem by stimulating with filter paper soaked in saturated salt solution, a method, that, however, does not work with the lantern muscles of

the echinoid used in the present investigation. Possibly for the same reason Millott and Yoshida have exclusively used photostimulation of the radial nerve in their work upon Diadema.

It has been shown that the muscles of the Echinodermata are sensitive to photic stimulation (Millott 1949, Pople and Ewer 1958). An analysis of the responses of the lantern muscles to photic stimulation has therefore been attempted and it is this study that constitutes the main section of the present investigation.

Material

The species of echinoid used in this investigation was Parechinus angulosus Mortensen. These echinoids occur commonly on the neighbouring coast and are largely to be found in rock pools. The animals are principally vegetarian and feed on the abundant algae in the pools. Most of the animals used were collected from Salt Vlei near Port Alfred in the Eastern Cape Province. Since the animals are to be found in the intertidal zone above mean low water neap tides they could be easily obtained, but the most profitable time for collection was at low spring tides, as animals living further down the tide line are usually bigger.

The following procedure was used in collection : the animals were removed from the rocks and placed in plastic buckets containing sea water. They promptly defaecated and before returning to the laboratory the water was changed. The journey inland did not harm the animals and once in the laboratory they were put in porcelain sinks with fresh sea water. Provided the sinks were covered with glass plates to keep evaporation to a minimum, the animals survived for up to three weeks. The water usually required changing after about 48 hours to rid the tank of faeces. Thereafter the accumulation of faeces was slight. The sea water was always vigorously aerated with compressed air. Failure to do this caused the animals to die in a short time. The animals were not fed in the laboratory since sea weed keeps badly in artificial conditions.

The Anatomy of the Lantern

The anatomy of Aristotle's lantern is well known and has been described in a number of text books (Bather, 1900; Chadwick, 1900; Delage and Hérouard, 1903; McBride, 1906; Hyman, 1955; Borradaile, Eastham, Potts and Saunders, 1958). All are agreed as to the parts that are found in the lantern although they are by no means agreed as to the functions of these various parts.

The lantern of Parechinus angulosus conforms very closely with the description of other well known and well developed lanterns found in the regular urchins. This lantern is both relatively larger and shows more complete development of the various ossicles than in the better known species such as Echinus esculentus. A description of the parts is necessary in order to follow the account of the experimental investigations and serves also to clarify certain minor obscurities in previous descriptions.

The skeleton. There are three functional units in the skeleton of the lantern; the jaws, the rotulae and the compasses. Within these functional units other entities may be recognised, but these are joined rigidly together to form a single structure.

The jaws. There are five jaws, each of which is built of five elements, namely the paired pyramids and epiphyses and the single tooth. These are all bound together with connective tissue to form the jaw; the pyramids and the epiphyses form the support for the tooth and serve as the structures to which are attached the muscles that work the teeth (fig. 1). The base of the jaw is formed from the pyramids which are joined interadially. The radial surface of each pyramid is strongly ridged and carries a comminator muscle which connects the pyramids of adjacent jaws (fig. 2). Above the pyramids are the epiphyses. Each is an L shaped structure with one arm lying above the radial face of the pyramid while the other forms one member of the arch between the pyramids over the interradius. A deep grove, formed at the junction of the two pyramids, carries the keeled tooth. As with other echinoid teeth the aboral part of the tooth is soft and is

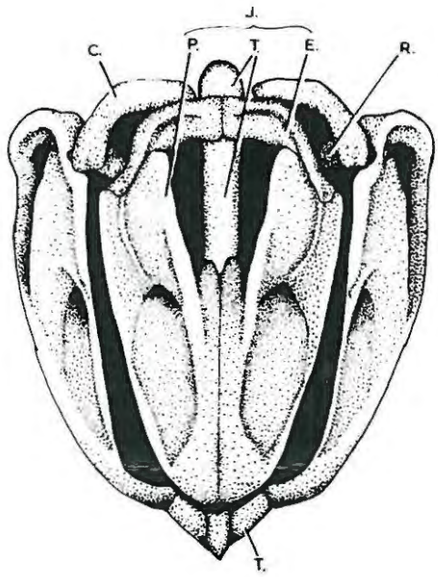

Fig. 1. Parechinus angulosus. Lateral view of the ossicles of the lantern. C. compass; E. epiphysis; J. jaw; P. pyramid; R. rotula; T. tooth.

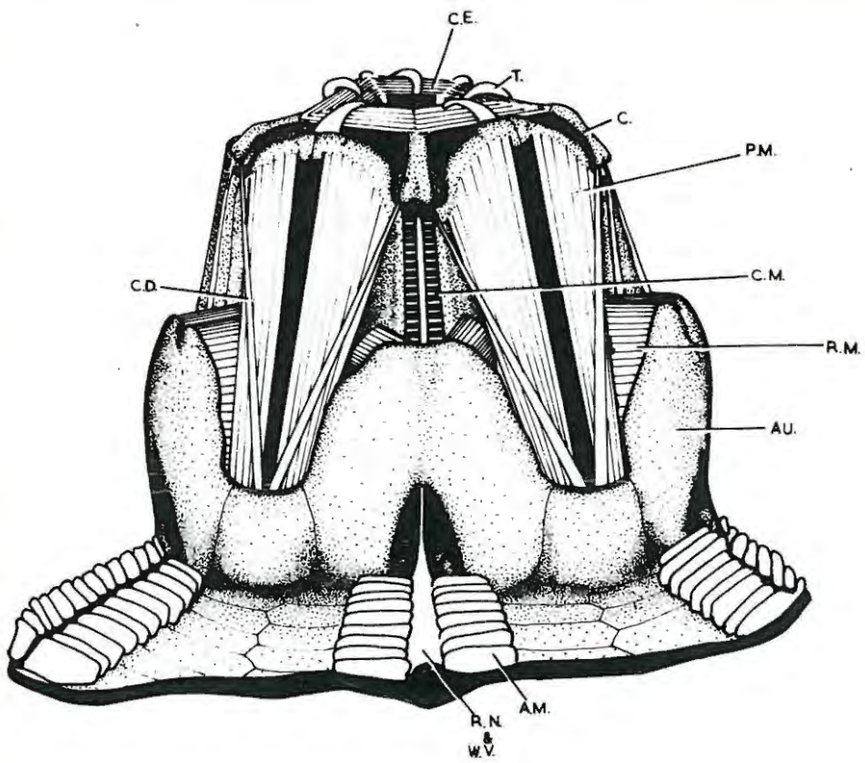

Fig. 2. Parechinus angulosus. Lateral view of the lantern showing the musculature. A.M. ampulla; AU. auricle; C. compass; C.D. compass depressor muscle; C.E. compass elevator muscle; C.M. comminator muscle; P.M. lantern protractor muscle; R.M. lantern retractor muscle; R.N. radial nerve; T. tooth; W.V. radial water vessel.

made up of a large number of small plates, which, as they grow down, fuse to form the hard tooth (Hyman, 1955).

The rotulae. These are oblong ossicles that lie in each radius between the epiphyses (fig. 3). On the lateral face of each rotula are slanting slots into which fit ridges carried on the epiphyses. They are further connected to the epiphyses by the connective tissue.

The compasses. These are slender ossicles which lie over the rotulae and are articulated with them at the innermost end (fig. 3). Each compass can be recognised as being constructed of two pieces, a more central and a more distal portion. These are firmly bound together with connective tissue and do not bend about their point of union. The compasses are slightly thickened in the middle region, where the muscles that are stretched between them are attached. At the distal end each is bluntly bifurcate, and it is here that the so called depressor muscles of the compass are inserted (fig. 2).

The lantern musculature. In the muscles operating the skeletal parts of the lantern, four elements may be recognised, namely : the lantern protractors, the lantern retractors, the compass elevators and the comminator muscles (fig. 3). There are also lip retractor muscles of the pharynx (fig. 4). Of these the lantern retractor muscle is of a highly complex shape since the muscle fibres run in different directions in the same muscle (fig. 5A). At the distal end the muscle is somewhat like a squashed crescent in cross section (fig. 5B) while at the pyramidal end the shape is that of an elongated oval (fig. 5C). The muscle fibres at the aboral end lie horizontal to the test, whilst those of the oral end nearest the radius are steeply inclined towards the mouth.

Before considering the function of the muscles of the lantern it is well to consider its activities. The primary action of the lantern is to chew off food and to pull it into the oesophagus. Other actions have been ascribed to the lantern of Echinus esculentus.

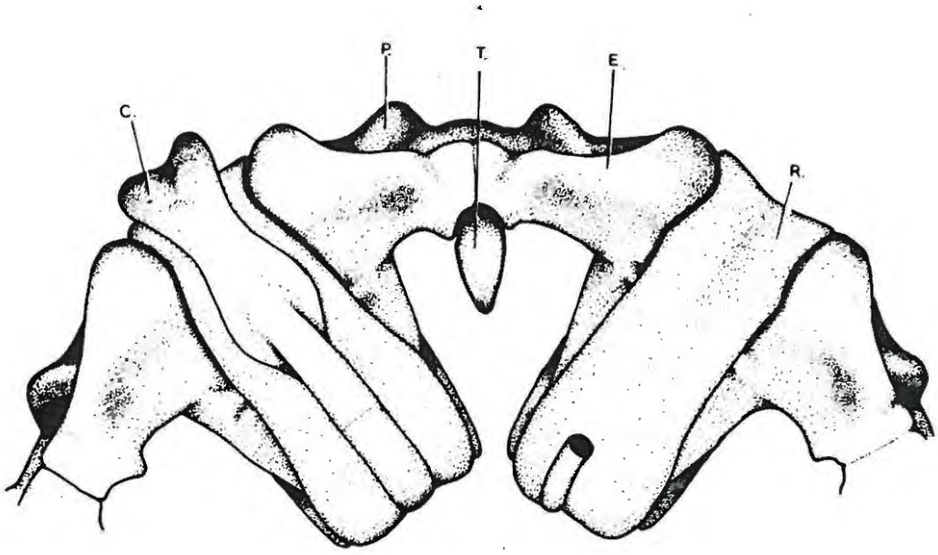

Fig. 3. Parechinus angulosus. Aboral view of the lantern ossicles. C. compass; E. epiphysis; P. pyramid; R. rotula; T. tooth.

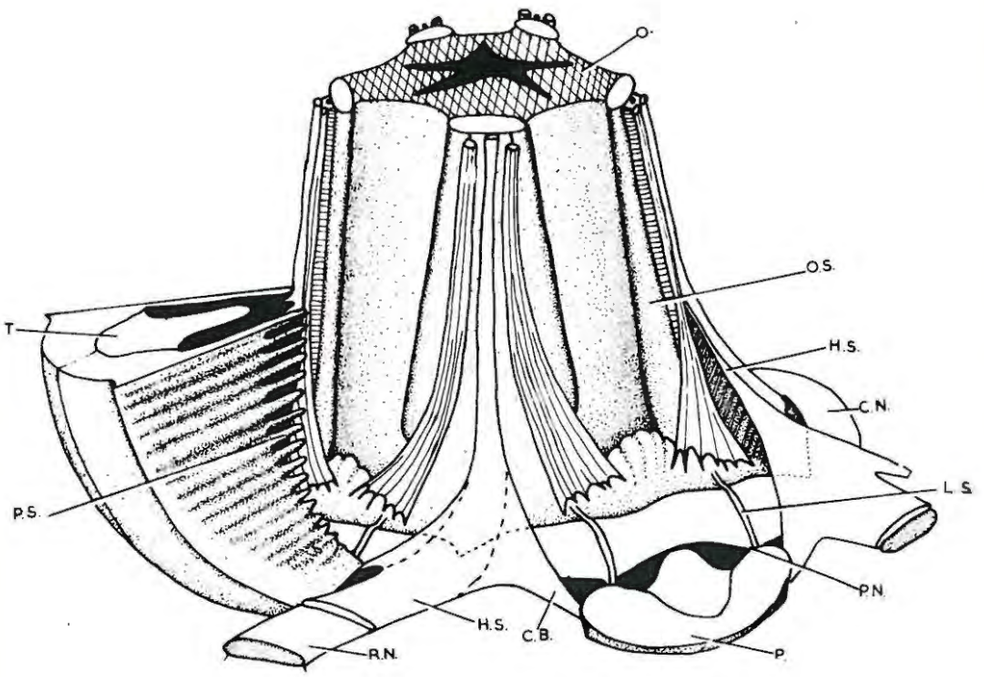

Fig. 4. Parechinus angulosus. Lantern dissected to expose the oesophagus in lateral view. C.B. connective tissue band; C.N. circumoral nerve ring; H.S. haemal strand; L.S. ligamentous strap; L.R. pharyngeal lip retractor muscle; O. oesophagus; O.S. oesophageal connective tissue support; P. pyramid cut down; P.N. pyramidal nerve; P.S. pyramid saw edge; R.N. radial nerve; T. tooth.

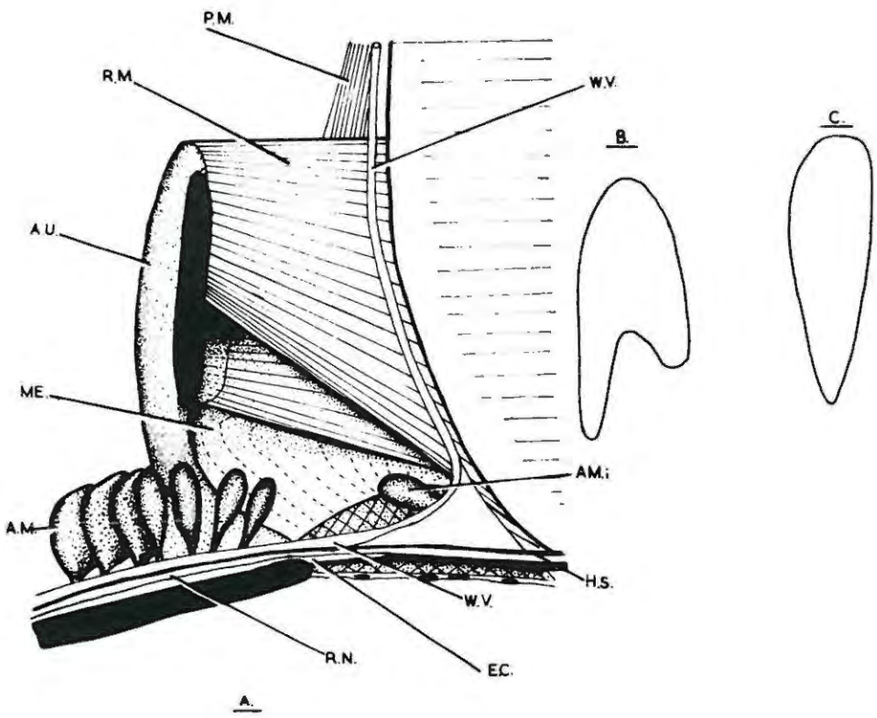

Fig. 5. Parechinus angulosus. Dissection of the lantern to show form of the lantern retractor muscle seen in lateral view.
A. A.M. ampulla; A.M.i. ampulla of buccal podia; A.U. auricle; E.C. epineural canal; H.S. haemal strand; ME. membrane to lantern retractor muscle; P.M. lantern protractor muscle; R.M. lantern retractor muscle; R.N. radial nerve; W.V. radial water vessel.
B. Cross section through the auricular end of the lantern retractor muscle.
C. Cross section through the pyramidal end of the lantern retractor muscle.

This animal is said to be able to pole itself along by means of its lantern, either in or out of water. (Gemmill 1912). I have never observed such behaviour in Parechinus angulosus

Parechinus feeds mainly on tough algae with relatively wide thalli. The food is pulled as close to the mouth as possible and pieces are then chewed off by means of the five teeth. To be able to reach the thalli the lantern must be pushed below the test and the short spines round the mouth. It seems very unlikely that the ten tube feet round the mouth would be strong enough to bend the thalli and pull them up to the mouth. Once the teeth have reached the food they may then open, catch hold of it and pull a piece away so that it may be swallowed. These actions must be carried out by the muscles available in the lantern. Protrusion of the teeth has been ascribed by all authors to the lantern protractor muscles, but some have also included the compass depressor muscles (Chadwick 1900; Delage and Hérouard 1903). The lantern protractor muscles (fig. 2 P.M.) insert on the epiphyses and originate on the interradial portion of the perignathal girdle between the auricles (AU). All authorities agree that these muscles protrude the teeth. They are fairly well developed and since the muscle fibres run in an oral-aboral direction they push the teeth out of the mouth. Delage and Hérouard (1903) have postulated that the lantern may be protracted by the compass depressor (fig. 2 C.D.) which runs from the compass to the perignathal girdle near the interradius. They admit that this is not the primary function of this structure and indeed they are not even certain that it is a muscle at all; they consider that it may be a ligament, a view expressed earlier by Chadwick (1900). Histological examination of the structure in P. angulosus shows it to be mainly built of connective tissue with a few muscle fibres. Mechanically the connective tissue more closely resembles a tendon than a ligament, having little elasticity. It contracts feebly when stimulated directly with electricity. The main objection to Delage and Hérouard's view is that the muscle fibres are few and hardly robust enough to push down the bulk of the lantern.

Chadwick (1900) had considered the compass depressor to be a ligament and believed that the force driving the lantern downwards came from the muscles in between the compasses, the compass elevator muscles (Fig. 2 CE). Once again these muscles are not particularly strong, and they would have to push the lantern downwards by decreasing the angle between the compasses. The compasses could only approach each other more closely by changing their initially horizontal position to a more vertical one with respect to the floor of the lantern. Since the distance between the ends of the compasses and the perignathal girdle will be fixed as the compass depressors are tendinous, the only way in which the compasses could approach each other would be by pushing the lantern down. In view of these considerations and the extremely inefficient way in which these muscles would have to exert their influence, I think it unlikely that they play any significant role in the extrusion of the teeth.

The function of the compass elevator muscles is obscure. Hyman (1955) considers that they are not part of the masticatory apparatus as such but are concerned rather with respiration. Von Uexküll (1896) reported that if the oesophagus of Sphaerechinus granularis was stimulated by pushing the head of a pin into it, the compasses would rise and thus increase the volume of the peripharyngeal cavity. Since the gills project into the water from the floor of the peripharyngeal cavity, the fluid in them would be withdrawn and when the compasses are pulled down they would force fresh fluid into the gills. Von Uexküll also reported that if the lantern were put in water with a raised carbonic acid content the compasses would perform pumping movements. Gemmill (1912), however, could not confirm von Uexküll's observations on Echinus, although he mentions that he did not have the opportunity to repeat the experiments with due care. He furthermore considered that the mechanism was unlikely to operate in Echinus since the structure of the gills of the two animals is very different. Echinus has gills with muscular walls that can contract,

whereas the gills of Sphaerechinus granularis have flaccid membranous walls.

In structure Parechinus is more like Echinus than Sphaerechinus. Pumping movements have not been seen in dissected lanterns in sea water. Further neither stimulation of the oesophagus nor immersion in water with excess carbon dioxide initiated pumping movements. One is therefore left with the problem as what the function of the compasses and the compass muscles in Parechinus may be. It seems unlikely that they are concerned with the protrusion of the lantern nor do they seem to be used in ventilatory movements as in Sphaerechinus. It is possible that they may be concerned with adjustment of the volume of the perioral cavity as the lantern is protruded.

Once the teeth are protracted they must be able to close together. Chadwick (1900), Delage and Hérouard (1903) and McBride (1906) are all agreed that this is brought about by the comminator muscle (fig. 2 CM) which stretches from one jaw to the other over the radius and beneath the rotulae. This muscle is built up of a series of flat plates with intervening spaces, each plate being attached to the peak of a ridge on the flange of the radial surface of each pyramid. The reasons for this "stacked" plate arrangement are possibly twofold. The first is that the spaces between the jaws are somewhat limited and to ensure a reasonable exchange of food materials and waste products, the muscle requires a good circulation of coelomic fluid. Probably more important is the need for sufficient space to allow the muscles to belly out as they contract.

Hyman (1955) says that the comminator muscles rock the teeth together. Exactly what is meant by the word "rock" is not clear, but if it implies that the jaws can rock at the top of the lantern on the parallel articulations over the radius formed by the rotulae and epiphyses to bring the teeth together at the oral end, the description is correct.

Delage and Hérouard (1903) are of the opinion that the main function of the comminator muscles is to maintain the "union" of the jaws. Since the jaws are articulated at the aboral end

with the rotulae and at the oral end by connections with the skin round the mouth their positions are likely to remain reasonably secure. The main functions of the muscles will then be to pull the jaws together and so clench the teeth. Both Chadwidk (1900) and Delage and Hérouard (1903) are agreed that clenching of the teeth is achieved by this muscle.

The teeth may be seen to take up two types of position when closed. They may either be closed symmetrically about the centre of the lantern (fig. 6A) or they may be twisted in a whorl (fig. 6B). This action must be brought about by three sets of muscles operating in conjunction with one another. Consider a pair of adjacent jaws (fig. 6); these will both articulate on the rotula between them. The musculature of a jaw is such that one shoulder may be raised while the other is depressed. This will mean that the oral - aboral axis of the jaw will be slanted with respect to the oral - aboral axis of the lantern. Since the rotulae articulate freely on the jaws the shoulder of one jaw may be lowered while that of the adjacent jaw is raised. If this should happen to each of the jaws round the lantern, with the teeth meeting around the central axis, the teeth will present a whorled appearance when viewed from the oral aspect. The action must be brought about by a differential pull of the lantern protractor on one shoulder of the jaw and the lantern retractor muscle at the oral end of the other side of the jaw. The comminator muscles would then pull the jaws together about the centre of the lantern. McBride (1906) states that successive contractions of the comminator muscles around the lantern give the jaws a rotating movement like that of an auger and that this is very efficient in grinding hard and calcarious matter. If by this he means the action described above, this can not be carried out by the comminator muscles alone. Further an auger-like action also requires pressure from above; this would involve protrusion of the lantern, so that the whole of the musculature would be in action during such activity.

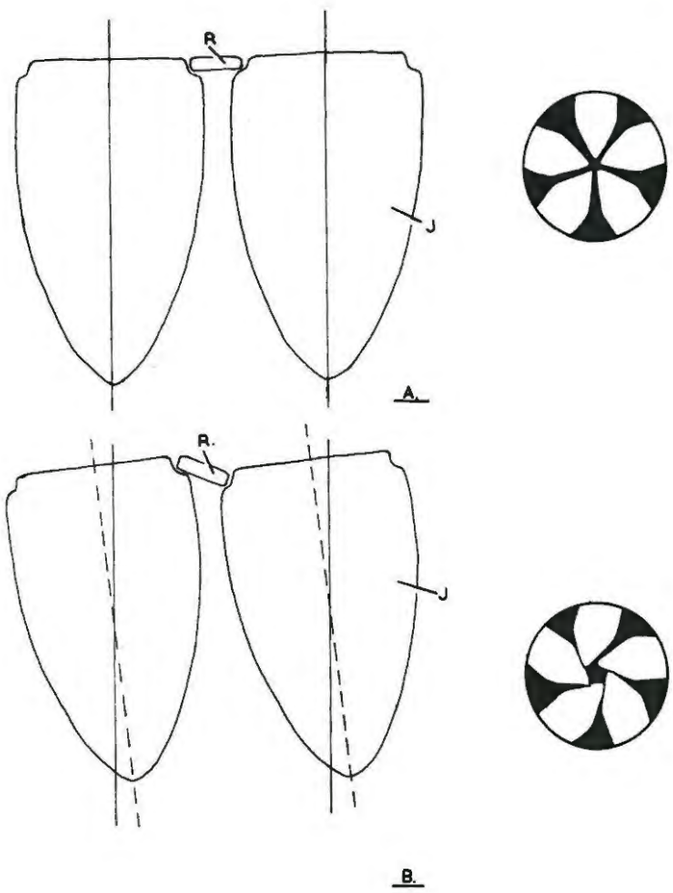

Fig. 6. Diagram to show two possible arrangements of the teeth of P. angulosus. The right hand drawings show the teeth in oral view, the left hand drawings two neighbouring jaws. For further explanation see text. J. jaw; R. rotula.

Chadwick (1900) considers that the comminator muscles may play a part in the trituration of the food in the lantern region of the pharynx. Certainly in Parechinus the ridges on the radial faces of the pyramids extend beyond the main body of the pyramid towards the centre, and look much like the teeth of a saw (fig. 4 P.S.). This idea seems, however, improbable for two reasons. Firstly, the jaws only come very close together at the tips of the teeth since the saw edges of the pyramids curve away from the central axis of the lantern. Furthermore, since the saw teeth are very delicate and easily broken, they could not help in trituration of the food; if they were strong enough to put pressure on the food in the oesophagus, they would easily penetrate its soft walls.

Delage and Hérouard (1903) have suggested that the lantern protractor muscles draw the teeth together, while parting the jaws at the aboral end. This cannot be achieved in P. angulosus since the distance between the jaws is fixed aborally by the tough connective tissue binding the rotulae to the epiphyses. It is furthermore difficult to see how the lantern protractor muscles could, in any controlled manner, bring the teeth together at the centre of the mouth since they are attached to the jaws aborally, far from the tips of the teeth.

All in all the lantern is a complex "floating" structure: that is to say that it is connected to the test, not by osseous articulations but only by the way of muscles. The jaws are firmly attached to the perignathal skin on either side of the radius by connective tissue.

Since the lantern is a floating structure capable of movement as a whole in all directions and also capable of intrinsic movements such as opening and closing of the teeth and twisting of the teeth in the mouth, the muscles cannot be thought of as having clear cut independent movements serving one function alone. To achieve any one set of movements there must be a high degree

of control and coordination between the various muscles.

The Nervous System

The gross structure of the nervous system of echinoids has been described by many authors (Cuénot, 1891; Chadwick, 1900; Delage and Hérouade, 1903; Hyman, 1955). In P. angulosus the system is very similar. Inside the lantern is a circumoral nerve ring round the oesophagus. Five radial nerves leave the ring and lie along the ambulacra. Aborally, the radial nerves lie beneath a hyponeural canal, a haemal strand and a radial water vessel (fig. 5). Below the nerve and between it and the test lies the epineural canal. Arising on either side of each radial nerve are nerves going to the tube feet. These are similarly accompanied by hyponeural canals, water vessels and haemal strands.

Starting at the perignathal girdle the nerves may be traced as they pass into the lantern by way of the arch formed by the auricles situated on either side of the radius. As the radial nerve enters the lantern, the hyponeural and epineural canals come to an end. The radial water vessel can, however, be traced to the distal edge of the comminator muscles where it leaves the radial nerve and travels aborally to the top of the lantern (fig. 5). Joining the oral face of the radial nerve two-thirds of the way from the centre of the lantern is a tough connective tissue strand attached to the test. This protects the radial nerve from stresses caused by movements of the lantern in the perioral region.

Just before passing between the jaws, and under the comminator muscle the radial nerve gives off a last pair of nerves which innervate the buccal podia (fig. 7 B.N.). The radial nerve then joins the circumoral nerve ring and at this point the haemal strand sweeps aborally up the side of the oesophagus. At the level of the circumoral ring the haemal strand is joined by connective tissue bands (fig. 4 C.B.) which are attached to the bases of the jaws. Just above the nerve ring

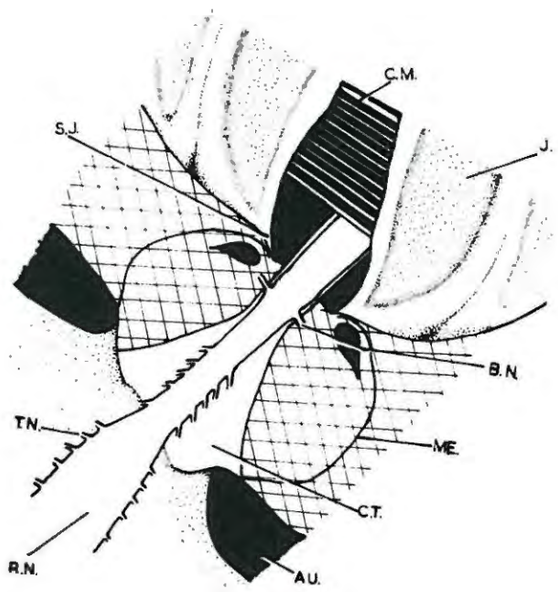

Fig. 7. Parechinus angulosus. Aboral view of radial nerve (R.N.) as it enters the lantern oral to the comminator muscle (C.M.). AU. auricle; B.N. buccal podial nerve; C.T. connective tissue; J. jaw; ME. membrane to lantern retractor muscle; S.J. connection of jaw to skin; T.N. podial nerves.

the haemal strand is attached to the oesophagus by means of muscle fibres and connective tissue.

Ten nerves, the pyramidal nerves, which run to the lantern, can be seen leaving the circumoral nerve ring where this structure comes in close contact with the radial faces of the jaws (fig. 8). Each nerve splits into two branches. One runs up in the groove between the tooth and the pyramid, the other runs aborally up the inter-radial aspect of the pyramid near the oesophagus. The nerves are quite thin, being approximately the same size as the nerves going to the tube feet. This is remarkable considering the bulkiness of the lantern musculature. The nerves to the retractor muscles of C. sykion (Pople 1952) are relatively much larger and can clearly be seen going to muscles. This is not the case in P. angulosus. Such connections have not definitely been traced to the musculature. While lanterns stained in reduced methylene blue (Smith, 1937) showed the tracts leading from the circumoral nerve ring and going onto the jaws, fixation of the tissue in ammonium molybdate in order to study the fine detail failed to preserve the tissue in a recognizable condition and so the eventual destinations of the tracts could not be seen.

In descriptions of the nervous system of echinoids with lanterns, mention is made of a hyponeural nervous system. It is said to consist of five plaques of nervous tissue situated on the aboral surface of the nerve ring in the radial position. It is from these structures that nerves are sent off into the lantern. A structure corresponding to this description has been seen in histological sections of P. angulosus, lying on the radial aboral portion of the nerve ring (fig. 9). The narrow "line" dividing the two can be seen as a continuation of connective tissue which lies on the aboral surface of the pyramidal nerve. This "line" can be observed most clearly at the origins of the pyramidal nerves (fig. 10). In other regions the dividing line disappears and the nervous tissue of the ring is continuous with the hyponeural nervous system (fig. 11).

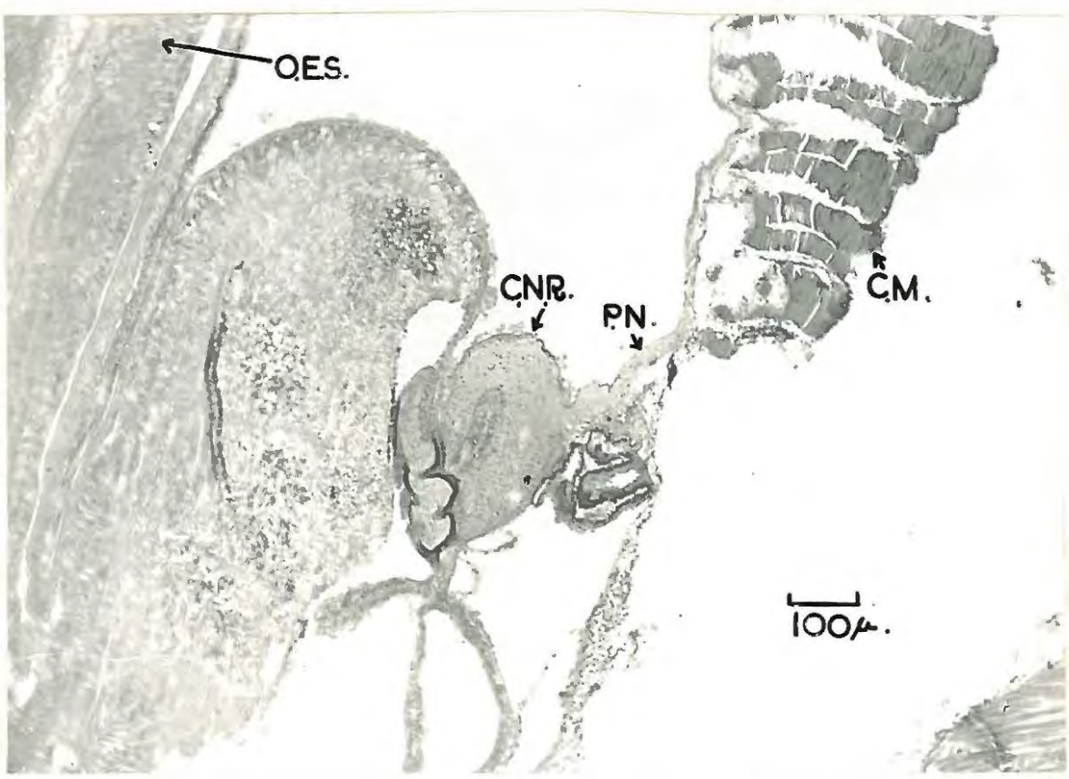

Fig. 8. Parechinus angulosus. Vertical section through lantern showing relations of the circumoral nerve ring (C.N.R.). C.M. comminator muscle; P.N. pyramidal nerve; OES. oesophageal wall.
Fixed: alcoholic Bouin. Stain: Masson trichrome.
Thickness of section 5 μ.
In this and all subsequent histological preparations initial decalcification was effected with dilute nitric acid.

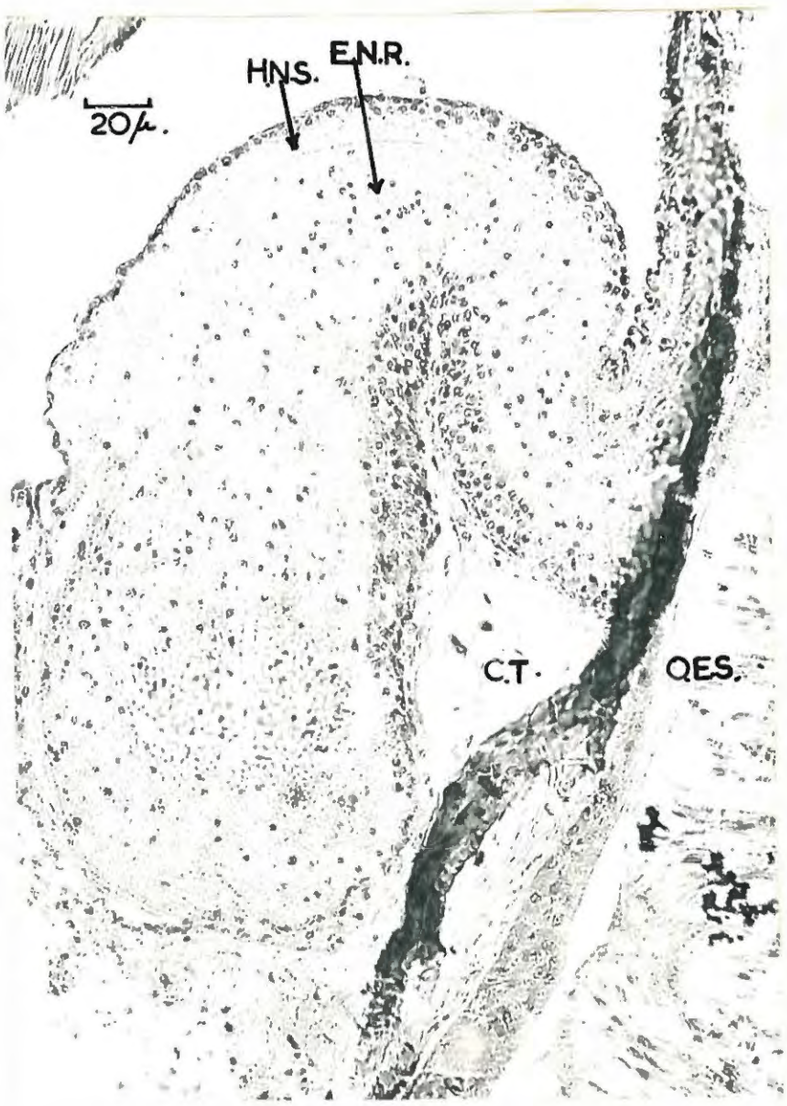

Fig. 9. Parechinus angulosus. Vertical section through the circumoral nerve ring (C.N.R.) to show the general relations of the hyponeural nervous system (H.N.S.). C.T. connective tissue; OES. oesophageal wall. Fixation, staining and thickness as in Fig. 8.

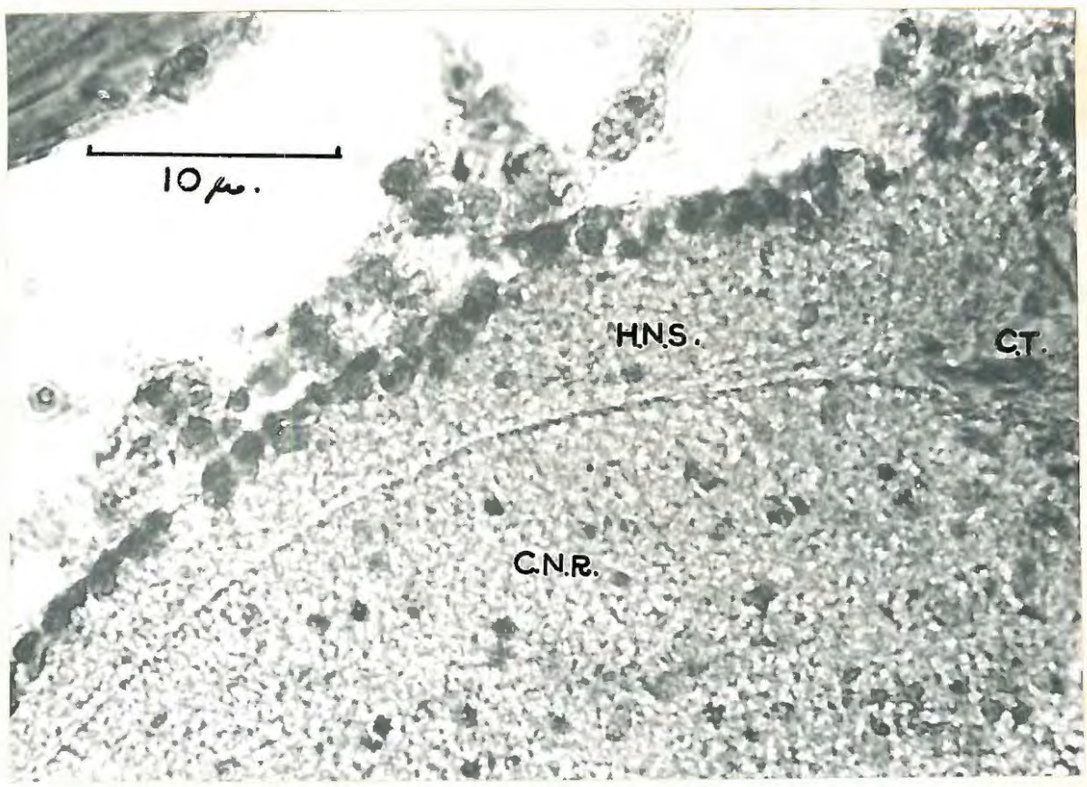

Fig. 10. Parechinus angulosus. Transverse section of circumoral nerve ring to show the invasion of connective tissue (C.T.) to separate the hyponeural nervous system (H.N.S.) from the main body of the circumoral nerve ring (C.N.R.).
Fixation, staining and thickness as in Fig. 8.

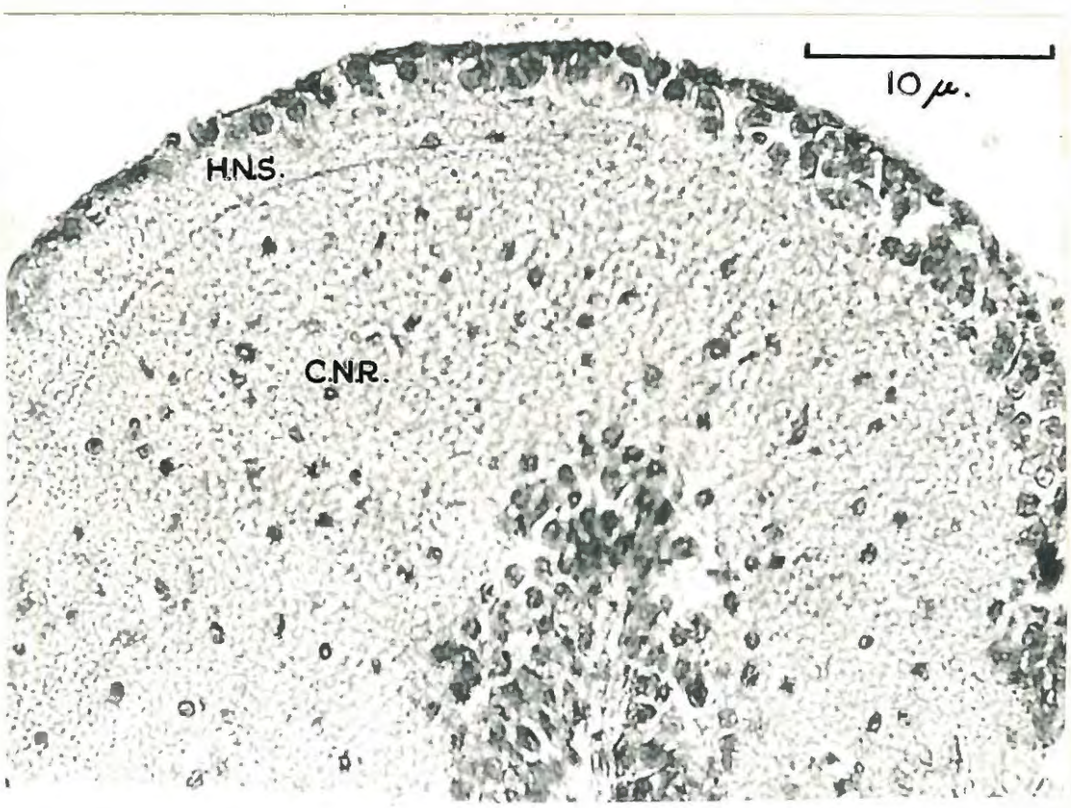

Fig. 11. Parechinus angulosus. Vertical section through circumoral nerve ring to show incompleteness of connective tissue layer separating the hyponeural nervous system (H.N.S.) from the body of the circumoral nerve ring (C.N.R.)
Fixation, staining and thickness as in Fig. 8.

In descriptions of other species it is stated that the hyponeural system is restricted to the radial sections of the circumoral nerve ring. This is not the case in P. angulosus. Although the hyponeural portion becomes somewhat attenuated in the interradial position it can still be recognised (fig. 12). However, it is in this region that the division between the two nervous systems disappears (fig. 11).

The detailed histology of the nervous systems is of interest. The radial nerve has a layer of large rounded nuclei on the oral surface (fig. 13). These are densly packed and it is impossible to see connections of a nervous character with other parts of the nervous system. Above this layer of nuclei lies the bulk of the nerve cord. In this region small, often oval nuclei are common. These bear suggestions of strands at either end and may be neurones, but they have not been seen with any great clarity. Sections stained in silver by Holmes' method (Gatenby and Beams, 1950) showed no more than did Masson's Trichrome stain. The general impression obtained from observations made by using phase contrast microscopy is that longitudinal nerve fibres run along the length of the radial nerve.

Vertical supporting fibres such as have been described in other echinoderm nervous systems (Smith, 1937) were also seen in the radial nerve cords with phase contrast (fig. 14). In the radial nerve, these structures did not stain with silver, but were stained in the circumoral nerve ring. Such fibres can be seen in both the hyponeural nervous system and in the main body of the ring itself (fig. 15 S.F.). In the dividing region of the nerve ring the fibres can be seen to arise from button-like objects. The fact that the supporting fibres do not stain in one part of the animal and do in another is not really surprising since silver stains are known to be very capricious.

Another interesting point is that in the radial nerve cords the oral edge has a dense layer of nuclei, while the aboral edge lacks this feature (fig. 13). The aboral edge of the hyponeural system bears a similar layer of large nuclei. This suggests that

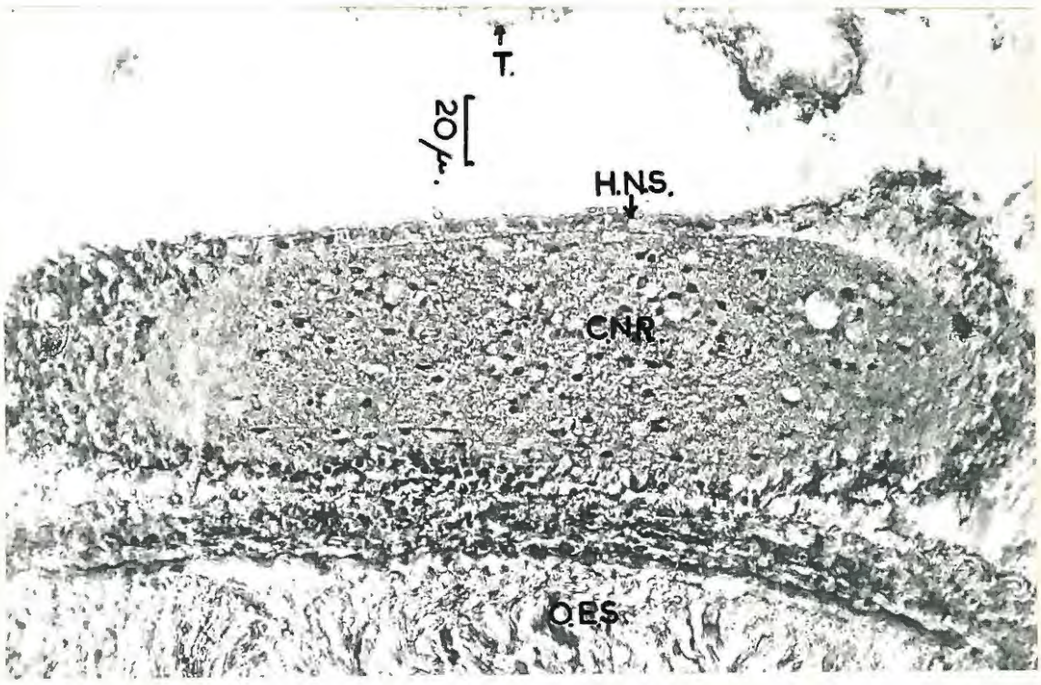

Fig. 12. Parechinus angulosus. Transverse section through circumoral nerve ring (C.N.R.) in an inter-radial position to show the continuation of the hyponeural system (H.N.S.) in the inter-radial region of the nerve ring. OES. oesophageal wall; T. membrane enclosing the tooth.
Fixation and staining as in Fig. 8. Thickness of section 10μ.

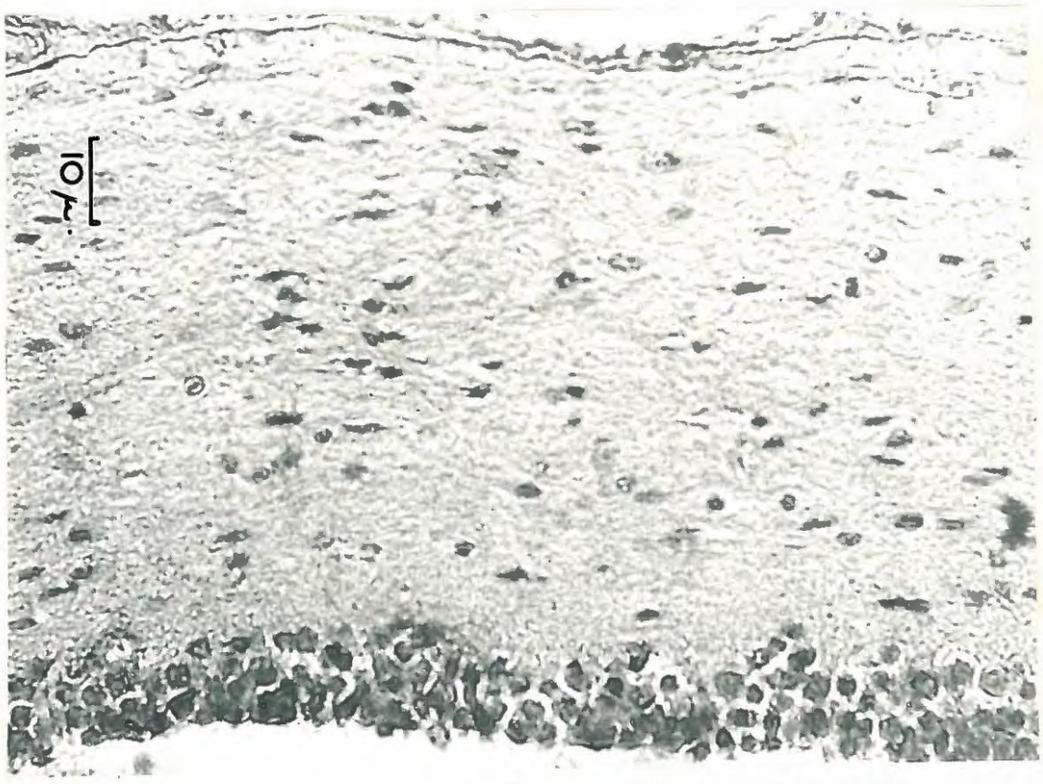

Fig. 13. Parechinus angulosus. Longitudinal section through a radial nerve to show the dense oral layer of nuclei.
Fixation, staining and thickness as in Fig. 8.

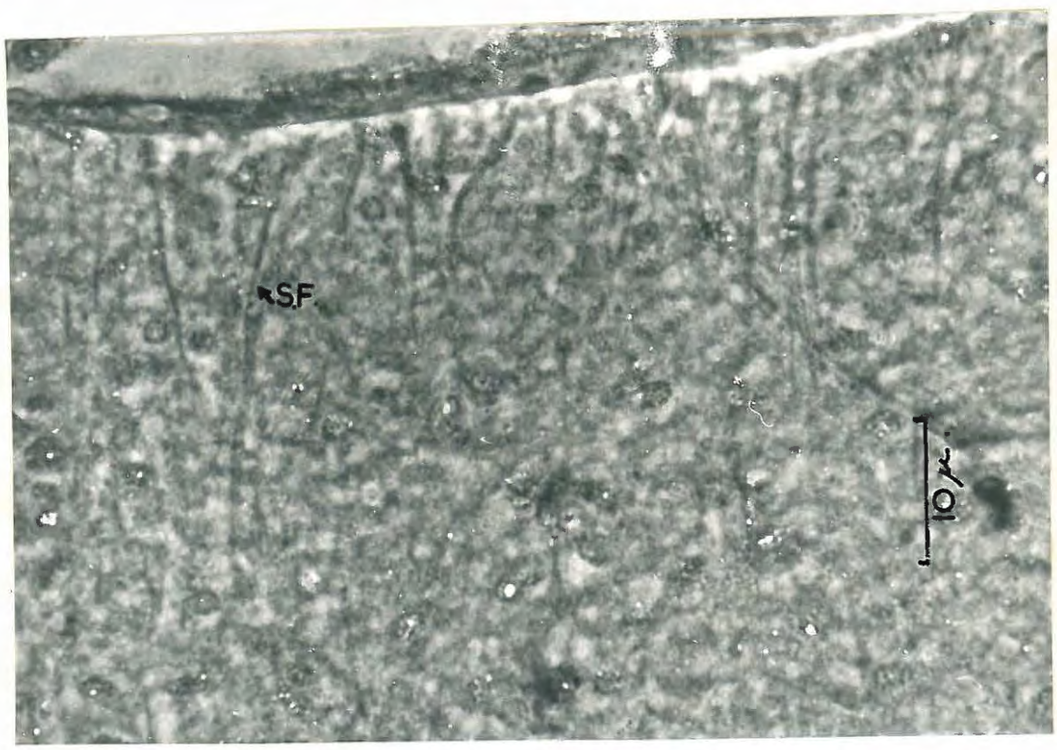

Fig. 14. Parechinus angulosus. Longitudinal section through the aboral edge of a radial nerve to show the supporting fibres (S.F.)
Fixation: alcoholic Bouin.
Staining: Holmes' Silver technique.
Thickness: 5μ. Phase contrast.

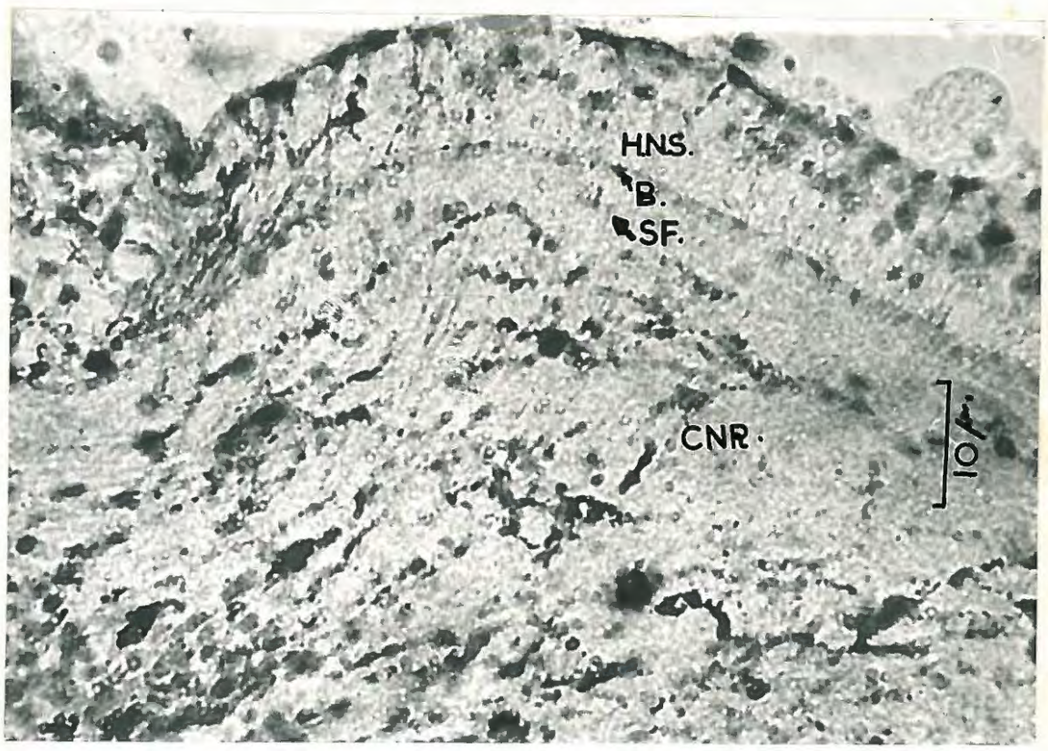

Fig. 15. Parechinus angulosus. Vertical section through the circumoral nerve ring (C.N.R.) and hyponeural nervous system (H.N.S.) to show the supporting fibres (S.F.) and the buttons (B) on the membrane separating the two nervous systems.
Fixation, staining, and thickness as in Fig. 14.
Not phase contrast.

the hyponeural nervous system is folded back upon itself so as to show the oral surface aborally on the circumoral nerve ring. A demonstration of a continuous layer of nuclei connecting the oral region of the nerve cord and the aboral region of the hyponeural nervous system would lend support to this idea. However, in serial sections, the layer of nuclei of the oral portion of the radial nerve stops before reaching the circumoral nerve ring and can only be found again in the nerve ring itself.

Motor complexes in muscles have been described for Marthasterias (Smith, 1937) and in the holothurian C. sykion (Pople, unpublished). Since neither silver staining not Novelli's stain revealed recognizable nervous tissue amongst the muscle fibres, the question as to whether P. angulosus has motor complexes remains to be solved.

The Histology of the Muscles

The histology of the lantern muscles varies from muscle to muscle. The lantern retractor muscle, with which this thesis is mainly concerned, consists of long fibres joined by means of connective tissue (figs. 16 & 17). In cross section the muscle fibres are collected roughly into groups (fig. 18). This has also been noted in C. sykion (Pople, 1952). The nuclei are to be found along the length of the muscle fibres. It has not been determined whether there is more than one nucleus per muscle fibre. No divisions along the length of a fibre have been seen; they appear to run the whole length of the muscle from its origin to its insertion. In the spaces between the muscles fibres lie cells with a fine fibrous processes (figs. 18 & 19) and with two sorts of nuclei. Cells with large, round nuclei are fairly common. More rarely smaller cells with oval nuclei have been seen, often with long, fine fibres connected to them (figs. 19 & 20). These latter cells may be bipolar neurones since they are of the same order of size as those found by Pople in C. sykion (unpublished). The bipolar neurones in C. sykion were demonstrated using silver

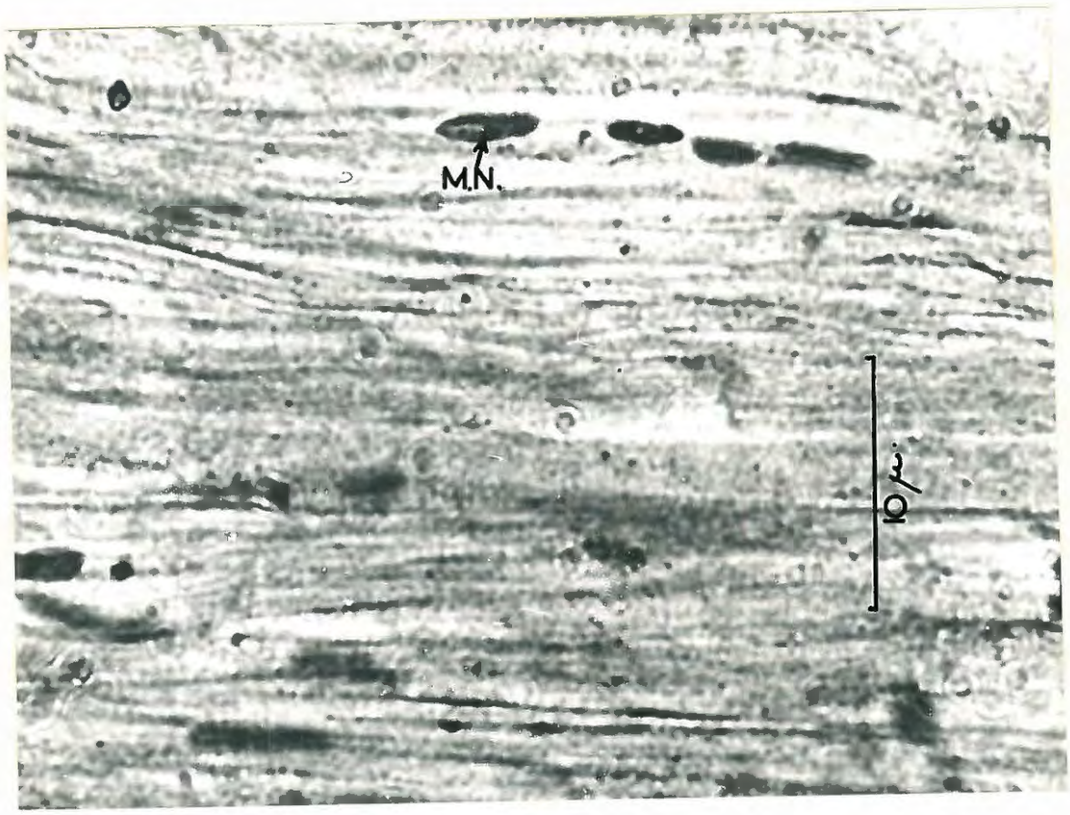

Fig. 16. Parechinus angulosus. Longitudinal section of the lantern retractor muscle showing oval nuclei of muscle fibres (M.N.).
Fixation, staining and thickness as in Fig. 14.
Standard illumination.

Fig. 17. Parechinus angulosus. Oblique section through the lantern retractor muscle showing the connective tissue (C.T.) between the muscle fibres (M.F.). Fixation, staining and thickness as in Fig. 14. Standard illumination.

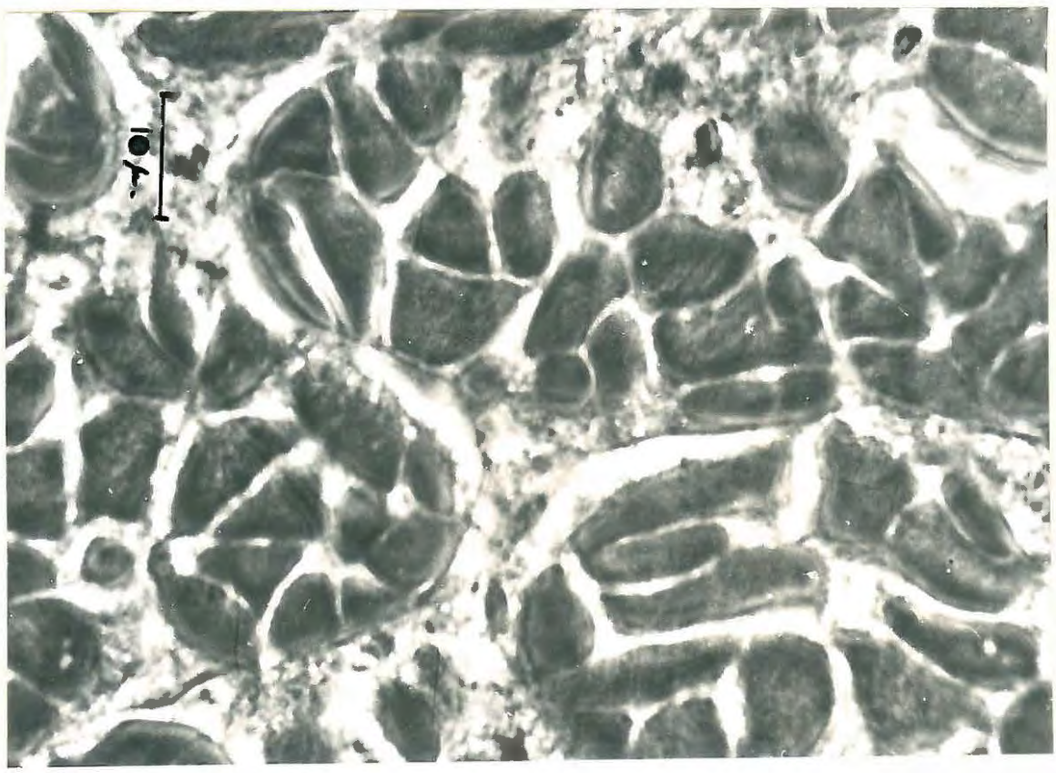

Fig. 18. Parechinus angulosus. Cross section of lantern retractor muscle showing the muscle fibres collected in bundles. Fixation, staining and thickness as in Fig. 14. Standard illumination.

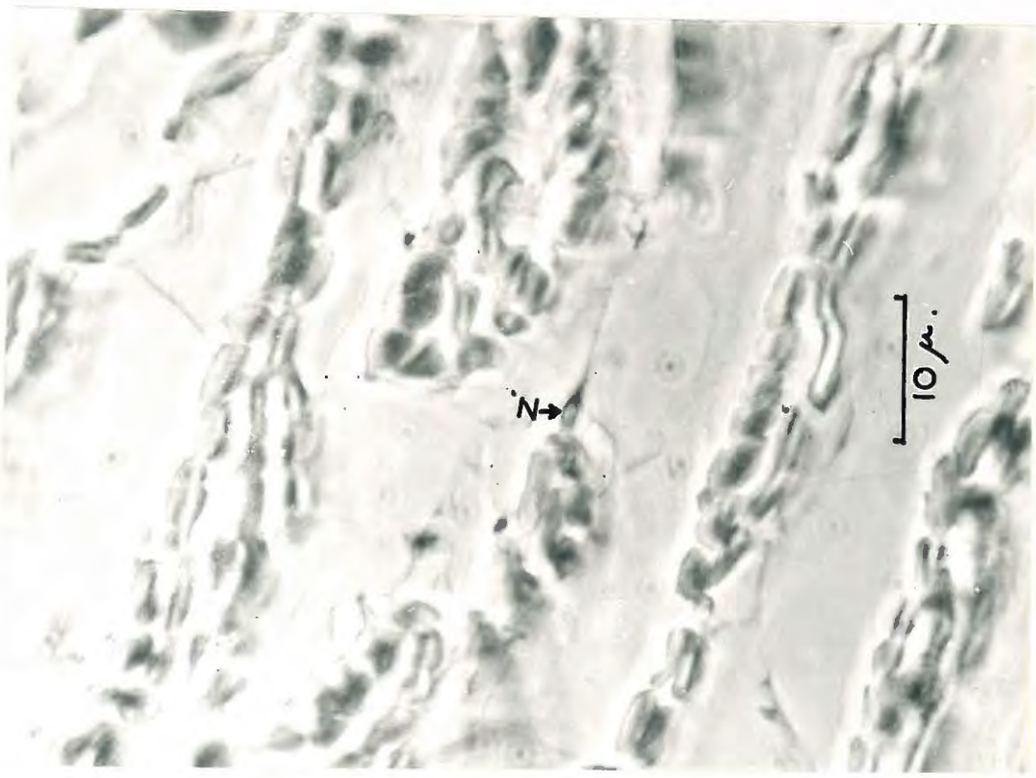

Fig. 19. Parechinus angulosus. Cross section of lantern retractor muscle fixed in Heidenhain's Susa without acetic and Novelli stain. The quality of the fixation of the muscle fibres is very poor and their appearance unnatural. This procedure does reveal details of connective tissue structure. Note cell with small oval nucleus (N) and filament. Thickness 10μ. Phase contrast.

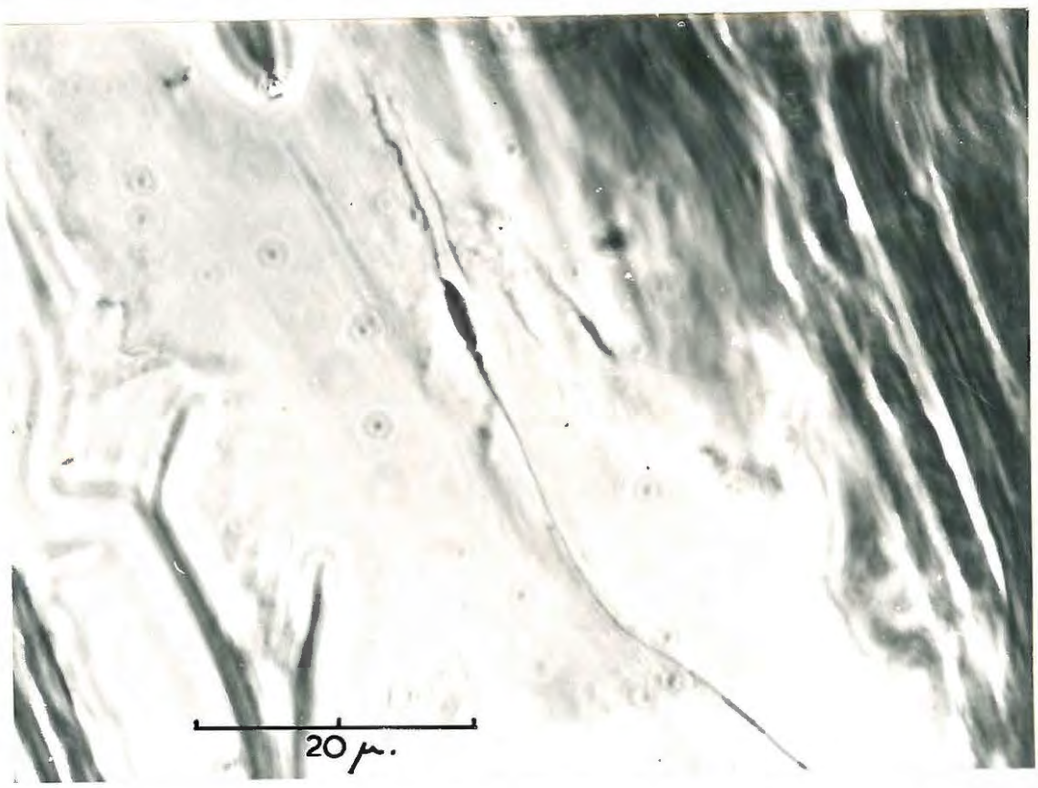

Fig. 20. Parechinus angulosus. Longitudinal section of lantern retractor muscle showing cell with resemblance to a bi-polar neurone in connective tissue.
Fixation, staining and thickness as in Fig. 19.
Phase contrast.

staining techniques. The present cells were found by the aid of phase centrast after staining the tissues with Novelli's (1952) method. Unfortunately the quality of the staining was not good enough to determine unquestionably whether these cells were bi-polar neurones or not.

The ribbon axons of the muscles in the echinoderms are well known. These have been demonstrated in Marthasterias (Smith,1937) and Cumcumaria sykion (Pople,1952). They have been stained with silver stains, Novelli's stain and methylene blue. Ribbon axons have not been seen in any muscle of P. angulosus, using the first two staining techniques. With methylene blue the entire muscle fibre is stained.

The compass elevator and lantern protractor muscles are also made up of long fibres stretching from origin to insertion. Aborally the compass elevator muscle is made up of fibres very similar to the lantern protractor and retractor muscles. Orally, however, there is a thin sheet of fibres separated off from the main bulk of the muscle by a thin membrane (fig. 21). The significance of this separation is not understood.

The comminator muscle is histologically highly unusual. As has been mentioned before, it is made up of a number of flat plates separated from each other by spaces. Each plate is enclosed in a densly nucleated membrane. Within each membrane lie but two layers of muscle cells. Each cell has an oblong cross section,(figs. 22 23),and stretches the whole distance between the pyramids (fig. 24). No nuclei have been identified in these muscle cells but their presence or absence is difficult to determine since they may lie close to the densly nucleated enclosing membranes. The nuclei of these membranes cannot belong to the underlying muscle cells for in surface view they are distributed in a closely packed mosaic arrangement bearing no relation to the position of the muscle cells. The two layers of muscle cells are separated by a thin membrane which lies between them. This

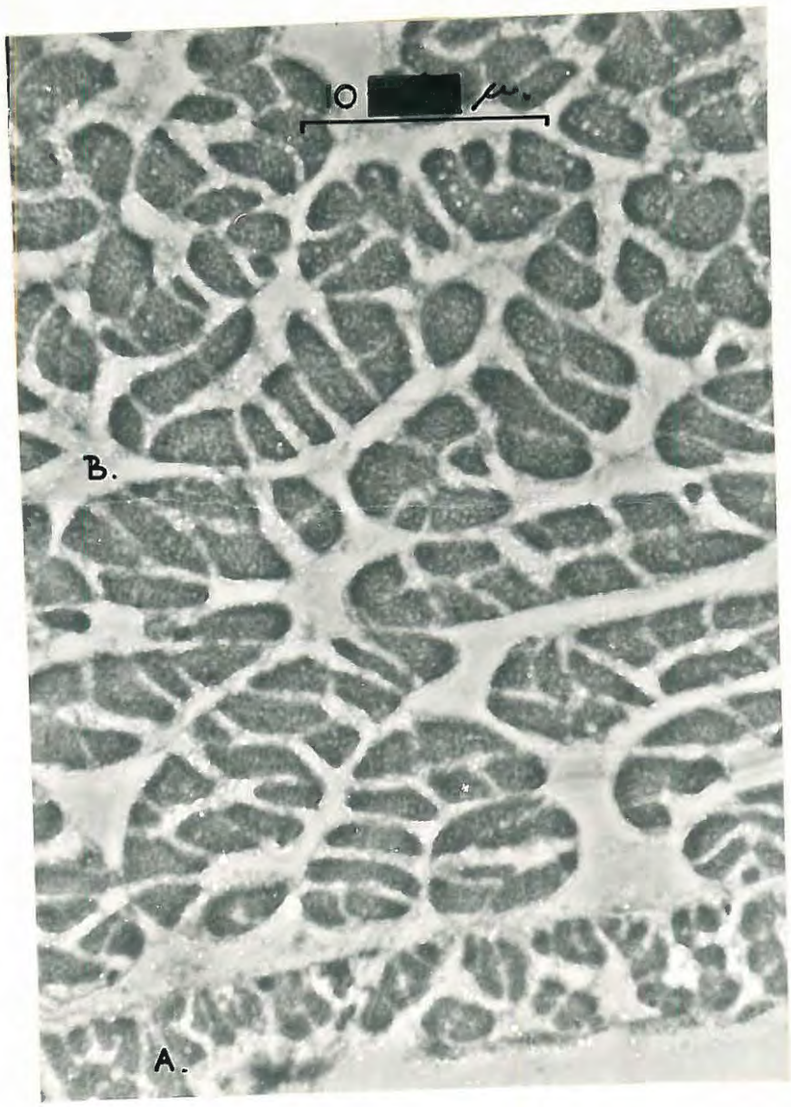

Fig. 21. Parechinus angulosus. Cross section of compass elevator muscle showing two regions, A and B, oral and aboral respectively.
Fixation, staining and thickness as in Fig. 14.
Phase contrast.

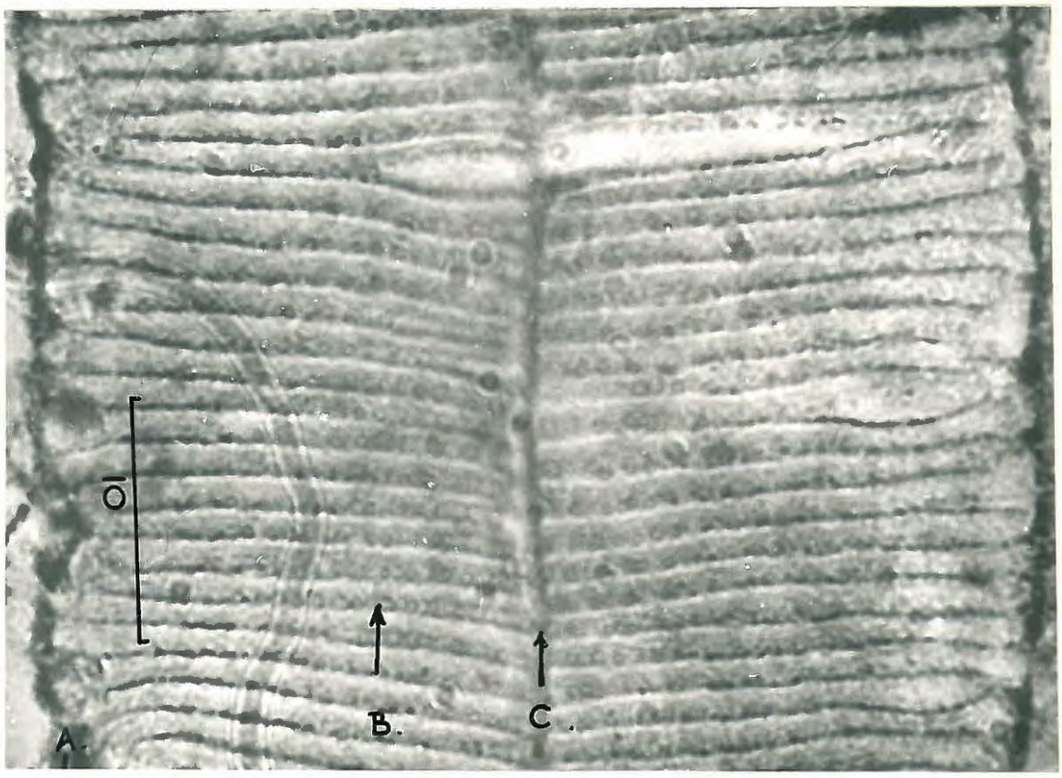

Fig. 22. Parechinus angulosus. Cross section of a comminator muscle plate. A, nuclei of enclosing membrane of the muscle plate; B, oblong cross section of muscle fibres; C, membrane on which two layers of muscle fibres rest.
Fixation, staining and thickness as in Fig. 14.
Phase contrast.

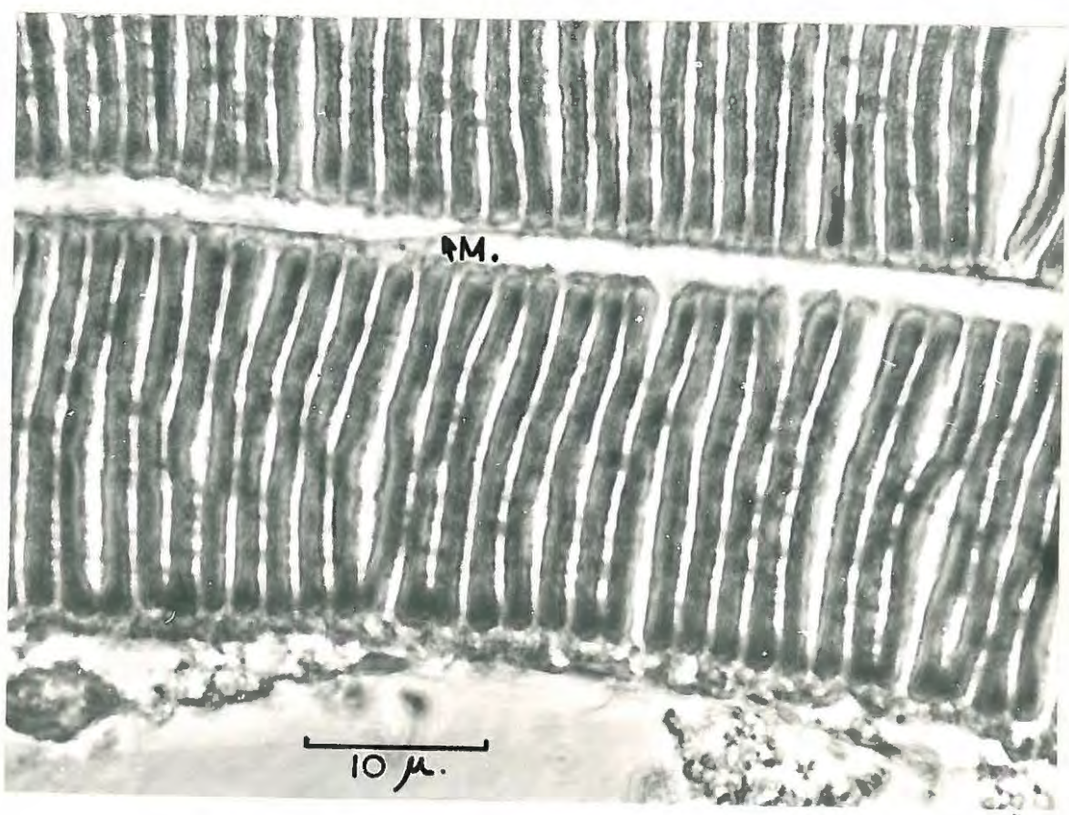

Fig. 23. Parechinus angulosus. Cross section of comminator muscle plate. Same preparation as in Fig. 22, but in a region of poor fixation. The shrinkage of the tissues shows clearly the membrane (M) separating the two layers of fibres.

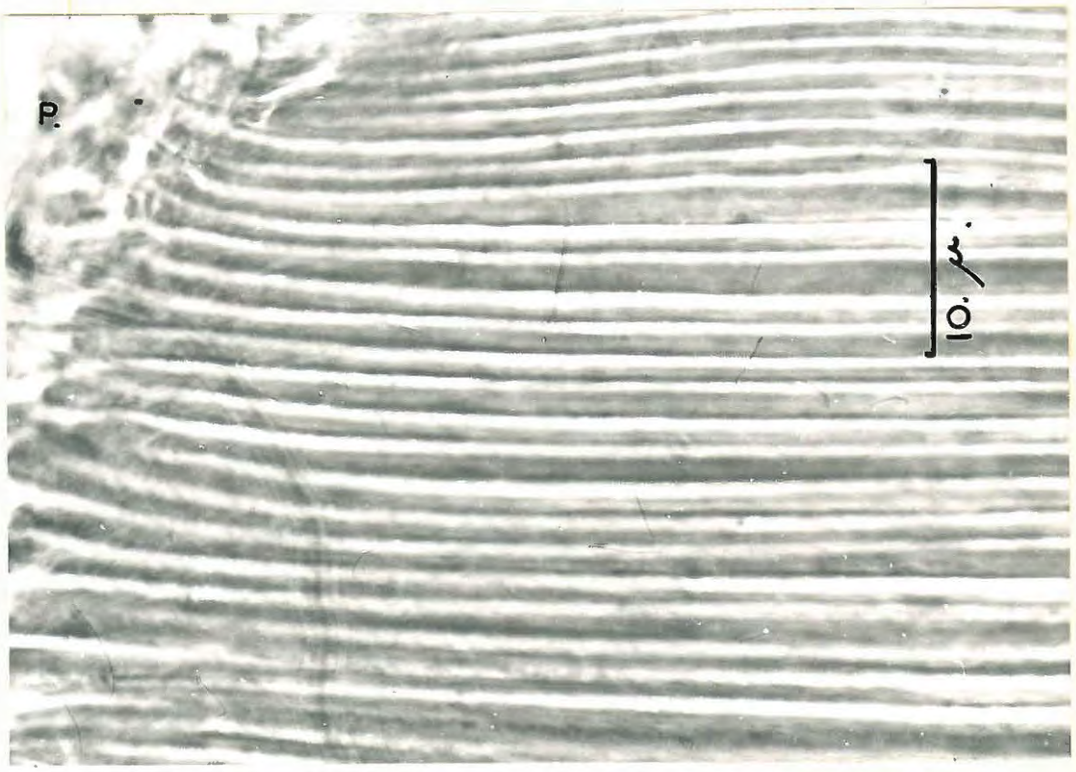

Fig. 24. Parechinus angulosus. Horizontal section of comminator muscle plate showing the fibres for about half their length. The fibres run directly from one pyramid (P) to the next.
Fixation and staining as in Fig. 8. Thickness 10μ.
Standard illumination.

does not appear to be part of the enclosing membrane as it is not nucleated.

The Physiology of the Lantern Retractor Muscle

In any study of a neuro-muscular system some form of excitatory stimulus has to be applied to the preparation. Electrical excitation is the stimulus of choice, but, as mentioned earlier, electrical excitation of the radial nerve of *Parechinus* failed to elicit any responses from the lantern musculature. This failure to excite the lantern muscles indirectly is not a matter of experimental technique as trials with *Cucumaria* showed that indirect responses of the retractor muscle could readily be obtained. Attempts were also made to excite the radial nerve by light beams in the hope that this might release responses from the pyramidal muscles, but again such experiments proved unsuccessful although Millott (1954) has shown that the nerves of *Diadema* are light sensitive. The lantern muscles do, however, respond to direct electrical stimulation and the lantern retractor muscle, but not the others, will also respond to direct light stimulation.

In view of these difficulties it was decided to concentrate attention upon the responses of the lantern retractor, partly because of its relatively greater development and accessibility, partly because it was considered that more might be learnt from a muscle capable of responding to two modes of stimulation. In the event the major study has been dedicated to the effects of photostimulation.

With one exception all the muscles of the lantern are delicate and unsuitable for making neuromuscular preparations. The exception is the lantern retractor muscle which is robust and since it is attached to large ossicles at either end is the easiest from which to make a preparation. The lantern protractor muscle may also be used, but it is very thin and has but few fibres in it. The resistance offered by a lever writing on

smoked paper is almost too great for the muscle to overcome and it tends to tear itself apart.

The single muscle preparation.

The test was cut round the ambitus midway between the oral and aboral poles. The gut was removed from the region just aboral to the lantern as rapidly as possible, and the lantern rinsed in fresh sea water. The compass ligaments were then cut through and the compass apparatus removed from the top of the lantern. A rotula was removed by slipping a pair of scissor forceps between it and the jaw and severing the ligaments between the ossicles. The rotula was approached from the centre of the lantern. The lantern protractor muscles were then cut away cleanly from their attachments to the epiphyses and the perignathic girdle. Care must be taken not to injure the lantern retractor muscle where the protractor muscle approaches it very closely. The epiphysis of the side of the jaw that was not to be used was then broken off, leaving the other half attached to the top of its pyramid. A pair of old straight scissor forceps was then placed beside the tooth on the side not to be used and the jaw split along the midline. The comminator muscles above the radial nerve were then cut down to the level of the radial nerve, care being taken not to injure the nerve itself. A groove was then scratched with a needle between the two auricles. This weakened the joint between the auricles so that one side could be broken away safely, leaving the other side intact. This was done by inserting the scissor forceps next to the auricle in the interradial position and breaking the auricle away from the perignathic girdle. The retractor muscle on that side was then cut away together with the attached auricle. The test was then broken on either side of the auricle with its attached muscle. Two ways of dividing the test were used. The better method was to cut the test with a dentist's diamond saw. The second was to make a groove in the test with a needle and thus weaken it. The test could then be cracked along these lines of weakness. The perioral skin was then cut towards the mouth and the segment of test and

jaw came away free. In this operation the circumoral nerve ring is cut. The spines on the segment of test thus removed were trimmed short and the segment inserted into a plastic clip (fig. 25). The jaw was tied by a thread just above the retractor muscle and connected to a flat watch spring. This in turn was attached to a gimbal lever which wrote on the smoked drum of a kymograph. The watch spring was found desirable as the preparation responds better when working in auxotonic conditions.

The double muscle preparation. A double muscle preparation was prepared either (a) by leaving the jaws on both sides of a radius intact and cutting the comminator muscles of the neighbouring jaws, or (b) by splitting the jaws on both sides of the radius which was to be used. In all cases the protractor muscles were dissected away.

The five paired muscle preparation. A preparation in which all the retractor muscles remained was set up in the following manner. The lantern was removed by cutting the test as close as possible to the perignathic girdle. The compass apparatus was dissected away. The lantern was placed aboral end down on a piece of sponge plastic. Mounted in a dish were three small perspex posts with holes drilled in them to take stainless steel needles. The posts were placed so that they came opposite three interadii in the lantern. The needles could be pushed into the lantern over the epiphyses and could thus pin the lantern down against the slight pressure offered by the plastic sponge. A cut was made in the perioral skin round the edge of the perignathic girdle. The perignathic girdle was then fractured along each of the radii. This left five pairs of muscles attached proximally to the lantern, but separated from each other distally. Threads, attached to levers and running over pulleys, were tied to the muscles (fig. 26).

A protractor muscle preparation. The epiphysis at the aboral end of the jaw was first broken off. The restraining membranes were then slit down to the perignathic girdle so as to

/free.....p.42.

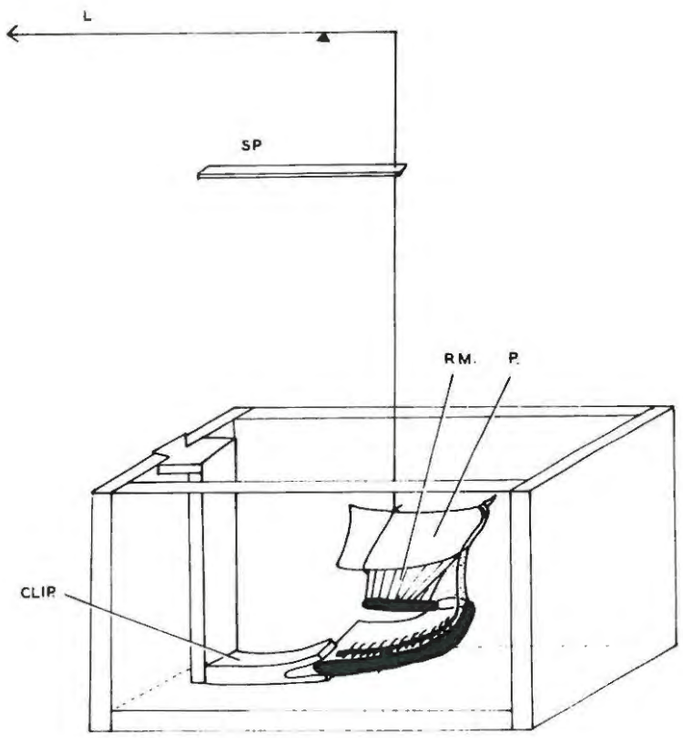

Fig. 25. Diagram of experimental arrangement used in recording activity of lantern retractor muscle preparation. L. writing lever; P. pyramid; RM. retractor muscle; SP. watch spring.

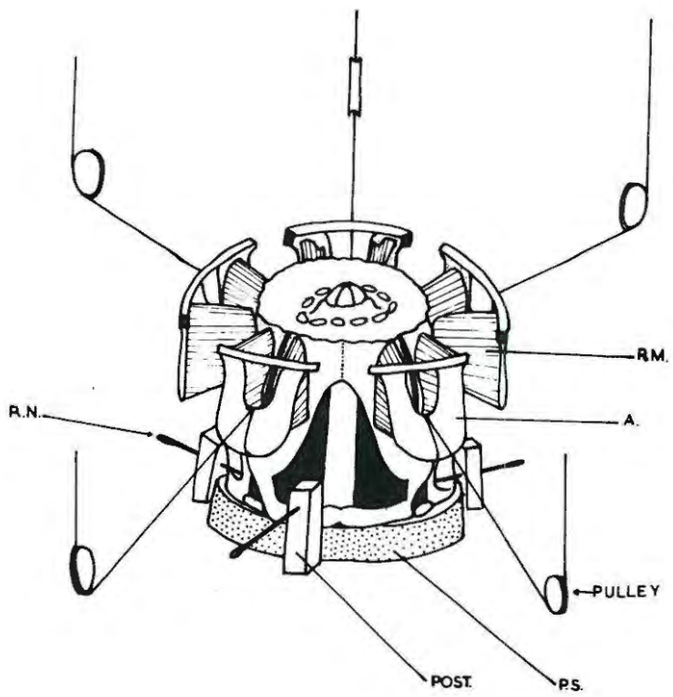

Fig. 26. Diagram to show arrangement for recording activity of five lantern retractor muscle pairs.
A. auricle; N. retaining needle; P.S. plastic sponge mat; R.M. retractor muscle.

free the muscle. The rest of the lantern was removed and the test bearing the muscle was broken away. The muscle was tied with thread at the aboral end. This was done either loosely just beneath the epiphysis, or more tightly, thus enabling the removal of the epyphysis. This ossicle was usually too heavy to remain supported by the muscle.

Since all preparations are sensitive to light the dissections were carried out in very dim general lighting conditions with a 15 watt bulb approximately two meters from the preparation. The dissections were made under a binocular microscope and were illuminated with a microscope lamp fitted with a red filter. The animals are insensitive to red light. Unless this precaution is observed preparations are, initially, only very weakly responsive to light stimulation and may take up to six hours to recover from the exposure to light during preparative dissection.

The preparations were kept in aerated sea water as the body fluids of echinoderms are isotonic with sea water (Pople 1952).

Recording apparatus.

All recordings were traced mechanically onto a smoked drum. A Palmer kymograph was used and the smoked paper fixed with shellac solutions. In the main Palmer gimbal levers were employed, though in a few experiments frontal levers were used.

Photostimulation apparatus.

Most of the stimulation by light was carried out using a Zeiss microscope lamp. Since the filament of the lamp takes an appreciable time to heat up a device was employed whereby the light could be exposed without having to heat the filament of the bulb. This was a Zeiss camera shutter mounted in front of the light source. The shutter was operated by means of a cable release.

The flash contact of the shutter was used as a switch to operate the circuit of a signal marker which, writing on the drum, showed the duration of stimulation. The delay between the opening of the shutter and the closing of the flash contacts

was measured in the following way. The leads from the selenium cell of a Seconic exposure meter were tapped and fed into one channel of a Cossor oscilloscope. The flash contacts were wired in series with a small power supply and the other channel of the oscilloscope. By placing the lamp with the shutter in front of the meter and releasing the shutter, a current was generated in the selenium cell of the meter. This sharply deflected one beam of the oscilloscope, while the other beam was deflected when the flash contact operated the other circuit. The time lag between the two deflections was less than one millisecond. This has been disregarded since there must be greater error in judging the actual onset of a contraction from a trace on smoked paper.

Pulsed light stimuli were provided in two ways. For short durations the exposure times offered by the shutter were used. These durations were calibrated in the following way. Film was run through an oscilloscope camera at a constant speed so that it would photograph a fifty cycle mains signal. The film was rewound and the front of the camera was screened off with light-proof black paper, in which a hole had been pricked. The microscope lamp with the shutter was placed in front of the camera. The film was run through again at the same speed and exposures were made by releasing the shutter in front of the lamp at various settings. The durations of any exposure could then be measured directly from the film.

The duration of time between these brief light pulses was controlled in the following manner. The head of a long cable release was mounted on the block of a knockdown key of a second kymograph. An eccentric cam on the spindle of this kymograph pushed the head of the cable release and so released the shutter. The time taken for the kymograph to complete one revolution would then be the time between stimuli.

As the shutter did not automatically give exposures of more than one second duration, another method had to be used to produce longer stimuli.

The duration of the pulse and the time between pulses was controlled in the following way. A variable duration electronic timer was built (fig. 27) to give impulses of 2 to 13 seconds. A Palmer time clock (fig. 28 S.M.) opened a relay (C.S.1) which started the action of the timer (D.R.T.). The timer pulled in a relay (C.S.2) which operated a solenoid (SOL) from the starter mechanism of a Chevrolet motor car. The plunger of this solenoid pushed the head of a cable release (C.R.) and operated the shutter The shutter mechanism was set on "time" and thus remained open so long as the solenoid was activated. At the end of the signal from the timer the circuit to the solenoid was broken and the plunger and cable release were returned by means of springs to their original positions (fig. 28).

Special Illumination.

In investigations of the effect of different wavelengths of light on the preparation, a mercury vapour lamp was used. The light was filtered with Wratten filters. The resulting transmission spectra were measured by means of a Hartridge reversion spectroscope.

Electrical Stimulation.

The preparations were stimulated electrically by means of square waves and also with direct current. Square waves were supplied by means of a stimulator modified from Ead's (1951) design. The pulse was reversed from positive-going to negative-going by putting the output on the cathode line of V5 rather than the anode. The output of the stimulator was approximately matched to the resistance of the preparation with R 23 (fig. 29).

Direct current was supplied from a dry cell battery connected with a series of resistors and potentiometers so that the current flow could be controlled; the direction of the current could be changed without altering the potential (fig. 30).

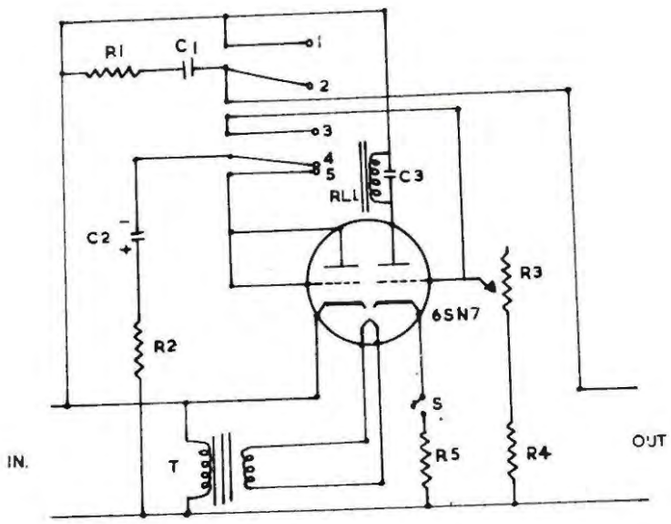

Fig. 27. Timer circuit. R.1. 500 ohms, ½ watt; R.2. 10 K, ½ watt; R.3. 100 K; R.4. 10 K, ½ watt; R5. 150 ohms, ½ watt; C.1. 0.1μf, 600 v.; C.2. 16μf, 450 v.; C.3. 4μf; 50 v; T.1. 6 volt transformer; RL.1. Post office relay, 6200 ohms winding. Input voltage, 250 volts A.C. The circuit is brought into operation by pushing the 'normally closed' switch, S. The contacts of the post office relay, 1 - 5, are shown in the operating position.

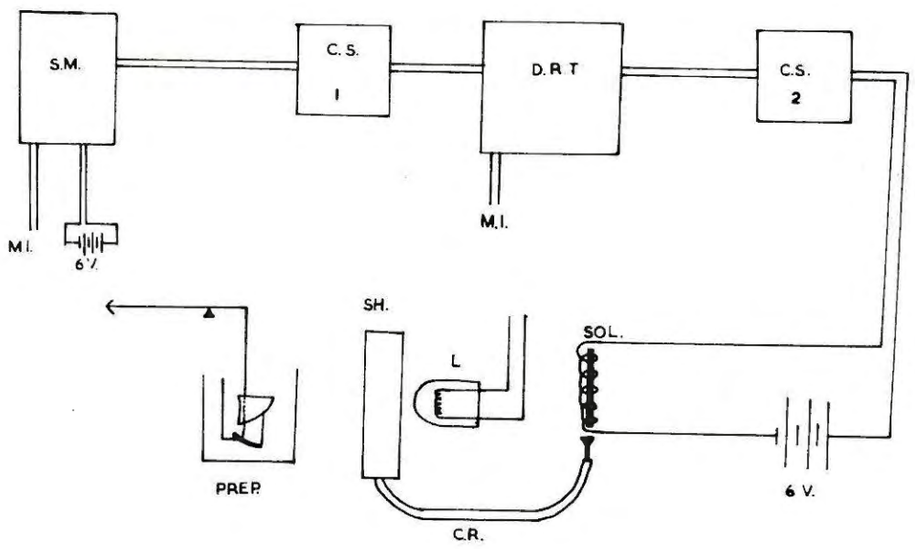

Fig. 28. Block schematic diagram of apparatus to deliver pulses of light whose duration is controlled by the timer (D.R.T.) operating solenoid (SOL) by way of a relay (CS 2) to control the movement of a camera shutter (SH) by way of a cable release (C.R.). The frequency of the light pulses is controlled by a Palmer Time Clock (S.M.) operating the relay (C.S. 1) which is normally closed and corresponds to the switch S of Fig. 27.
L. lamp; M.I. 250 volts A.C.; PREP. retractor muscle preparation.

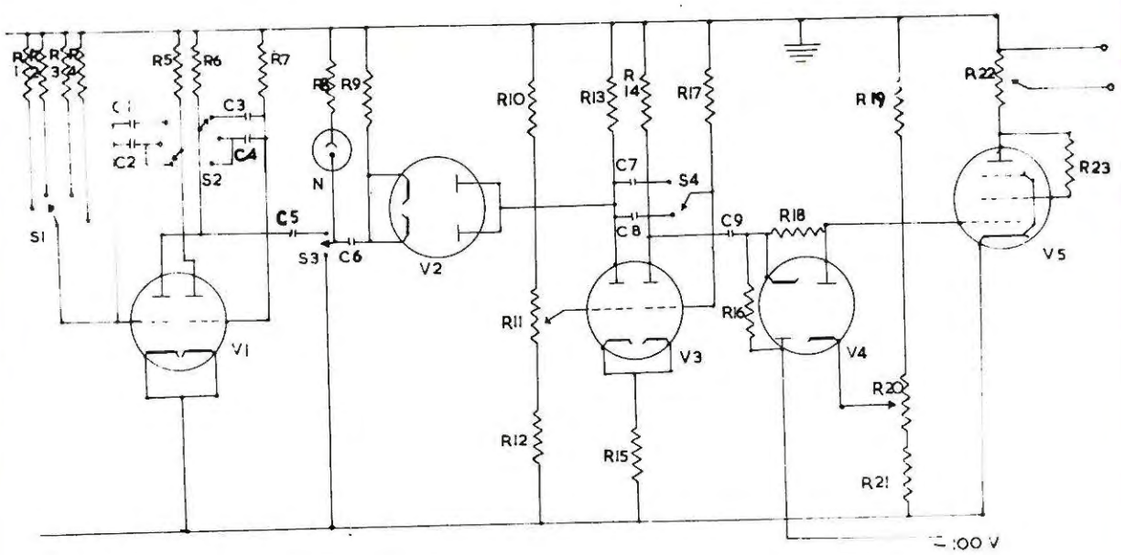

Fig. 29. Circuit of Ead (1951) square wave stimulator. The circuit has been modified to give negative going pulses.

R.1 10M; R.2 4.3M; R.3 1.7M; R.4 20M;
R.5 270K; R.6 1M; R.7 250K; R.8 25K; R.9 25K;
R.10 20K; R.11 10K; R.12 20K; R.13 4.4M; R.14 1M;
R.15 47K; R.16 1M; R.17 5M; R.18 47K; R.19 2M;
R.20 1M; R.21 1M; R.22 1K; R.23 100 ohm.
C.1 2μf; C.2 0.5μf; C.3 0.5μf; C.4 0.001μf;
C.5 1μf; C.6 100pf; C.7 0.1μf; C.8 0.01μf;
C.9 1μf; V.1 6SN7; V.2 6H6; V.3 6SN7;
V.4 6H6; V.5 6L6.
N. neon.

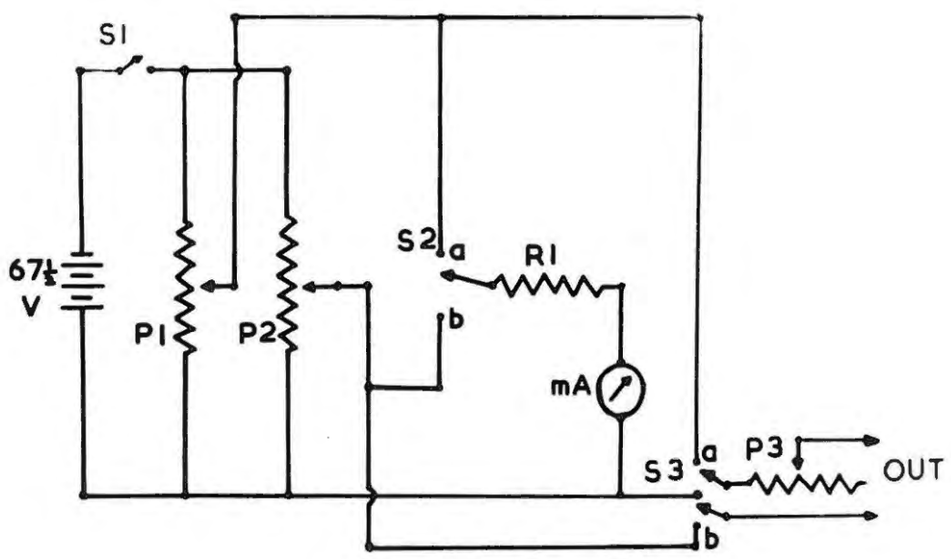

Fig. 30. Circuit diagram of D.C. stimulation apparatus.
R.1 19K; S.2 double pole, single throw switch;
S.3 double pole, double throw switch; P.1 - 3 25K.

The Properties of the Lantern Retractor Muscle Preparation.

1. Spontaneous Activity.

Some preparations of the lantern retractor muscle display spontaneous movement. This is a rhythmic activity characterised by a slowly rising tension followed by a fairly rapid fall (fig. 31). If left undisturbed such preparations will maintain a constant rhythm over long periods. It seems probable that this activity is a facet of the feeding pattern of the entire animal, for direct observation shows the teeth of Parechinus to make rhythmic movements during feeding. The teeth open slowly and close together quickly. The opening movement, as has already been indicated, is effected by the lantern retractor muscles, the closing by the comminator muscles between the pyramids. Here, of course, only the activity of the retractor muscles is being observed.

Spontaneous rhythmic activity was only displayed by a limited number of the preparations studied and no certain way of stirring an inactive preparation to maintained activity has been found. If a preparation is contracting feebly, its level of activity may be enhanced by a long light stimulus (fig. 32). Similarly an increased stretch upon the muscle may enhance the activity (fig. 33). Mechanical stimulation of the lips of the pharynx may increase or decrease the level of activity. These various influences appear primarily to affect the amplitude of the contractions recorded, not the activity rhythm itself.

Spontaneous activity can be interrupted by light stimuli. Exposures to light lasting 20 seconds may cause a maintained contraction of slowly increasing tone. After the cessation of stimulation the rhythmic activity will be resumed after a pause (fig. 34). The type of response shown in these conditions to the light stimulation is unaffected by the point in the cycle of spontaneous activity at which the stimulus is applied. With longer light stimuli, of five minutes duration, the rhythmic activity may be re-established during the stimulation and in these circumstances a new activity rhythm appears to be set from the time of cessation of stimulation (fig. 32).

Fig. 31. Parechinus angulosus. Spontaneous activity of the lantern retractor muscle. Time mark: 10 seconds.

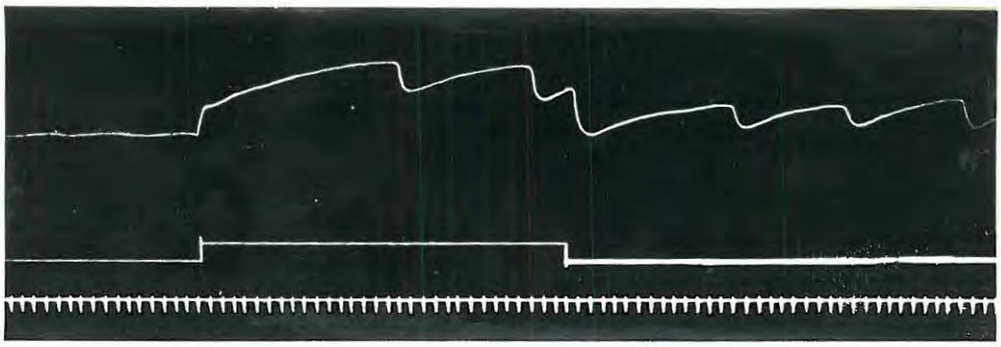

Fig. 32. Parechinus angulosus. Activity of a retractor muscle preparation. Upper signal indicates onset and duration of light stimulus. Time mark: 10 seconds. In this and subsequent traces, unless otherwise stated, light stimuli were all of the same intensity at a transformer rating of 8 volts.

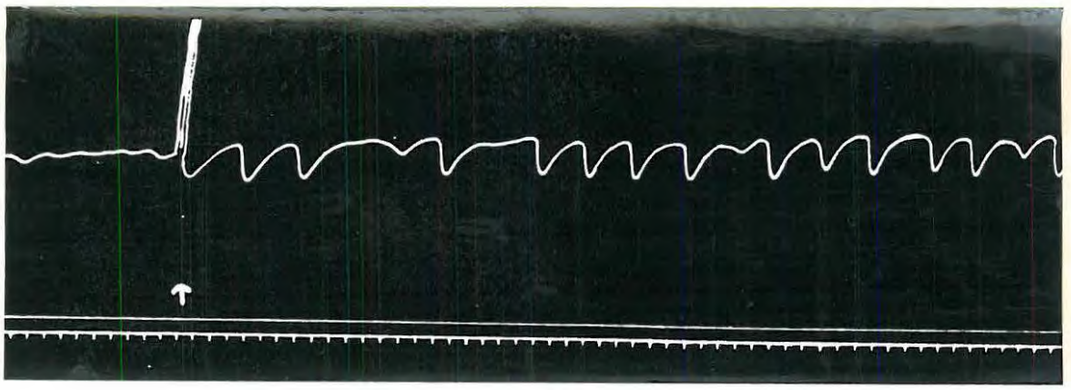

Fig. 33. Parechinus angulosus. Retractor muscle preparation. At arrow stretch on preparation increased. Time mark: 10 seconds.

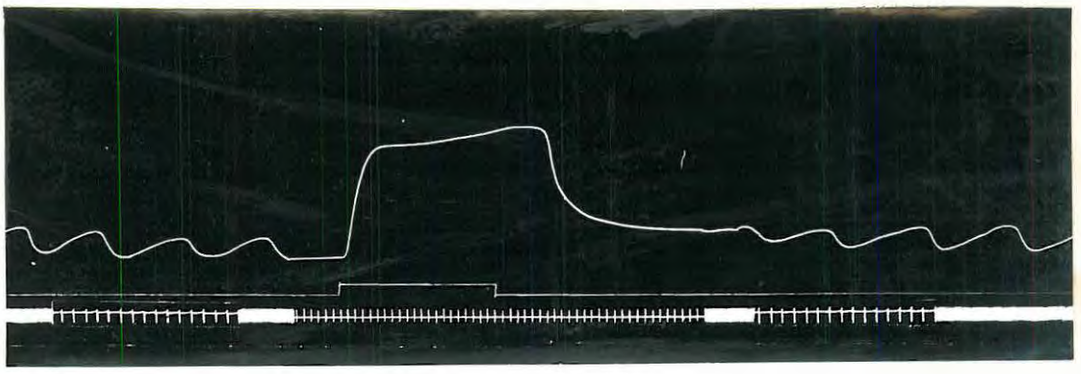

Fig. 34. Parechinus angulosus. Retractor muscle preparation. Upper signal indicates light stimulus. Time mark: 1 second.

beneath the light stimulus and ten seconds on either side.

Fairly brief light pulses may stop a spontaneous contraction which is developing and a new cycle is again commenced at the cessation of stimulation (fig. 35).

2. Stimulation with White Light.

The response of the retractor muscle to light stimulation has three characteristics that are reasonably consistent from one experiment to the next. There is an initial quick contraction which is followed by a slow rise in tension. After a variable and usually considerable time the muscle then begins rhythmically to relax and contract (fig. 36). The details of the different phases of the response vary considerably from preparation to preparation. Thus in some cases a preparation, when stimulated with white light, shows a clear separation between the quick and the slow components of the response (fig. 37). Where these are well separated the form of the record produced is characterised by an initial rapid rise in tension followed by a plateau and then a relaxation before the development of the second or slow response. The general form of the first, quick response is unaffected either by the intensity or duration of the stimulation. This may be seen by comparing the response shown in fig. 37 elicited by maintained stimulation with that in fig. 38, the response to a photographic electronic flash of 10^{-3} second duration. A number of experiments were undertaken to see whether this clear double response could be regularly elicited by subjecting the preparation to some particular treatment. The action of the following different factors was studied:- light intensity, wave length, stimulus duration, slow narcotisation of the muscle with carbon dioxide and finally addition of adrenaline at various concentrations. In no case did the treatment modify the responses of different preparations in a regular manner.

It must be emphasised that the various records presented in the account which follows have been obtained from preparations of widely different physiological state. When a preparation is initially set up it is generally inactive and insensitive and inhibitory phenomena, presently to be described, are most easily

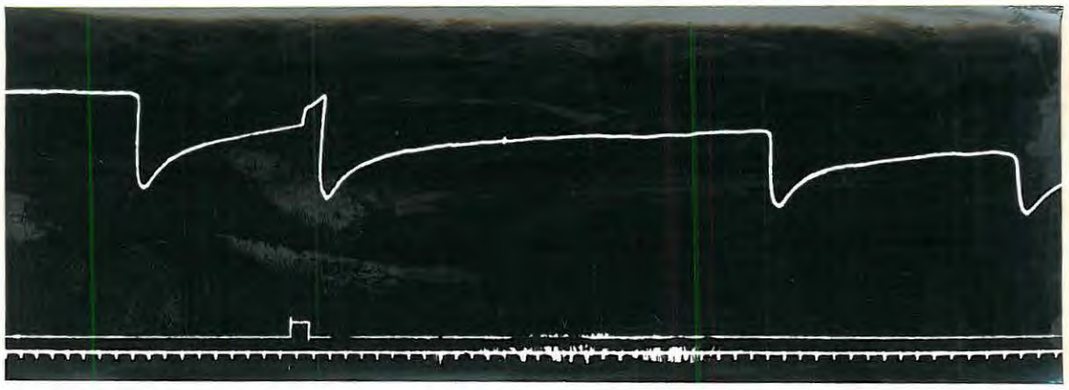

Fig. 35. Parechinus angulosus. Retractor muscle preparation. Influence of a light stimulus of 15 sec. duration upon a spontaneously active preparation. Time mark: 10 seconds.

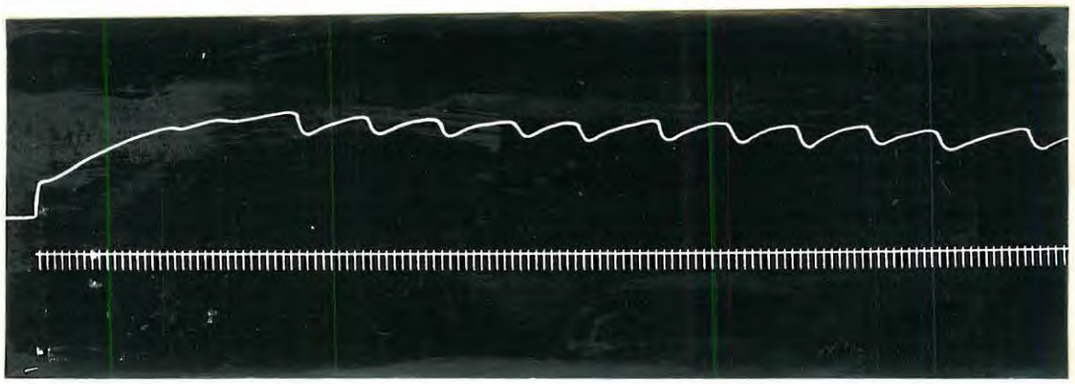

Fig. 36. Parechinus angulosus. Typical response of lantern retractor muscle to a prolonged light stimulus. Onset of stimulation indicated by downward movement of upper signal marker. Time mark: 10 seconds.

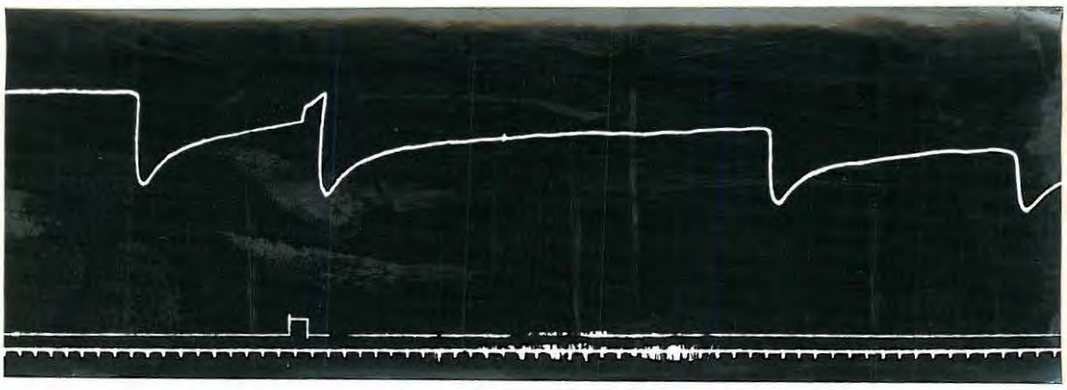

Fig. 35. Parechinus angulosus. Retractor muscle preparation. Influence of a light stimulus of 15 sec. duration upon a spontaneously active preparation. Time mark: 10 seconds.

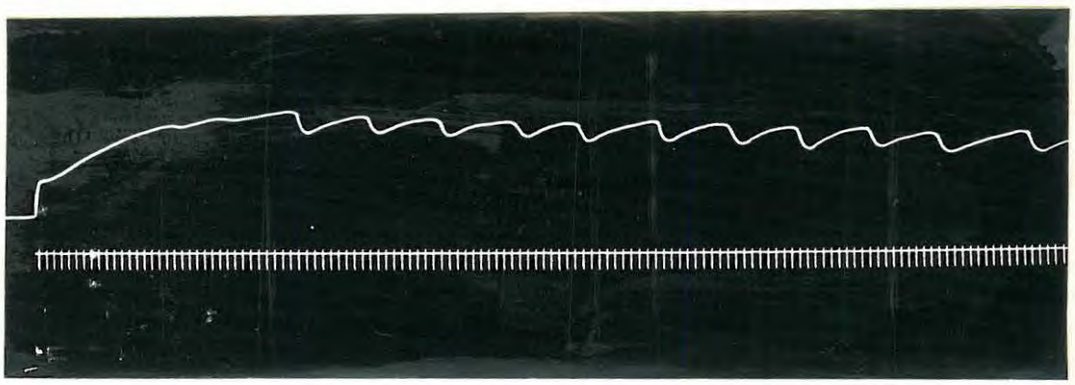

Fig. 36. Parechinus angulosus. Typical response of lantern retractor muscle to a prolonged light stimulus. Onset of stimulation indicated by downward movement of upper signal marker. Time mark: 10 seconds.

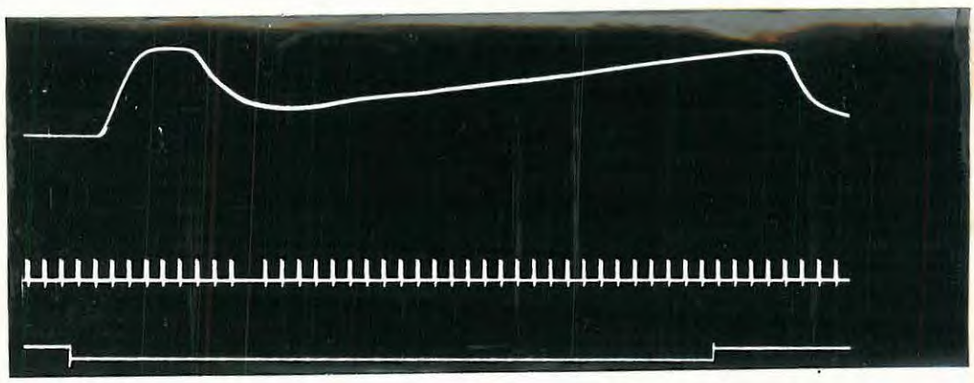

Fig. 37. Parechinus angulosus. High speed tracing of retractor muscle contraction showing differentiation into 'fast' and 'slow' responses. Duration of stimulation 27 seconds. Time mark: 1 second.

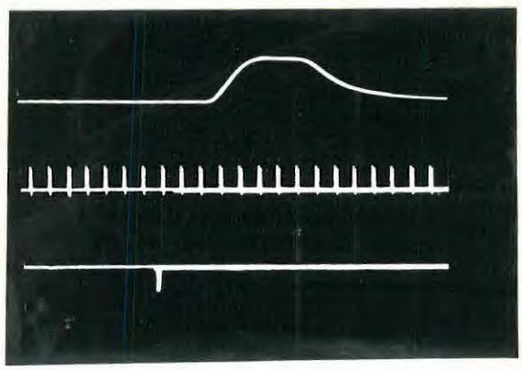

Fig. 38. Parechinus angulosus. Retractor muscle preparation. Response to electronic flash of a millisecond duration given at mark on lower line. Time mark: 1 second.

demonstrated at this time. Later the preparation becomes dominantly excitatory and inhibitory effects are only demonstrated with difficulty. A comparable "dying away" of inhibition has been found by Ewer (1960) in circular muscle preparations of Calliactis.

(a) The Effect of Intensity of White Light.

(i) Latency.

The effects of the intensity of white light on the preparation may be assessed by its influence on the latency and the amplitude of the quick response. As the intensity of the stimulus is increased the latent period of the response decreases. Fig. 39 A and Table I show the results from one preparation where two series of latencies were determined twelve hours apart. Each stimulus was followed by a 20 minute rest. In all cases the duration of the stimulus greatly exceeded that of the latent period. From the Table it can be seen that, although the latencies are shorter during the second series, in both cases the latent period falls as the stimulation intensity is increased.

(ii) Amplitude.

The tension developed by the muscle during the quick response increases with increasing intensity of stimulation. This may be seen in fig. 39 B and Table II which are records of the same preparation as before. In Table II the effect of a twelve hour rest is shown. There is a suggestion that the magnitude of the response in the second period is greater.

(iii) The effect of intensity on the slow response.

The effect of light intensity on the slow response cannot be assessed quantitatively since the final tension developed may be obscured by the onset of rhythmic activity. It is also not possible, because of the variable character of the response, to make a meaningful assessment of latency, nor to determine comparable rates of tension development.

(b) The Effect of Duration of Stimulation.

(i) Latency.

The longer the duration of the light stimulus the shorter

T A B L E I

Effects of Light Intensity upon Latency of Quick Response.

Sets of values for the latency of the first response taken from the same preparation twelve hours apart. 20 minutes rest allowed between each stimulation.

Series 1			Series 2	
Lamp Voltage	Latency		Lamp voltage	Latency (sec.)
6	2.2		5	2.1
5	3.0		8	1.8
8	2.0		5	2.6
8	2.2		6	2.2
5	3.2		8	1.8

Note: The lamp intensity at different filament voltages was calibrated with a photoelectric light meter whose readings could be converted to candle power.
This showed that the three filament voltages offered by the lamp transformer represented in fact a linear increase in luminous intensity. In these experiments absolute values are of no significance as no easy allowance can be made for absorbtion by, and reflection from the apparatus. The light intensity falling upon the preparation will however be directly proportional to the filament voltage.

TABLE II

Effects of Light Intensity upon the Tension Developed by the Quick Response.

The same preparation as in Table 1 was used. The readings for series 1 and 2 were taken twelve hours apart. Tension measured as height of response in mm. using an auxotonic lever.

Series 1			Series 2	
Lamp voltage	Tension		Lamp voltage	Tension
6	13		5	14
5	7		8	19
8	14		6	13
8	12		5	8

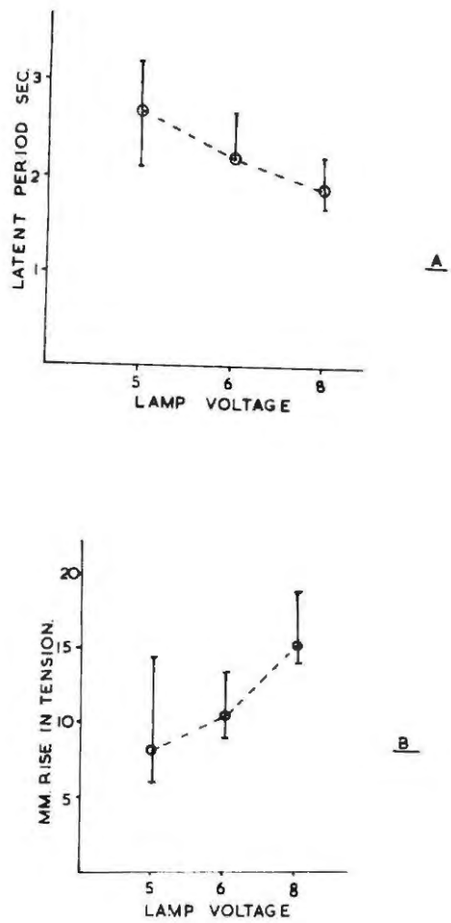

Fig. 39. Parechinus angulosus. Graphs showing relationship of latency and amplitude of contraction to intensity of stimulating light. For further explanation see text.

In these graphs and in fig.40 each point represents the mean of a number of readings whose range is shown by the vertical line through the point.

is the latent period as may be seen by reference to Table III and Fig. 40. Comparison of the latencies recorded in Series 1 and 2, separated by an interval of twelve hours, show no consistent trend of variation.

(ii) Amplitude of the quick response.

Over a short period the amplitude of the quick response of any particular preparation is fairly constant when tested at various durations of stimulation provided these are greater than one second. But for shorter stimuli, the amplitude of the response falls as the duration of stimulation decreases (Table IV).

(iii) The effect of duration upon the second response.

The effect of duration of stimulation upon the second response cannot be expressed quantitatively for the reasons given above. In preparations which show a marked separation of fast and slow responses, the slow response may not be clearly shown following short stimulations. This is illustrated in fig. 41 where a series of traces obtained from a single preparation in response to stimuli of durations varying from two to ten seconds have been superimposed. As is to be expected with these exposure times, the first response is hardly affected by the duration of the stimulus. The second response, however, becomes clearly marked only when the duration of stimulation is greater than seven seconds.

In other preparations where the fast and slow responses are not markedly separated, the slow response will be shown following briefer periods of stimulation.

(c) The Effects of Repetitive Light Stimulation.

The effects of repetitive light stimulation are highly variable. The results obtained depend on whether the preparation responds to a long light stimulus with a separate quick and slow response, or whether the two responses merge one with the other. Fig. 42 shows one type in which there is a smooth increment of amplitude. This appears to be simple summation and is displayed by preparations showing a smooth response to a single long

T A B L E III

Effect of Stimulus Duration upon the Latency of the Quick Response.

The light intensity was kept constant at 8 volts. A rest of twelve hours was allowed between series 1 and 2. A ten minute interval was allowed after each stimulation within the series.

	Duration of light stimulus (sec.)	Latency (sec.)
Series 1	0.04	1.9
	0.55	1.2
	0.33	1.3
	0.15	1.5
	0.09	1.5
	0.04	1.9
	0.04	1.8
	0.09	1.7
Series 2	0.04	2.3
	0.09	1.6
	0.55	1.4
	0.33	1.2
	0.15	1.2
	0.09	1.7

T A B L E IV

Effect of duration upon the Size of the Quick Response.

Duration of stimulation (sec.)	Increase in tension (mm.)			
	Preparation			
	1	2	3	4
0.1			4.0	
0.2	3.0	4.0	7.0	
0.5	4.5	7.0	8.5	
1.0	7.0	8.0	9.0	4.0
2.0				4.0
3.0				4.1
4.0				4.0
5.0				4.0
6.0				4.0
7.0				4.0
8.0				4.0
9.0				4.0
10.0				4.0

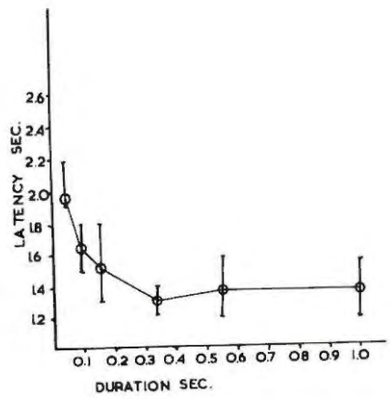

Fig. 40. Parechinus angulosus. Graph showing the relationship between duration of stimulation and latency of response.

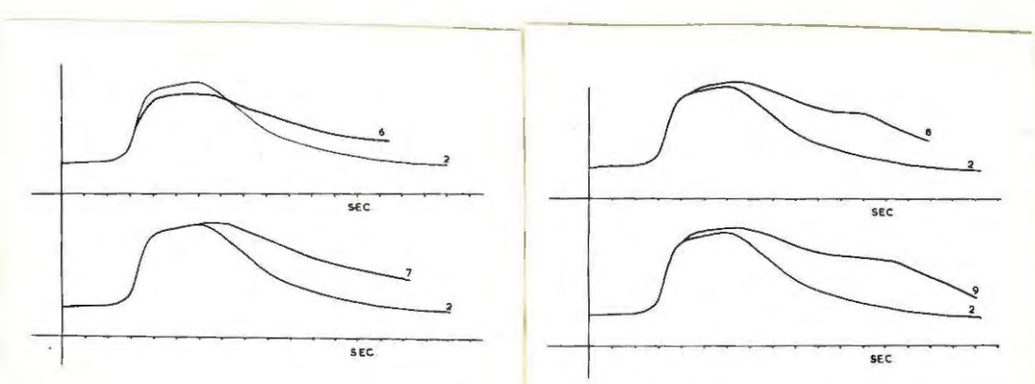

Fig. 41. Parechinus angulosus. Superimposed traces of responses of a retractor muscle preparation to light stimuli of different durations. The numbers indicate the duration of the stimulus in seconds.

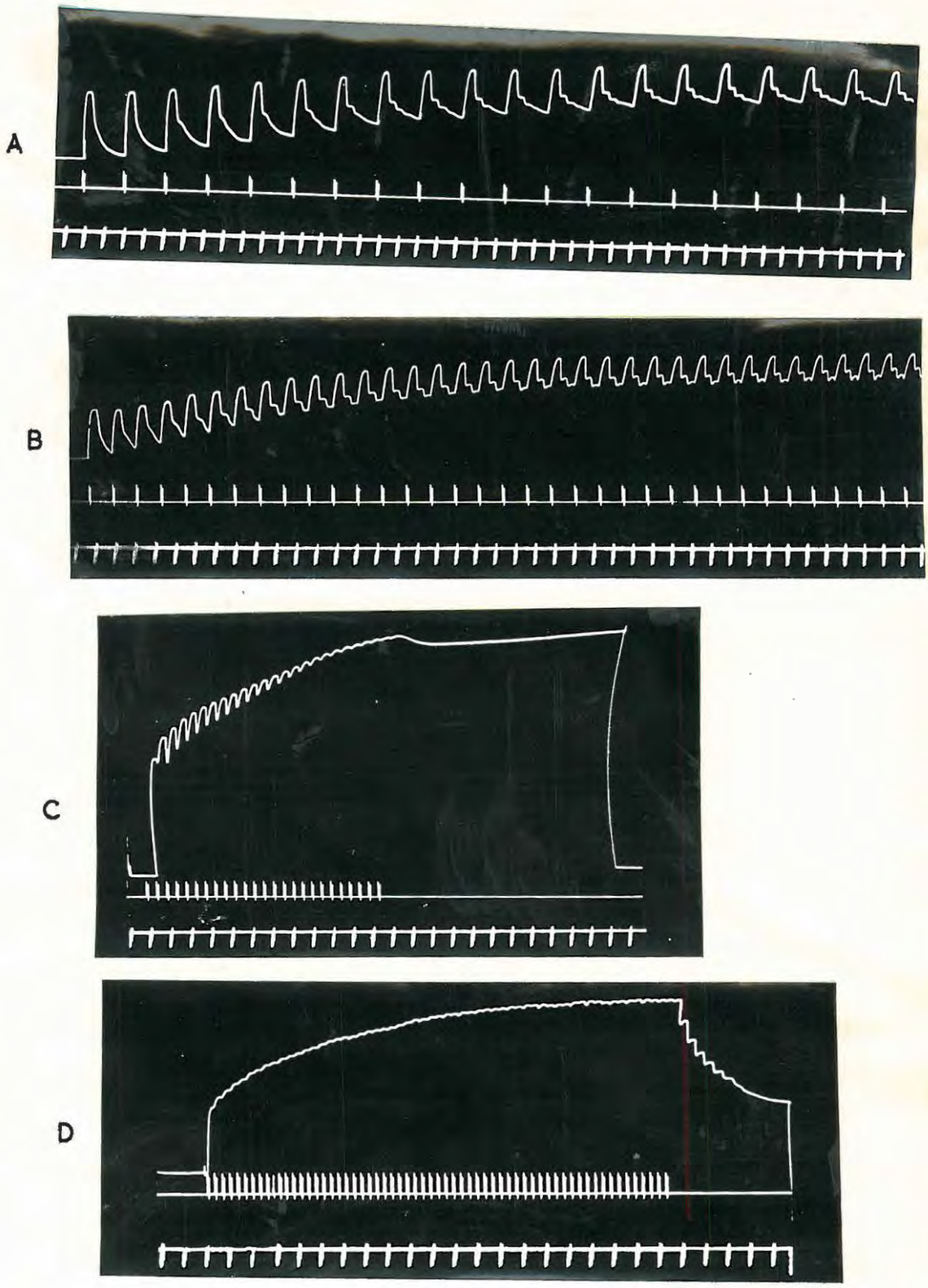

Fig. 42. Parechinus angulosus. Responses of a retractor muscle preparation to light pulses of 0.55 seconds duration at various frequencies. A.1/20 seconds; B. 1/10 seconds; C. 1/5 seconds; D 1/2.5 seconds. Time mark: 10 seconds.

stimulus. However, in preparations that show well separated quick and slow responses, the second and subsequent responses may be considerably modified and smaller than the first (fig. 43).

This phenomenon may be studied quantitatively and can be shown to depend upon the interval between the stimuli. The effect is illustrated by the results of experiments summarised in fig. 44. In this graph the ordinate represents the difference in tension between the first and second responses to a pair of stimuli while the abscissa represents the interval between the first and second stimulus of a pair. Where these are separated by about 10 seconds the magnitude of the response to the second stimulus is depressed, but by 30 seconds the depression, albeit still present, is small.

When longer pulses are used so as to evoke the slow response as well as the fast, there is evidence that the depression of the fast response may last for considerably longer periods. Thus fig. 45 shows the effect of two long light pulses separated by an interval of 25 seconds. The second contraction shows clearly a depression of the fast response.

But with short light pulses a second long duration depression is to be seen. This is shown in the results summarised in fig. 46. Here is recorded the magnitude of the first response to trains of brief light pulses given at varying intervals of time. Graph A relates to trains of five pulses at one pulse every five seconds, graph B to five pulse trains with one light pulse every ten seconds. In both graphs the short duration depression reflected in fig. 44 may be seen. But at intervals of more than one minute between stimulations a second depression occurs which persists for about 20 minutes.

Finally fig. 47 shows a unique phenomenon. Here the preparation was stimulated for periods of 30 seconds alternating with periods of 30 seconds darkness. In this case no depression but an enhancement of both quick and slow responses is to be seen.

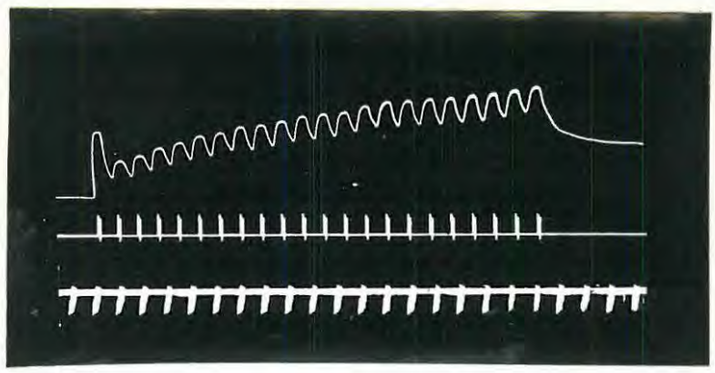

Fig. 43. Parechinus angulosus. Response of retractor muscle preparation to repeated stimulation. Duration of light pulses 0.55 seconds. Frequency of pulses 1/10 seconds. Time mark: 10 seconds.

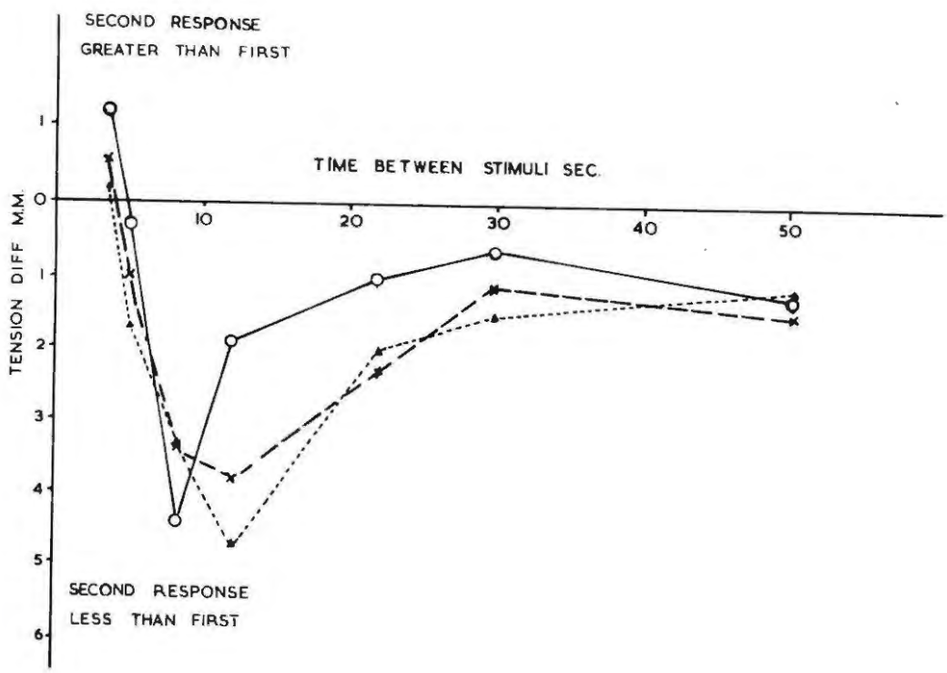

Fig. 44. Parechinus angulosus. Graph showing difference in size of response to the first and second stimuli of pairs of light pulses of 0.55 seconds duration at various time intervals. Open circles: lamp intensity, 8 volts; crosses: lamp intensity, 6 volts; triangles: lamp intensity, 5 volts. For further explanation see text.

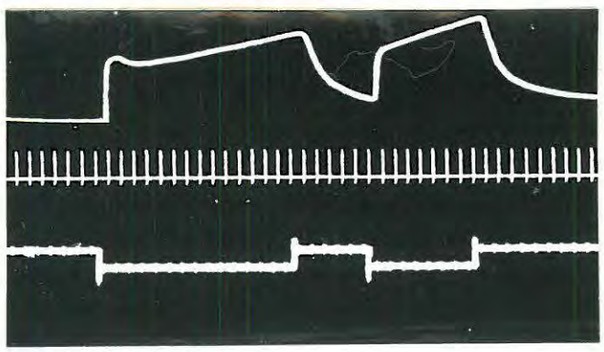

Fig. 45. Parechinus angulosus. Response of retractor muscle preparation to two light stimuli, the first of 75 seconds duration and the second of 40 seconds. Interval between stimuli 25 seconds. Time mark: 5 seconds.

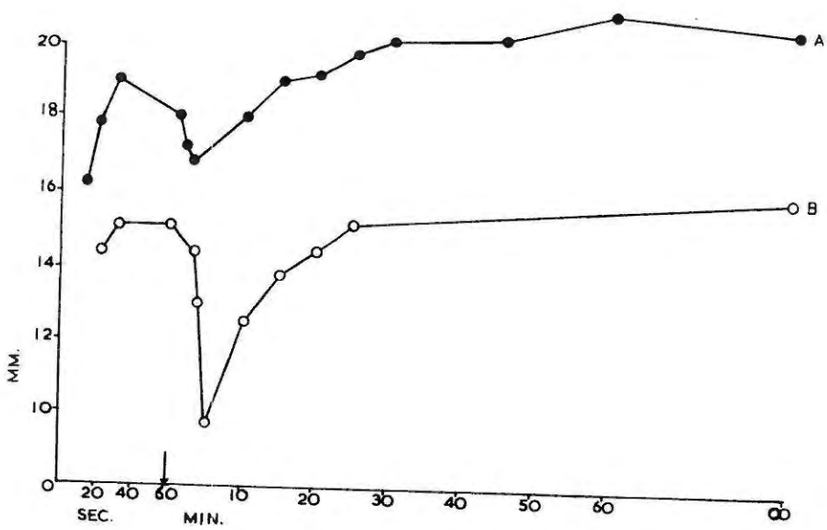

Fig. 46. Parechinus angulosus. Graph showing the magnitude of the first responses to trains of five light pulses of 0.55 seconds duration at varying times between stimulations. A. Frequency of pulses 1/5 seconds; B. 1/10 seconds. For further explanation see text.

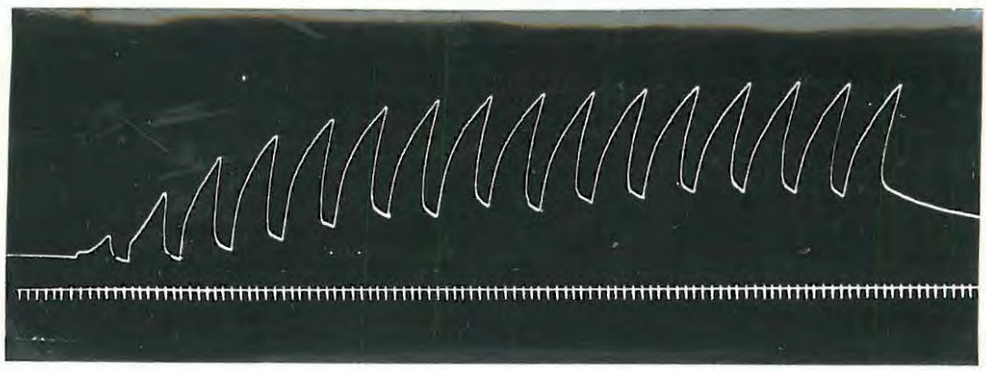

Fig. 47. Parechinus angulosus. Responses of lantern retractor muscle to alternating periods of 30 seconds light and 30 seconds dark. Time mark: 10 seconds.

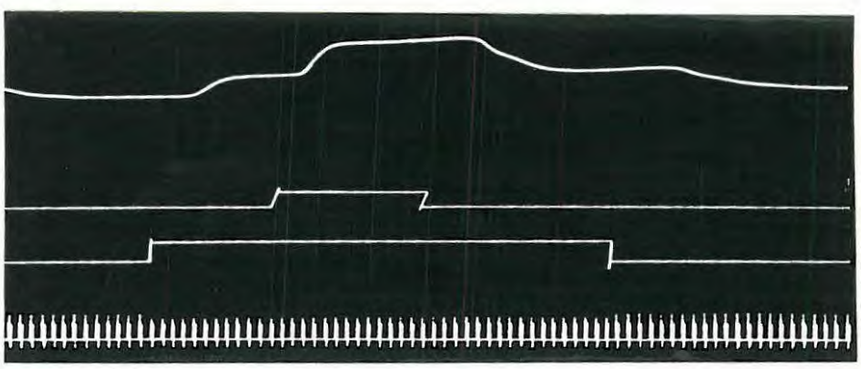

Fig. 48. Parechinus angulosus. Response of retractor muscle to superimposed light stimuli. Lower signal, background illumination. Upper signal, superimposed illumination. Time mark: 1 second.

(d) The Effect of Superimposed Stimuli.

The effect of two light stimuli superimposed one on the other varies according to the duration of the superimposed stimulus. If this is long there is merely summation (fig. 48). On the other hand if the superimposed stimulus is short, that is of the order of a second or less, a different effect, illustrated in fig. 49, may be shown. Here it will be seen that a brief additional stimulus is inserted at A. This is followed by a quick contraction and a relaxation in which the tension falls below that achieved by the background illumination and then rises again. A second series of superimposed stimuli at B show the same effect, the tension developed by the later responses not achieving the value of the first and the relaxation between stimuli falling below that characteristic of the background response. The tension then rises slowly once more until the background stimulation is removed.

(e) Effects of Sustained Light Stimulation.

Sustained light stimulation causes the preparation to go into rhythmic contractions (fig. 36). These bear a striking resemblance to spontaneous contractions (fig. 31). The general form of these contractions is constant. There is a slow development of tension followed by a fairly rapid relaxation. The time between the contractions is very variable both from one preparation to the next and at various times during the life of a single preparation.

If, during the development of one of these regular contractions, a short light stimulus is superimposed upon the background illumination, the contraction ceases and a new contraction cycle starts when the superimposed stimulation ceases. This is similar to the effect observed with spontaneous rhythmic activity in the dark.

(f) Variations in the Relaxation of a Preparation.

A preparation may relax in one of two ways. It may relax smoothly or there may be one or more hesitations during the

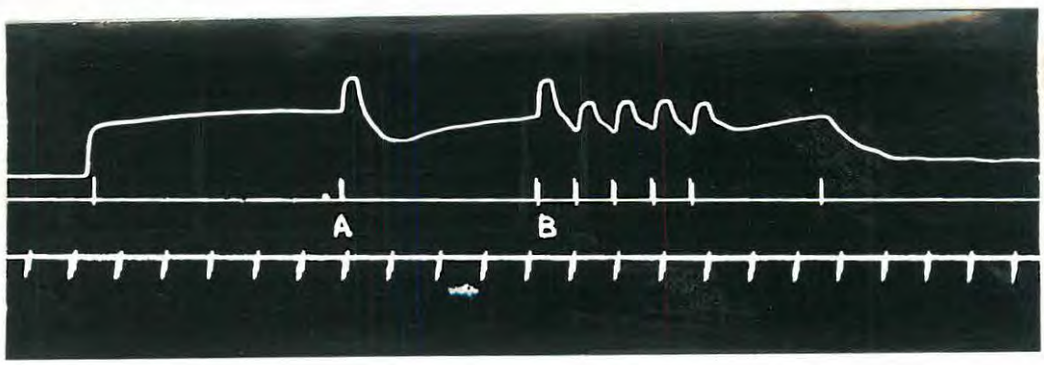

Fig. 49. Parechinus angulosus. Responses to superimposed light stimuli. Background stimulus on at first deflection of signal. At A one light pulse of 0.55 seconds duration. At B five light pulses of 0.55 seconds duration and a frequency 1/10 seconds. Background stimulus off at last signal deflection. Time mark: 10 seconds.

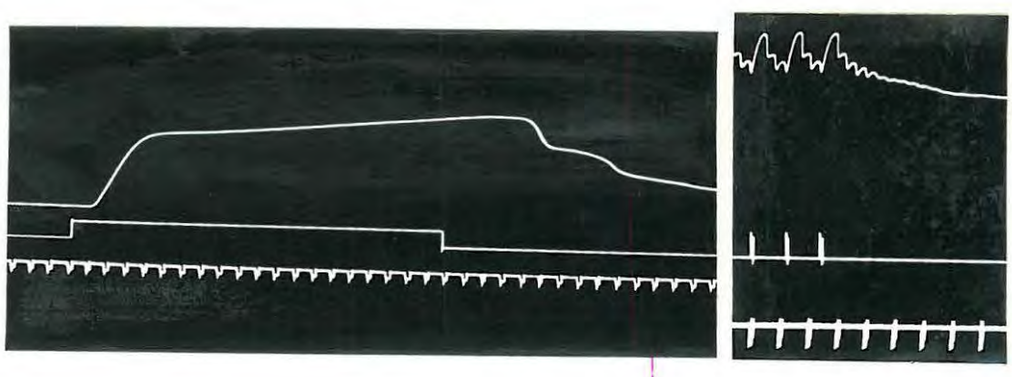

Fig. 50. Parechinus angulosus. Responses of retractor muscle preparations showing irregular relaxation. A. Duration of stimulation 20 seconds. Time mark 1 second. B ~~and C~~ relaxation curves of responses shown in Fig. 42 B ~~and D.~~

relaxation (fig. 50).

Again after stimulation the preparation may either relax to its original level of tension quite quickly or hold a much higher tension than it did originally (fig. 51 A). Similarly light stimulation during spontaneous activity will often give rise subsequently to a heightened resting tonus after the stimulus has ceased (fig. 51 B).

(g) Inhibitory Effects of White Light.

There is a considerable number of lines of evidence which suggest that white light is not only stimulatory to some system causing the muscle to contract, but also excites some inhibitory system. Of these the most compelling are the following.

(i) In a few preparations light stimulation given within the first hour of setting up the preparation causes a fall rather than a rise in tone (fig. 53 A).

(ii) In a spontaneously active preparation a brief light stimulus applied during a period of rising tone will be followed by a sharp relaxation and a new slow increase in tonus (fig. 35).

(iii) The same effect of a short light pulse superimposed upon a steady tonic contraction produced by photostimulation is shown in fig. 49. The brief pulse is followed by marked loss in tone which is subsequently regained.

(iv) Mr. Pople reports (in litt.) that white light stimulation of the pharyngeal retractor preparation of Cucumaria stimulates both excitatory and inhibitory elements. These he has shown to be sensitive to lights of different wave lengths.

I have repeated these observations in the following manner. Shortly after being first set up a preparation is stimulated with white light. As the contraction develops it is illuminated also by a beam from a mercury vapour lamp. There is a fall in tension and then a rapid rise when the mercury vapour lamp is screened (fig. 52). This contrasts markedly with the behaviour of preparations exposed to a superimposed beam

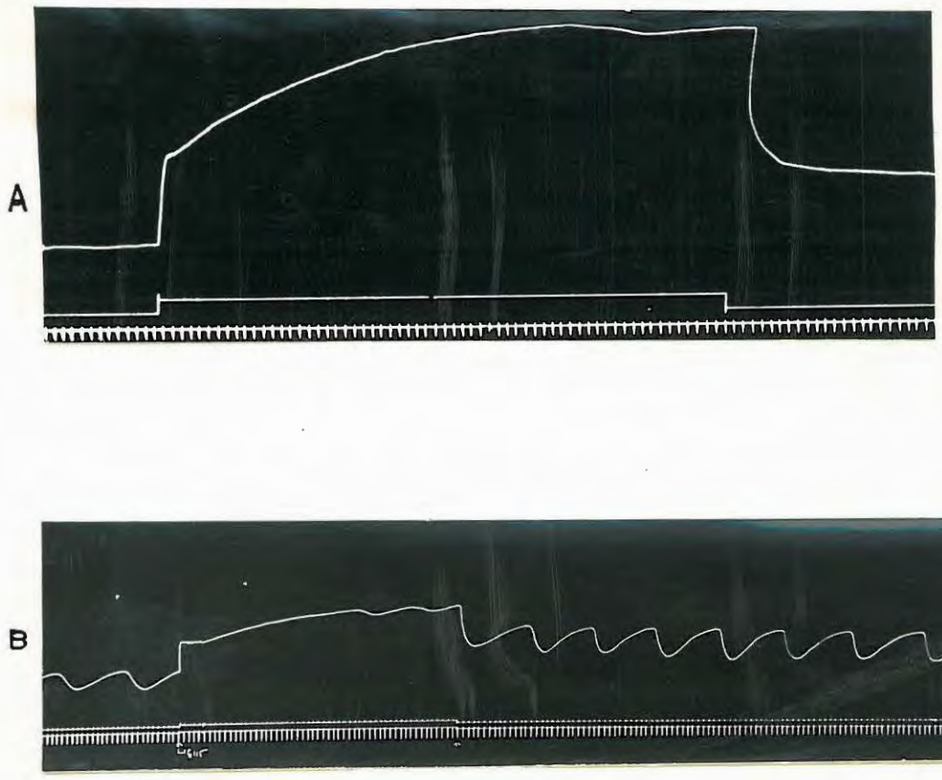

Fig. 51. Parechinus angulosus. Responses of retractor muscle preparations to long light stimuli. Duration of stimulation 620 seconds in A, 520 seconds in B. Time mark: 10 seconds.

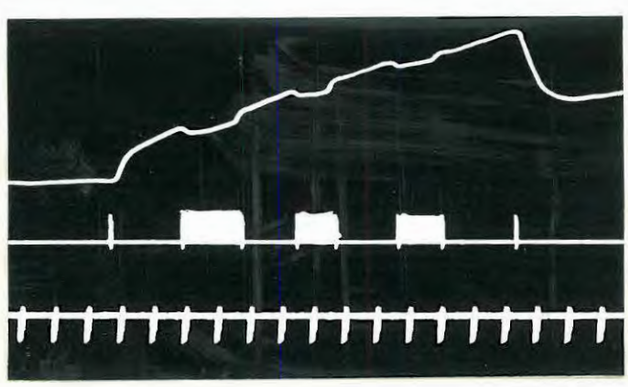

Fig. 52. Parechinus angulosus. Response of a retractor muscle preparation to stimulation from a mercury lamp against a background of white light stimulation. At first signal background illumination started. At three subsequent blocks mercury lamp beam exposed. Final signal, background light off. Time mark: 10 seconds.

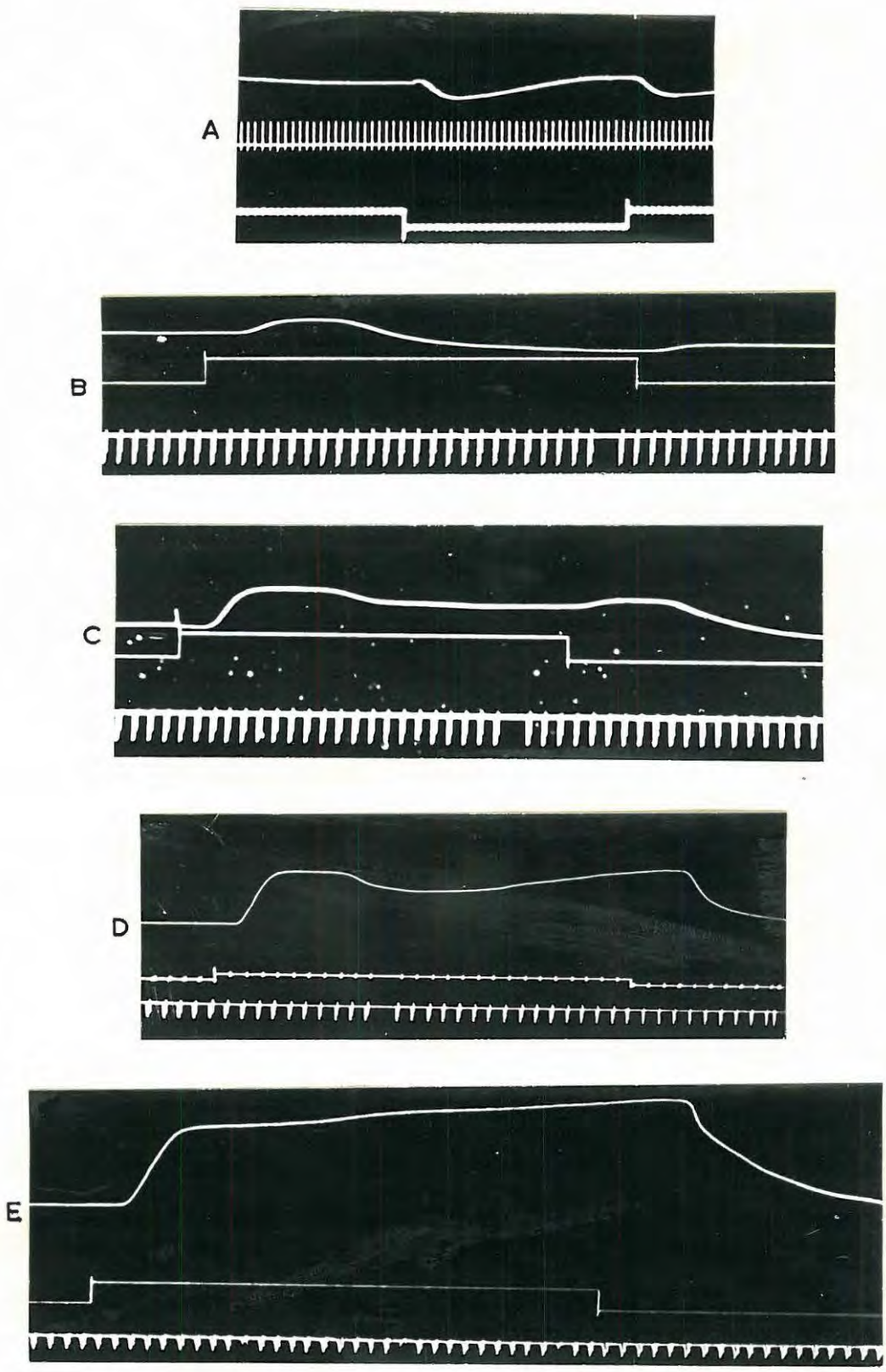

Fig. 53. Parechinus angulosus. Various types of response of retractor muscle preparations. Time mark: 1 second, except for trace A in which the time mark is 5 seconds.

of white light: this invariably produces a further contraction.

Attempts to identify the active wavelengths were unsuccessful. The pure yellow doublet from the mercury lamp is neither excitatory nor inhibitory, while the inhibitory action of the mercury lamp is not prevented if a photographic ultra-violet filter or thick glass plates are placed across the beam. Both the excitatory and inhibitory wave lengths lie between green and violet but attempts at separation using Wratten filters were unsuccessful as these allow too broad a bandwidth to pass.

3. An Interpretation of the Effects of Light Stimulation.

It is convenient at this stage to attempt an analysis of the results which have been obtained in the hope of reaching some provisional conclusions. The most immediate question which arises is whether light is stimulatory to the nerves, the myo-neural junctions or the muscle fibres of the lantern retractor muscle.

Firstly it is clear that the excited systems lie within the muscle. Evidence for this conclusion stems partly from the fact that if a light beam is not directed accurately at the muscle, the tension developed in response to photo-stimulation is never as great as that which the muscle can develop when fully stimulated by the beam. Again if the muscle itself is shaded and light directed at the surrounding structures no response is obtained from the muscle.

It seems possible that the light is stimulating nervous tissue within the lantern retractor rather than the muscle fibres. The reasons for this conclusion are two-fold. Firstly the complexity of the responses recorded differs markedly from the simple responses obtained by direct electrical stimulation of the muscle. Secondly it is found that as a preparation ages the response to light gradually diminishes and finally fails. At this time the muscle fibres are still directly excitable by electrical stimulation, suggesting that the photosensitive structures are distinct from the muscle fibres themselves. While

no histological evidence for a motor complex relating to this muscle has been found, the possibility that such a structure exists cannot be excluded.

Attempts to make a finer localisation of the photosensitive areas were unsuccessful. Experiments using fine light guides failed to elicit any responses from the muscles, possibly because the luminous intensity was too low, the tissues themselves rapidly dispersing light from a limited area.

It has been found that the amplitude of the initial response and its latency are affected by the intensity and duration of the light stimulation. Both vary in a manner typical of photosensitive systems. Since, however, there is evidence that light stimulation has both excitatory and inhibitory effects and that the balance of these varies with the physiological state of the preparation, detailed quantitative studies appeared of little value and have not been undertaken.

The response to light can be regarded as three fold, a quick initial response followed by a slow development in tension and subsequently a regular rhythmical rise and fall in tension. In attempting an analysis of these events attention will initially be directed upon the first two.

An examination of responses made by the lantern retractor to photostimulation shows that a wide range of different patterns may be obtained. Some of these are illustrated in fig. 53. They have been arranged in a series in which the inhibitory action of the light appears to become decreasingly significant. In fig. 53 A the influence of inhibition is clearly marked. The light stimulus causes a short initial contraction of about five seconds duration but then the tone immediately drops, slowly recovers and drops once again when the light stimulus ceases. In fig. 53 B the initial contraction is again of about five seconds duration, but the subsequent loss of tone is less striking than in fig. 53 A. There is a slight increment in tone after stimulation has ceased but this appears simply to be a return to the original resting level

of the preparation. In fig. 53 C the initial contraction is once again of about five seconds duration but the subsequent tone of the preparation is higher than the resting value. At the end of stimulation there is a transient increment in tension. Fig. 53 D shows the characteristic double response, while fig. 53 E shows only a slight but a distinct check in the development of tension. There are then two distinct rates of development of tension and one could consider them to reflect two distinct processes, both modified by a third, an inhibitory event. Indeed this interpretation has been implicit in the preceeding account of the behaviour of these preparations. But a simpler hypothesis would be that there is a single excitatory event modified in its expression by an inhibitory process, the extent of contraction at any time being the resultant of the difference between the levels of excitation and inhibition.

A number of assumptions were tested to see whether in fact such difference curves could be obtained using relatively simple functions to express the growth of the two states. The most satisfactory of these involved the postulate that both excitatory and inhibitory states developed after the manner of a first-order reaction, but that the inhibitory condition did not normally reach an equilibrium - rather if it were relatively high it continued to increase, if low it waned. The results of such calculations are shown in Fig. 54 and it can be seen that the difference curves do fairly closely reflect the types of response shown by the retractor muscle. While this idea offers the attraction of relatively simple assumptions, it cannot be regarded as acceptable. The plateau which is characteristic of so many preparations can only be imitated by a careful choice of parameters for the reactions and even then, although there is a levelling in the difference curve, it is not a true plateau but a curve of great radius.

Such a simple dual mechanism does not provide a satisfactory answer and the further pursuit of an analysis which will account for the wide diversity of phenomena observed can be

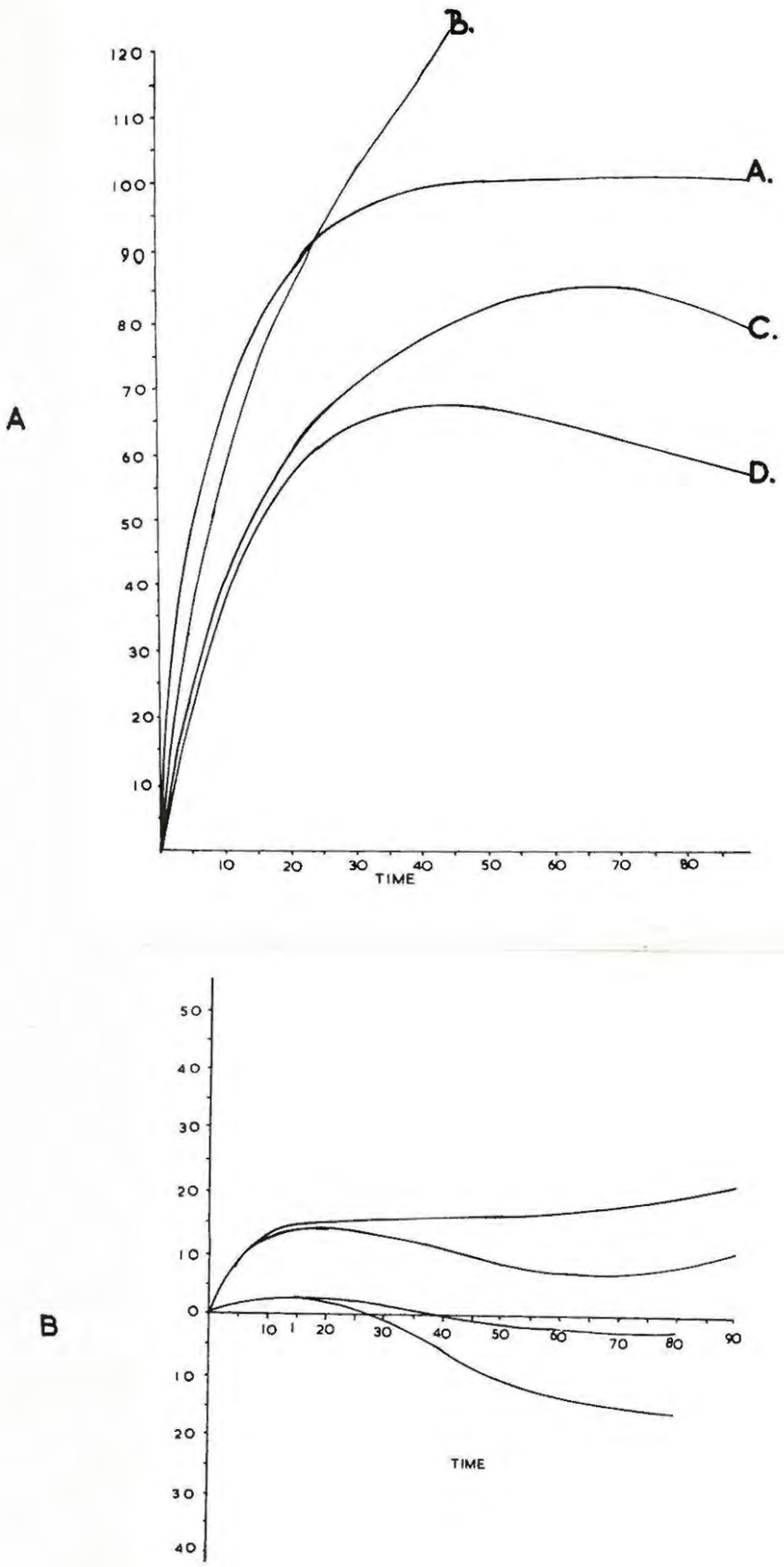

Fig. 54. A. Curves of excitation, A, and various levels of inhibition, B, C and D. All curves initially follow a first order reaction. B. Difference curves between the standard excitatory curve A and various inhibitory curves. Compare form of curves with responses shown in Fig. 53. Time scale in arbitary units.

presented either as a long argument involving the erection of trial hypotheses and their subsequent rejection and modification or by a logical presentation of the conclusion finally reached. The latter, being briefer, has been followed here.

The retractor muscle of Parechinus shares with the pharyngeal retractor muscle of Cucumaria the property of spontaneous activity. Pople and Ewer (1958) have presented evidence that the spontaneous activity of the retractor muscle of Cucumaria arises from an interplay of excitation and inhibition in the motor complex of this muscle and the assumption is made here that this is true also for the retractor muscle of Parechinus. The form of the spontaneous activity of the two preparations is very different, albeit each is part of the feeding pattern of the animal. In Cucumaria a spontaneous contraction occurs every 10-15 minutes, in Parechinus typically every two or three; in Cucumaria a contraction consists of a relatively rapid rise in tension followed by a slow relaxation, in Parechinus there is a slow rise in tension by a fairly rapid fall.

The genesis of spontaneous activity in Parechinus can be represented by a model (fig. 55) which includes an excitatory cyton, the S cyton, which, by a steady outflow of impulses, causes the muscle gradually to develop tone. A colateral from the S cyton feeds back to an inhibitory cyton, the I cyton; the steady arrival of impulses on the dendrites or soma of the I cyton effects a gradual depolarization and after about 120 seconds the I cyton discharges a train of impulses which, relaying back to the S cyton, inhibits the output of motor impulses for about 20 to 30 seconds causing the muscle to relax. The cycle then starts again. This proposal for the control of a rhythmic activity finds justification in the fact that Eccles, Sears and Shealy (1962) have demonstrated a similar slow depolarization leading to a burst of impulses in the motoneurones of the external and internal intercostal muscles of the cat, albeit the rhythm in the mammal is more rapid. The S cyton may be compared with the "pattern centre" postulated by Pople and Ewer (1958), the I cyton

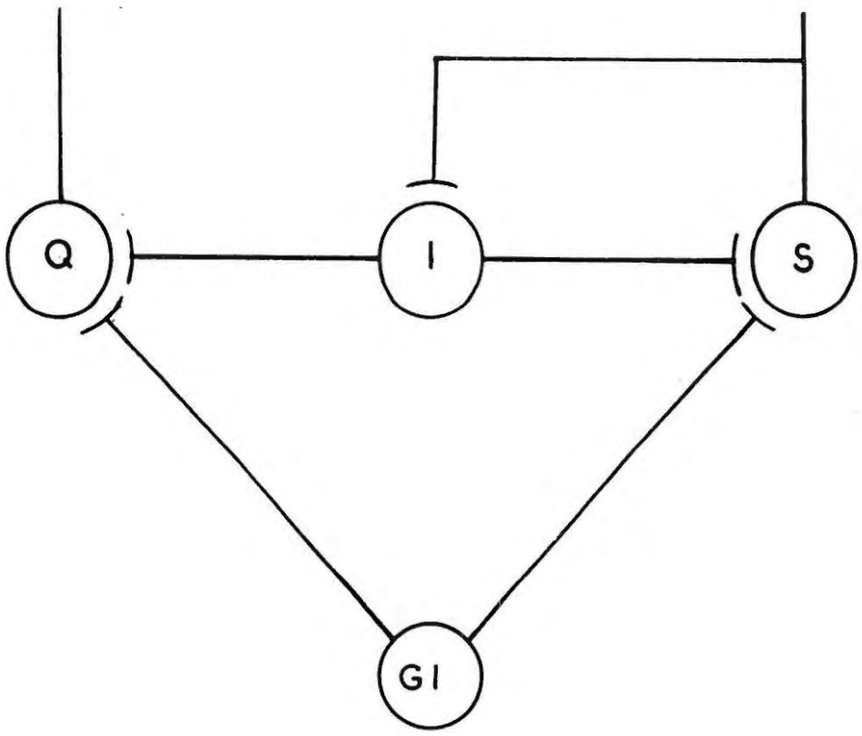

Fig. 55. Hypothetical arrangement of neurones in a motor unit of the motor complex of Parechinus.

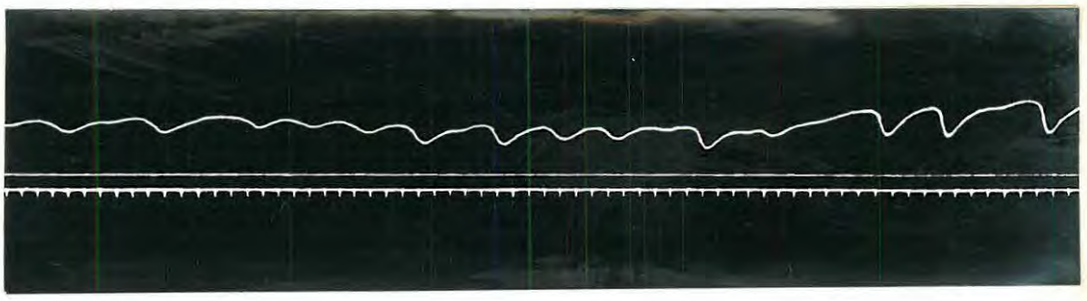

Fig. 56. Parechinus angulosus. Retractor muscle preparation showing irregular spontaneous activity. Time mark: 10 seconds.

with the "inhibitory centre".

The model here proposed can be regarded as the organisation of a single motor unit within the motor complex of the retractor muscle. Commonly the muscle displays a regular rhythmic activity, so that all motor units must be in phase or nearly so. This implies that some general coordinatory mechanism must exist within the motor complex. It is possible that this depends upon the existance of a ramifying neuropile system, so that all motor units are effectively inter-connected and a single unit may serve at any one time as pace-maker for the whole complex. In some preparations the spontaneous activity is arhythmic (fig. 56), which could be explained on the present hypothesis as a result of several cells acting independently as pace makers.

Pople and Ewer further postulate the presence in the motor complex of Cucumaria of tracts responsible for the quick and delayed responses shown by this muscle. The general pattern of the quick response of this animal closely resembles that of Parechinus showing a similar plateau lasting for about five seconds. It is suggested that, in Parechinus, there are cytons, Q cytons, in the motor complex which are photosensitive. These are responsible for the initial 'quick' contraction and its plateau.

The slow response may be seen as arising from the S-cytons which are also normally photosensitive.* The S-cytons differ from the Q cytons in requiring exposure to far longer light pulses to stimulate them to activity. Thus, with a very brief light pulse, only a Q type response is obtained (fig. 38). The discharge of the S-cytons continues for a short while after the cessation of photostimulation as may be seen in figs. 53 D and E.

The reason for regarding the slow response and the

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* It is a matter of debate whether the slow response of the retractor muscle of Parechinus should be regarded as homologous with the delayed or the slow response of Cucumaria retractor. Pople (personal communication) regards the delayed response of the Cucumaria retractor as an experimental artifact, but it is possible that structures comparable with S cytons exist in the motor complex of Cucumaria but are not normally spontaneously active.

spontaneous slow contraction as being of the same neuronal origin lies in their great similarity. This is especially clearly seen in fig. 32 where the rates of development of tension of the two types of contraction are almost identical.

We have earlier rejected the hypothesis that the varying forms of response to photostimulation could be accounted for by a single excitatory event differentiated by an inhibitory action. They can, however, be satisfactorily regarded as the product of the activity of Q and S cytons modified by an inhibitory influence. This idea is illustrated in fig. 57 which shows hypothetical outputs from Q and S cytons at different levels of inhibition.

In this figure diagram A represents the condition in a preparation showing negligable inhibition; Q represents the quick response, S the slow and T the two responses summed. This type of response can be obtained from a preparation which has been quiescent and has not been stimulated for at least 12 hours. An example of such a response is shown in fig. 58. It is important to note that the drum speed of this record is far slower than that used for most of the records in fig. 53.

In conditions of inhibition, the Q cyton starts to discharge and then its activity is, in some manner, stabilised so as to produce a plateau. The higher the level of inhibition, the sooner this stabilisation occurs. The inhibition also affects the S cyton, influencing both its level of activity and its latency. These conditions are represented in fig. 57 B and C in which the T curves are based upon the recorded responses shown in fig. 53 D and E. It will be seen that, according to this interpretation, the duration of the plateau associated with the Q discharge becomes greater when the level of inhibition increases, an expectation which is confirmed by observation. In preparations with an inhibitory system in a high state of photosensitivity, the light stimulus may cause a fall in the activity of the S cyton so that tone is lost as in fig. 53 A and B.

The range of types of response shown in fig. 53 may thus

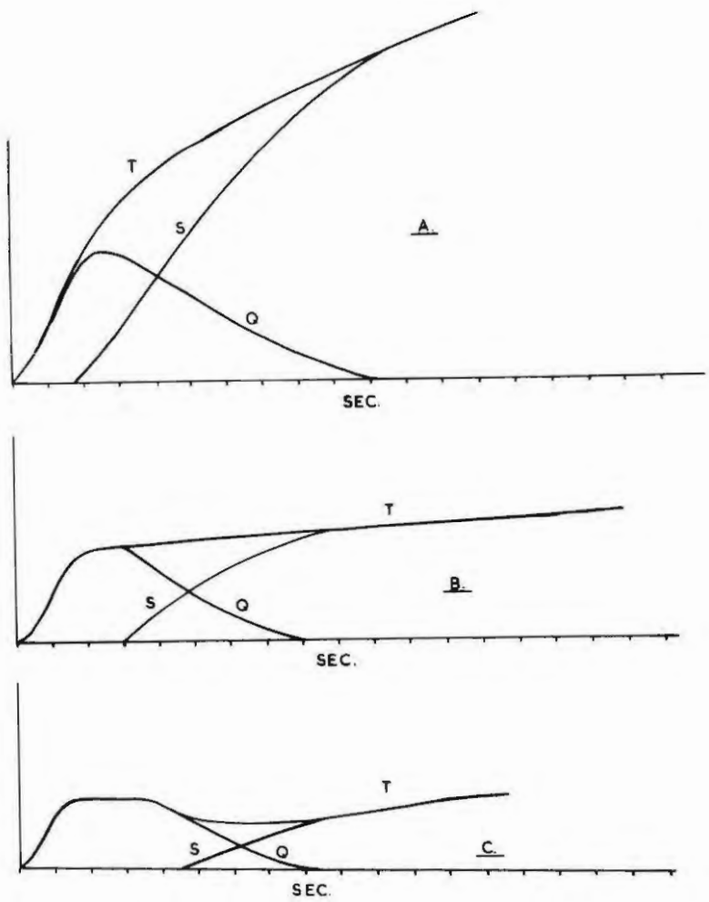


Fig. 57. Hypothetical reconstructions of the development of tension by the quick and slow mechanisms of the retractor muscle in different conditions of inhibition. For further explanation see text.

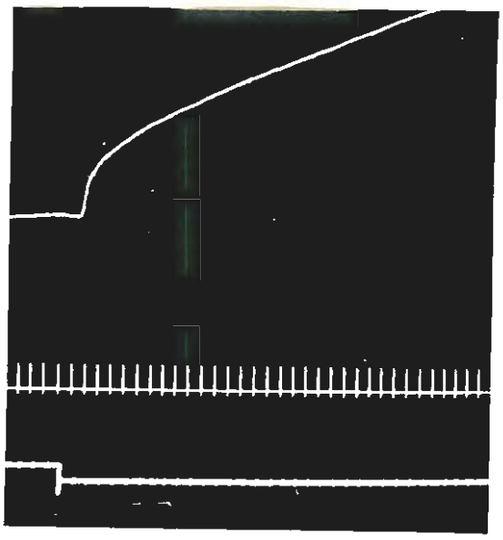


Fig. 58. Parechinus angulosus. Response of lantern retractor preparation. Time mark: 5 seconds.

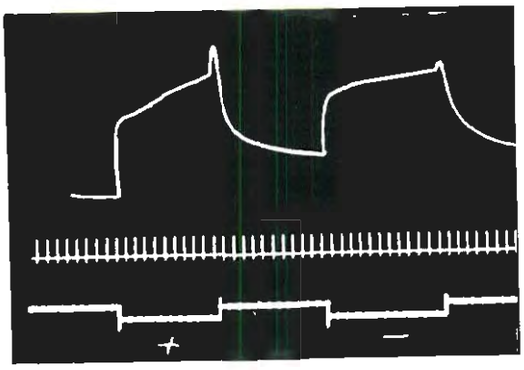


Fig. 59. Parechinus angulosus. Response of lantern retractor muscle to D.C. stimulation. In this and subsequent figures a + beneath the signal indicates that the anode was at the pyramidal end of the muscle, a - that the cathode was pyramidal. In certain cases the polarity of the stimulation has been abruptly reversed. Time mark: 5 seconds.

be accounted for by the assumption that the activity of both Q and S cytons is liable to inhibition.

The simplest hypothesis would be to attribute this inhibition to the I-cyton. There are several reasons for believing this not to be the case. We have already postulated that this cyton responds to depolarization by a volley of impulses. Such an output might account for the varying levels of inhibition reflected in fig. 53.

But if we attribute this inhibition to photostimulation of the I cyton, we would also expect rhythmical activity to be regularly expressed during the course of prolonged light stimulation. This we do not observe. Thus in fig. 51 B rhythmic activity does not commence until 340 seconds from the start of stimulation. Alternatively, if we postulate that the I cyton discharges continuously during photostimulation we would expect one of two things to occur. Either there should be no rhythmical activity displayed during photostimulation or any rhythm displayed would be markedly different from that shown in the absence of any stimulation. Neither of these expectations is fulfilled as may be seen by inspection of fig. 51 B where the frequency of rhythmic activity towards the end of photostimulation is very similar to that shown subsequently. This leads us to conclude that there is some second photosensitive inhibitory system, the GI cyton, which acts on both the Q and S cytons. This cyton may be continuously discharging, thus setting a general level of inhibitory tone, but the arguments advanced above give reason to believe that its activity may be enhanced by illumination.

There is evidence that the photosensitive inhibitory action of this cyton does not persist long after the cessation of illumination. Thus in fig. 53 C there is a rebound of tension at "off" which can be attributed to a cessation of the specific photostimulation of the G.I. cyton. Again the experiment illustrated in fig. 52 shows a fall in tone on illumination from a mercury lamp: this is considered to be due to enhanced photostimulation of the GI cyton. In this case also tone recovers

immediately the stimulus ceases.

But there is a different inhibitory phenomenon which is reflected in the fall of tone following brief photostimulation. This is seen clearly in fig. 49 at A. It is this inhibition which is responsible for the effect seen in fig. 49 at B in which the second and subsequent responses to a series of light pulses are smaller than the first. The time course of decay of this inhibitory effect has been assayed by the relative depression of the second response of a pair of stimuli and the results are shown in fig. 44 where it can be seen that the discharge has a latency of about 5 seconds and decays in about 30. The slight positive upswing at intervals less than five seconds is probably due to mechanical summation. This inhibition is considered to be a reflection of the activity of the I cyton which is stimulated to discharge, not by the onset of illumination, but by its cessation. In other words it shows an "off discharge" which will affect both quick and slow responses.

This "off inhibition" may be recognised as following brief light stimuli to spontaneously active preparations (fig. 35) or in preparations which have become spontaneously active during prolonged photostimulation (fig. 32 and 51 B). It is probably also reflected in the fall in tone at the end of a stimulation of 85 seconds seen in fig. 53 A.

The question now arises as to why spontaneous activity does not express itself earlier in conditions of maintained illumination. The most reasonable assumption is that the I cyton, rather than being stimulated by light to discharge, actually hyperpolarises if the light stimulus is maintained. Analogous behaviour is shown by a photosensitive neurone in Spisula in which a low background discharge is inhibited by light, a long volley of impulses following when the light stimulus is removed (Kennedy, 1960). The level of hyperpolarization of the I-cyton will, however, be reduced by the activity of the recurrent fibre of the S-cyton until the I cyton can once again fire and spontaneous activity is resumed.

It is possibly noteworthy that, comparing figs. 32 and 51 B, the former which shows a briefer time to the establishment of spontaneous activity, shows also a more effective relaxation. Further it is clear from the events following cessation of photostimulation in fig. 32 that the "off" discharge of the I cyton can occur very shortly after a spontaneous discharge. This is not surprising as the mechanisms of depolarization are probably distinct. In some preparations, however, repetitive stimulation results in simple summation of contractions (fig. 42). In such preparations we must assume the I cyton to be insensitive to photostimulation.

Using long light pulses, there is also the clear evidence of facilitation shown by the rare type of event recorded in fig. 47. Here the duration of exposure to light is probably sufficient to paralyse the I cyton, if it is active at all. What appears to be taking place is a photoadaptation of the GI axon so that both quick and slow responses grow with succeeding stimuli. The facilitation, on this view, is not a facilitation of excitatory effect, but simply a dying away of inhibitory action. It is of interest to note that the forms of the first three responses broadly resemble those of the contraction types shown in figs. 53 D, E and 58. It is possible that during prolonged photostimulation such an adaptation as is here postulated may normally occur, for it is striking that the envelope of the contractions recorded is very similar to the growth of a slow contraction. This would further offer an explanation of the heightened tone often seen following prolonged stimulation, (fig. 51 A and B): the implication is that the adaptation of the GI cyton has resulted in a lowering of the level of background inhibition so that inherent activity of the S cyton is now greater. This effect must be contrasted with the long period depression of the quick response to light which is shown in fig. 45. Here it is possible that we are dealing with the need for resynthesis of photosensitive material as there are some indications that the effect is more marked after long light trains than brief ones.

Lastly, during the relaxation process some preparations

show a step like fall in tension or an oscillation imposed upon a falling tone (fig. 50). The genesis of this is uncertain, but it is possible that it represents a low level "off discharge" by the I cyton.

The present hypothesis has the interpretative advantage of a considerable number of degrees of freedom, the disadvantage of offering only the greatest difficulty in decisive experiment. However, two further modes of stimulation have been examined, namely responses to D.C. currents and to various drugs, and it is at least possible to see whether the present hypothesis conflicts with these observations.

4. Responses to Direct Current Stimulation.

While it was clear that little could be learnt about the properties of the preparation using pulsed electrical stimulation, it was considered of interest to study the effect of direct current (D.C.) stimulation as this has been shown to have striking, though complex, effects upon central nervous structures.

Using the circuit described earlier D.C. was applied to the preparation using wick electrodes. One electrode rested on the extreme auricular end, the other on the extreme pyramidal end of the muscle. To reduce short circuit effects, the sea water around the preparation was usually drained away before stimulation.

The response shown to D.C. stimulation is characterised by a rapid upswing followed by a slow change in tone and then, frequently, a further sharp but transient rise in tension on breaking the circuit. The initial rise in tension may sometimes show a distinct make response. The form of the response depends upon the polarity of the electrodes. In general a make shock occurs only when the cathode is on the pyramid while the break shock is greater when the anode rather than the cathode is on the pyramid (fig. 59). This observation suggests that make and break responses arise from some structure near the pyramidal end of the muscle. The rate of tension development during maintained D.C. stimulation is characteristic of the slow response to light stimulation.

It is likely that D.C. stimulation will act upon both nerve and muscle. It has been found that in this preparation adrenaline at a concentration of 1×10^{-6}M acts as an effective blocking agent. Addition of adrenaline at this concentration produces a complete block to light stimulation and the response to direct current stimulation is only a slight and relatively slow rise in tension. No make or break shocks are apparent (fig. 60).

More may be learnt from a study of preparations in which a tonic background is provided by light stimulation. At low D.C. stimulation intensities, changes of tone are produced without the confusion of make and break responses. One such result is shown in fig. 61. Here it is clear that with the cathode at the auricle the D.C. is producing a very slight increase in tone, while with the cathode in the pyramidal position there is a marked loss of tone. The simplest interpretation which can be offered is that the cathode is stimulating inhibitory centres within the admittedly hypothetical motor complex.

With more intense stimulation the picture is confused by make and break responses while the effective intensity for any stimulation is not known. The results shown in fig. 61 were obtained with the preparation under sea water and at a high intensity of stimulation. Nevertheless the effective intensity is low owing to the short-circuit offered by the sea water. When the preparation was stimulated in air, it was found that the details of a response were markedly affected by the precise position of the electrodes which were easily disturbed when draining and flooding the preparation. Further, the extent of residual sea water almost certainly varied from one exposure of the preparation to the next. Both these factors make clear statements about stimulation intensity impossible.

With this limitation in mind results from other preparations may be studied. Considering first responses with the anode on the pyramid, fig. 62 A broadly confirms the previous result. There are clear make and break responses and between these the tension in the muscle is somewhat increased. The tension development returns

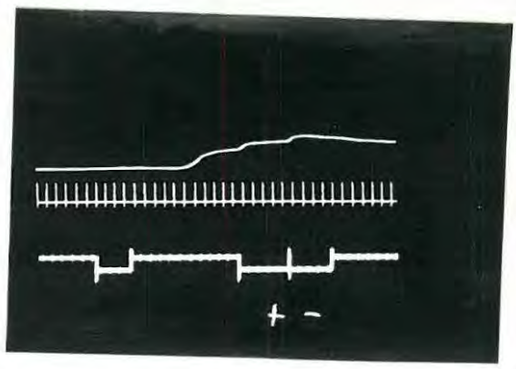


Fig. 60. Parechinus angulosus. Responses of retractor muscle preparation in the presence of 1×10^{-6} M adrenaline. At the first signal the preparation was stimulated by light, at the second by D.C. Note that the increase in tension at the arrow and before the application of D.C. stimulation is an artifact due to draining sea water away from around the preparation. Time mark: 5 seconds.

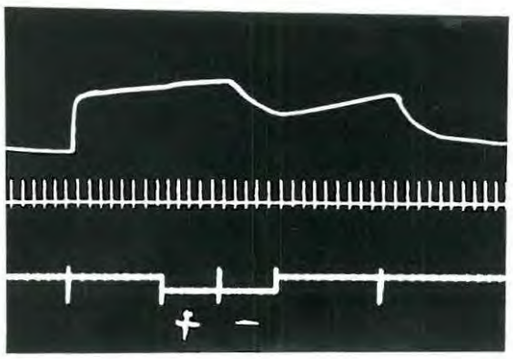


Fig. 61. Parechinus angulosus. Responses of a retractor muscle preparation to weak D.C. stimulation achieved by leaving the preparation in sea water during stimulation. Time mark: 5 seconds.

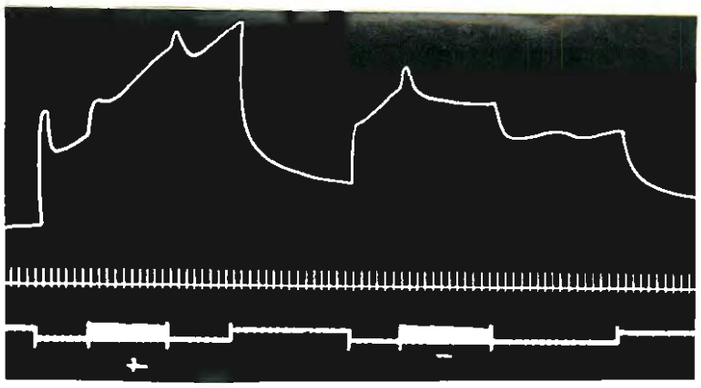


Fig. 62. Parechinus angulosus. Response of a retractor muscle preparation to D.C. stimulation with a background of light stimulation. In both responses the first signal indicates the onset of illumination, the white block the duration of D.C. stimulation and the final signal the end of light stimulation. Time mark: 5 seconds.

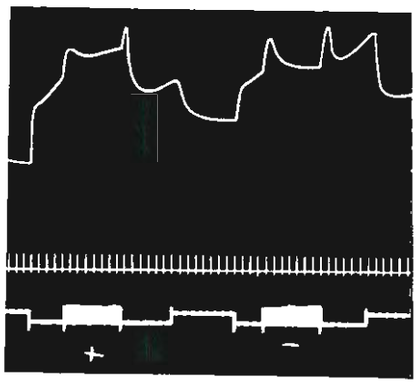


Fig. 63. Parechinus angulosus. As Fig. 62. Further responses from the same preparation. Time mark: 5 seconds.

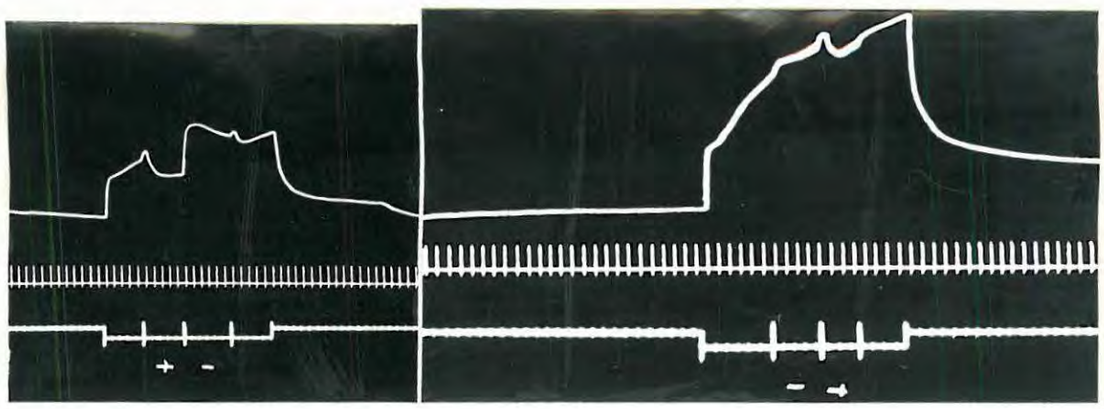
to normal at the end of D.C. stimulation. Fig. 63 A shows an apparent fall in the rate of tension development after the make twitch, while fig. 64 A shows a clearly marked fall in tension; the same effect but less distinct may be present in fig. 64 B. Further, there is a striking fall in tension following the break response in fig. 63 A. Thus the nature of the response to anodic stimulation of the pyramid is variable.

The same effect, in the reverse sense, is to be seen with cathodic stimulation. In fig. 62 B, after the make response there is a fall in tension and no subsequent recovery, in fig. 83 B a fall in tension but, after the well marked break response a rise in tension. In fig. 64B there is at most a slowing of the rate of tension development while in fig. 64 A a suggestion of a transient rise in tension, but the picture is confused by the response when polarity is reversed.

Figs. 65 and 66 show the results of the opposite type of experiment in which a light stimulus is imposed upon a background of D.C. stimulation. In fig. 65, with the pyramid anodic, the additional light response is a typical fast contraction followed by a slow. Fig. 66 is more complex. After an initial make response, the rate of tension development is high. The light stimulus now produces a quick twitch which rapidly subsides and the rate of tension development is very low indeed. On switching off the light there is a marked rebound and the subsequent rate of tension development is closely similar to the original value.

Before proceeding to an analysis of the present data it is of interest to consider the results of similar experiments by Hughes (1952) upon cockroach thoracic ganglia. Direct currents affect the flexor-extensor muscle balance of the legs causing them either to bend or stretch. The result of stimulation depends on the intensity of the stimulus as well as the direction of the applied current. Hughes shows that the site of action of the D.C. lies in the ganglion and not in the motor axons.

The responses recorded from Parechinus similarly show a



A B

Fig. 64. Parechinus angulosus. As Fig. 62. Responses from a different preparation and with abrupt change of polarity of stimulus. Time mark: 5 seconds.

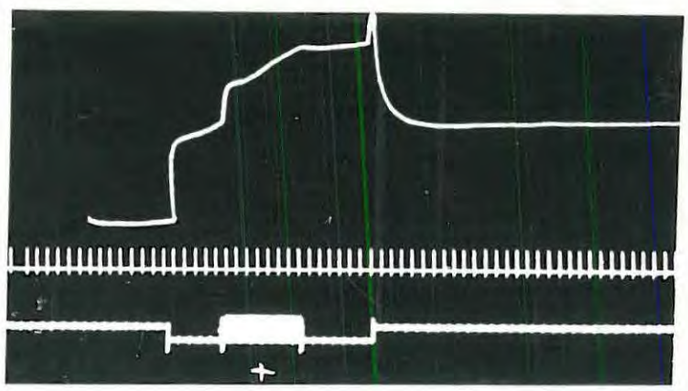


Fig. 65. Parechinus angulosus. Response of a retractor muscle preparation to a light stimulus applied against a background of D.C. stimulation, the pyramidal end of the muscle being anodic. At the first signal D.C. stimulation is applied, the white block indicates the duration of light stimulation and the final signal the end of D.C. stimulation. Time mark: 5 seconds.

difference with the direction of current flow, while there is some evidence that the same pattern of stimulation may produce different effects: thus anodic stimulation of the pyramid may cause either a rise or a fall in tone. Whether this is due to different intensities of stimulation is uncertain.

Hughes attempts an explanation of these effects on the assumption that the action of D.C. is at the synapses and that synaptic transmission at inter-nuncials depends upon electrotonic potentials rather than chemical transmitters. In this way he is free to construct an hypothesis in which the intensity and direction of direct current flow will modify the electrotonic level of the central synapses. Hughes' basic assumption stems from the work of Brooks and Eccles (1947) on the Golgi cells of the vertebrate spinal cord and on recordings of electrotonic potentials in the ocellar nerve of Locusta by Parry (1947). Unfortunately neither of these have stood the test of time. Brock, Coombs and Eccles (1952) have shown that the original suggestions about the action of the Golgi cells is untenable, while Parry's observations have not been confirmed, more recent work (Hoyle 1955, Ruck 1961) showing the ocellar nerve to function like any other. Indeed the site of action of D.C. stimulation upon ganglionic structures is yet unresolved, but it is clear that its action depends upon the presence of internuncial neurones. It is therefore reasonable to assume that a primary action of D.C. upon the preparation studied here will be upon the cells of the motor complex, but the possible action of the anode in producing an axonal block must also be remembered.

In attempting an interpretation in terms of the hypothesis we have developed from an analysis of responses to photostimulation, it will be assumed that the action of D.C. stimulation is two-fold. At make and break of the D.C. stimulus there will be an abrupt change of electrotonus which will be stimulatory to axonal structures, while during the maintained stimulus there will be an electrotonic focus which may affect the soma of different cells within the motor complex to different extents.

We have already seen that make and break responses are more easily elicited from cathodic stimulation of the pyramidal end of the muscle and it seems likely that the contractions are twitch responses obtained by stimulation of the motor axons running from the motor complex to the muscle fibres. The experiments using adrenaline eliminate the possibility of these responses being due to direct stimulation of the muscle cells themselves.

Low intensity cathodic stimulation causes a fall in tone from which, in figs. 61 and 63, there is a rapid recovery. This is most easily interpreted as due to catelectrotonic stimulation of the GI cyton. The effect is seen also in fig. 66 where, against a background of cathodic stimulation, photostimulation causes a quick response followed by a fall in tone and a marked post-stimulatory rebound. This, though here exaggerated, is what we have already seen in fig. 53 C. At what may be possibly higher intensities of cathodic stimulation there is slight evidence for an increase in tone; this could be due to electrotonic stimulation of the S cytons.

With low intensity anodic stimulation (figs. 61, 62) there is an increase in tone which can be attributed to electrotonic block of the GI cytons. Again recovery is rapid. With what is possibly more intense stimulation the response is reversed and anodic stimulation causes a fall in tone. Such an effect can be attributed to an electrotonic block of the S cytons.

It is thus possible to accomodate most of the present results within the hypothesis already elaborated if we assume that the GI cytons have a lower electrotonic threshold than the S cytons. Weak anodic stimulation will depress the output of the GI cyton leading to greater activity by the S cyton, strong anodic stimulation will partially block the S cyton. Weak cathodic stimulation will excite the GI cyton leading to a partial inhibition, strong cathodic stimulation possibly excites the S cyton.

But there are clearly further effects of D.C. stimulation expressed in the response of the preparation of the end of stimulation. Thus in figs. 62 and 63 the break shock is followed by a

marked fall in tone from which, in fig. 62, there is no recovery. Although these effects can be accomodated within the bounds of the present hypothesis, their interpretation is too arbitary to merit elaboration.

5. Responses to Drugs.

The action of a limited number of pharmacological agents was tested upon the lantern retractor preparation, particular attention being directed to acetylcholine and its antagonists as this drug has been shown to be active upon a wide variety of echinoderm muscle preparations (Ambache and Sawaya, 1953; Bacq 1935, 1939; du Buy, 1936 a; Florey and McLennan, 1959; Pople, W. (unpublished); Prosser and Judson, 1952; Sawaya, 1951; Wyman and Lutz, 1930) including the lantern retractor muscle of Echinus esculentus (Bacq, 1937).

Acetylcholine (Chloride, B.D.H.). As Bacq has reported, the preparation is very sensitive to the action of acetylcholine. At a concentration of 1×10^{-5}M there is almost invariably a powerful contraction, the tension developed being held almost unchanged for at least 80 minutes (fig. 67). At 1×10^{-6}M the magnitude of the response is variable from one preparation to another. If a preparation is already rhythmically active there may be an increase in background tone with no significant alteration in the pattern of the spontaneous activity (fig. 68). If a preparation is treated with the drug at a concentration less than that which will produce a maximal contraction, the increase in tension is invariably accompanied by rhythmic activity regardless of whether the preparation was initially active or not (fig. 69).

The response of a preparation to a light pulse is unmodified by the drug provided the concentration applied is not sufficient to cause a maximal contraction (fig. 69).

Eserine. (B.D.H.). This drug acts as an anti-cholinesterase and would therefore be expected to enhance the action of acetylcholine. Bacq (1937) found it to lower the threshold of the lantern retractor

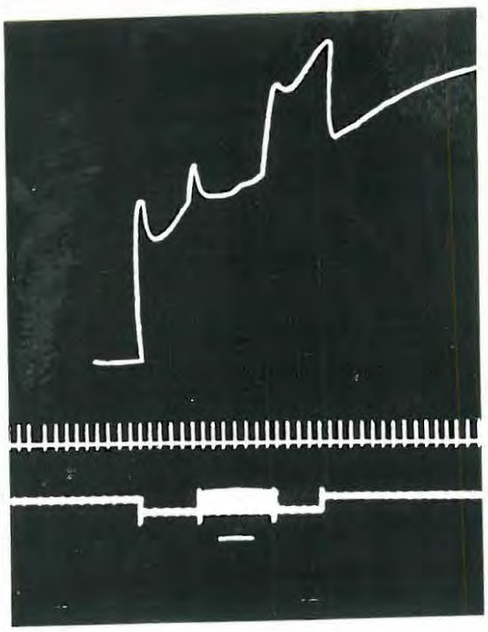


Fig. 66. Parechinus angulosus. As Fig. 65, but with cathodic stimulation at the pyramidal end of the muscle. Time mark: 5 seconds.

Fig. 67. Parechinus angulosus. Response of a retractor muscle preparation to acetylcholine at 1 x 10^{-5}M. Drug added at first signal and washed out during time between two final signals. Time mark: 30 seconds.

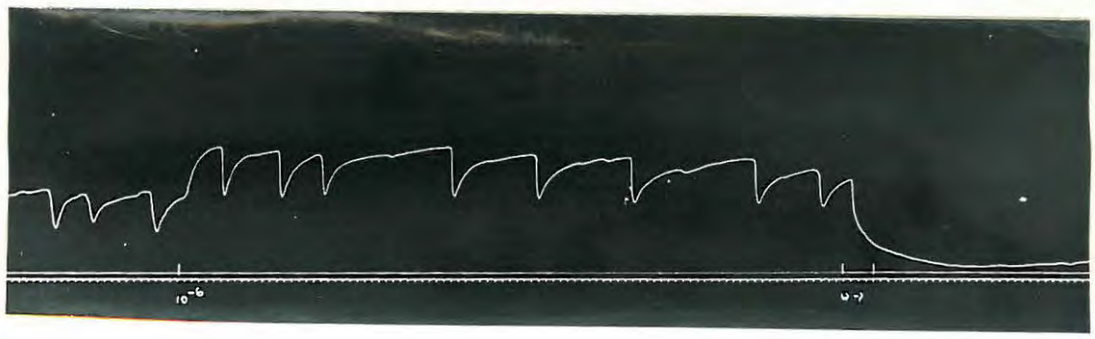


Fig. 68. Parechinus angulosus. Response of a spontaneously active preparation to acetylcholine at 1 x 10^{-6}M. Signals as in Fig. 67. Time mark: 30 seconds.

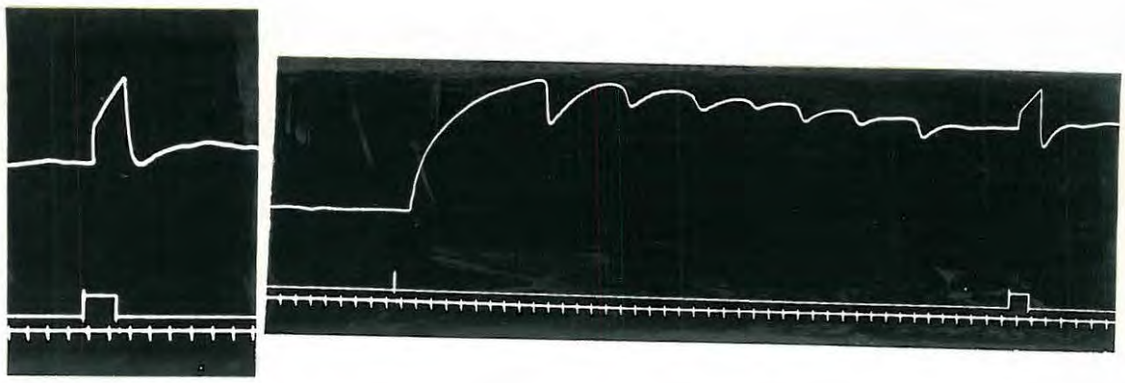


Fig. 69. Parechinus angulosus. Response of an inactive preparation to acetylcholine at 1 x 10^{-6}M. The first trace shows the response of the untreated preparation to a light pulse for comparison with the response after treatment shown by the signal block. Time mark: 30 seconds.

Fig. 70. Parechinus angulosus. Response of a retractor muscle preparation to eserine at a concentration of 1 x 10^{-6}M. The drug was added at the first signal, the preparation was washed at the first pair of signals and again at the second. Time mark: 30 seconds.

muscle to acetylcholine from 10^{-6} to 10^{-9}.* When applied to a Parechinus preparation showing slight spontaneous activity, there is a slow gain in tension followed by the development of rapid rhythmic activity (fig. 70). On washing out the drug, the tone rises further and the spontaneous activity, although falling in frequency, becomes greater in amplitude, but eventually, after about two hours, it dies away.

Atropine (sulphate. Merck). The mode of action of atropine as an antagonist of acetylcholine is probably that of competitive inhibition. In mammals it is only effective in those regions of the autonomic system which are stimulated by muscarine: nicotine type responses are not abolished by atropine. With the present preparation atropine at 1×10^{-6}M appears to have no effect upon the response of the preparation to acetylcholine, nor does it modify the response of the preparation to light pulses.

Nicotine. In keeping with the previous observation, nicotine is stimulatory. At 5×10^{-7}M it stimulates a preparation to spontaneous activity (fig. 71) and at higher concentrations causes a sharp increase in tone. It does not modify the response of the preparation to light pulses.

Curare. (Tubarine. Burroughs Wellcome). The preparation is very sensitive to curare, a dosage of as little as 1×10^{-6}M enhances the tone and depth of activity of a spontaneously active preparation without affecting the frequency of the contractions (fig. 72). The action of the drug ceases when the preparation is washed clean. With inactive preparations addition of the drug may stimulate the preparation to action. This is, however, not invariable; some preparations are not activated by the drug. The response of the preparation both to brief and long light pulses is slowly depressed by curare, the action becoming more effective with time.

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* Unfortunately Bacq never makes clear in any of his numerous publications the precise unit in which his dilutions are stated.

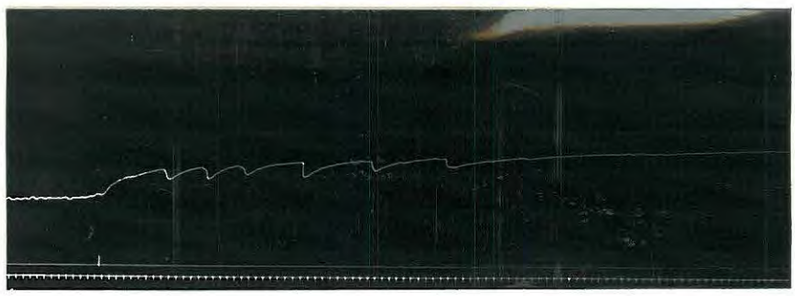

Fig. 71. Parechinus angulosus. Response of a retractor muscle preparation to nicotine at 5 x 10^{-7}M. Time mark: 30 seconds.

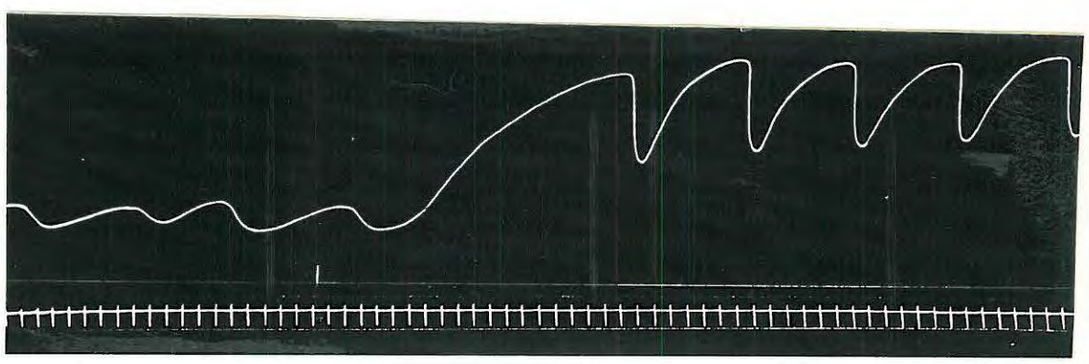

Fig. 72. Parechinus angulosus. Response of an active preparation to curare at 1 x 10^{-6}M. Time mark: 10 seconds.

Adrenaline. (tartarate. B.D.H.). The action of adrenaline is depressant. Thus Fig. 73 A illustrates a recording from a preparation of high tone and slight irregular spontaneous activity. Addition of adrenaline at 1 x 10^{-5}M causes a rapid loss of tone and, as has already been described, a loss of response to light stimulation, although a small on-response which lasts about 10 sec. may be shown while the drug is establishing its action (fig. 73 B).

dl-Nor-adrenaline. (Light). This drug has the same action as adrenaline, but is effective at far lower dosages; a spontaneously active preparation was inhibited both in tone and activity at 1 x 10^{-9}M, a concentration at which adrenaline is completely ineffective (fig. 74).

γ - amino-butyric acid. This material, whose inhibitory action has been demonstrated on a variety of preparations, caused at 1 x 10^{-6}M a slight depression of the response to light pulses. The effect is more strongly marked at 1 x 10^{-5}M.

5-hydroxy-tryptamine. (creatine sulphate. Light). This drug is without action upon the preparation.

Interaction between acetylcholine and adrenaline. These drugs, having opposite actions, were tested against each other. They were found to be antagonistic. Thus if a preparation is brought to a contracture by treatment with 1 x 10^{-6}M acetylcholine, subsequent treatment with adrenaline at the same concentration will result in a gradual lowering of tension achieved as a series of fairly abrupt falls, each resembling the relaxation of a pattern of spontaneous activity. After each fall in tension, the new level would be maintained steady (fig. 75 A). With a higher concentration of adrenaline (1 x 10^{-5}M) there follows the typical spontaneous activity pattern of a slow rise in tension followed by a more abrupt fall (fig. 75 B). The same effect is observed if the preparation is first treated with adrenaline and subsequently with acetylcholine.

Interaction between acetylcholine and curare. Although curare stimulates the present preparation to activity, it acts,

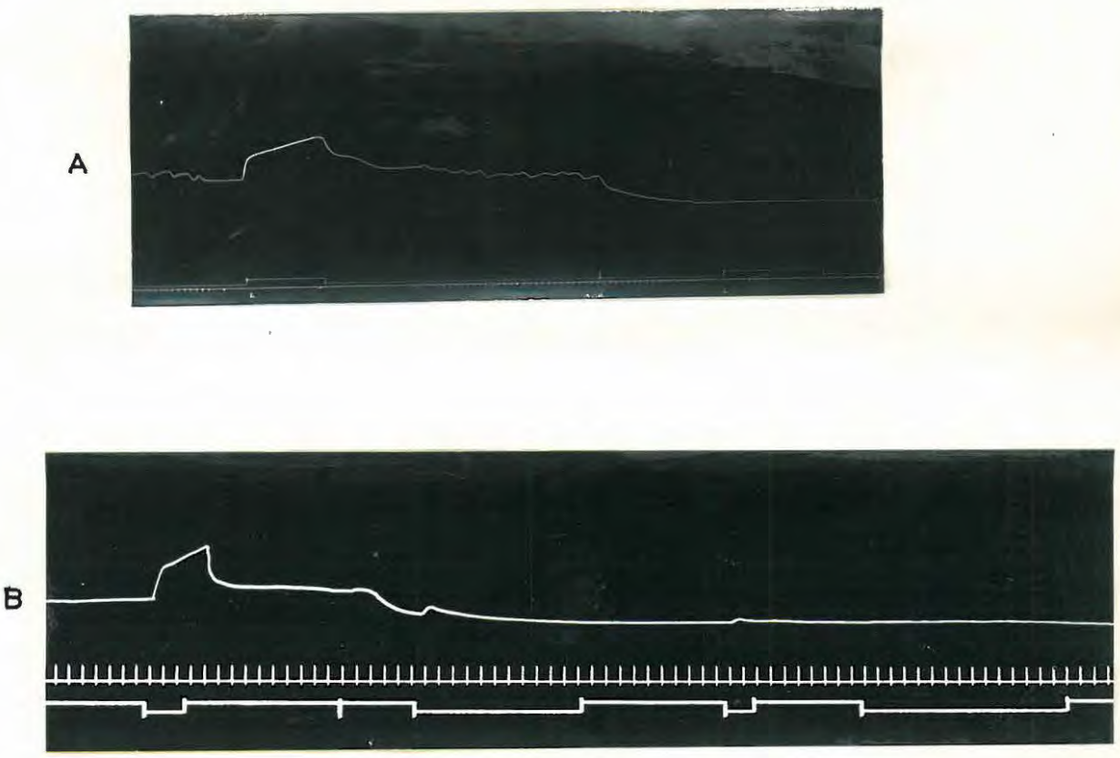

Fig. 73. Parechinus angulosus. Responses of retractor muscle preparations to adrenaline.
A. Response of an active preparation to a concentration of 1 x 10^{-5}M. Two light stimuli, indicated by the signal, were given, one before and one after the addition of the drug. Time mark: 30 seconds.
B. Light responses of a preparation before and shortly after treatment with a concentration of 1 x 10^{-6}M. Time mark : 10 seconds.

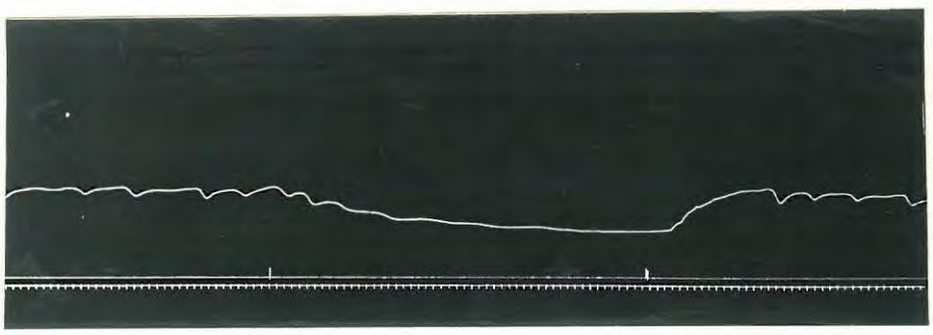

Fig. 74. Parechinus angulosus. Response of a retractor muscle preparation to nor-adrenaline at a concentration of 1 x 10^{-9}M. The drug was washed out at the second signal. Time mark : 30 seconds.

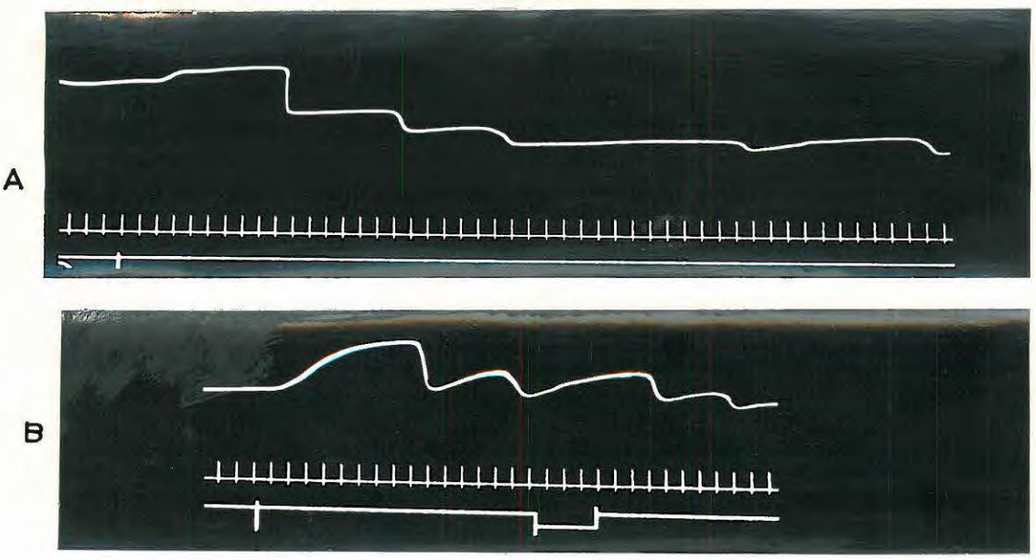

Fig. 75. Parechinus angulosus. Response of a retractor muscle preparation to adrenaline during treatment with acetylcholine at a concentration of 1 x 10^{-6}M. The adrenaline was added by syringe to the bath to obtain the required concentration in the presence of the acetylcholine.
A. Activity following addition of adrenaline to produce a concentration of 1 x 10^{-6}M.
B. Activity after a further addition of adrenaline to produce a concentration of 1 x 10^{-5}M.
Time mark : 10 seconds.

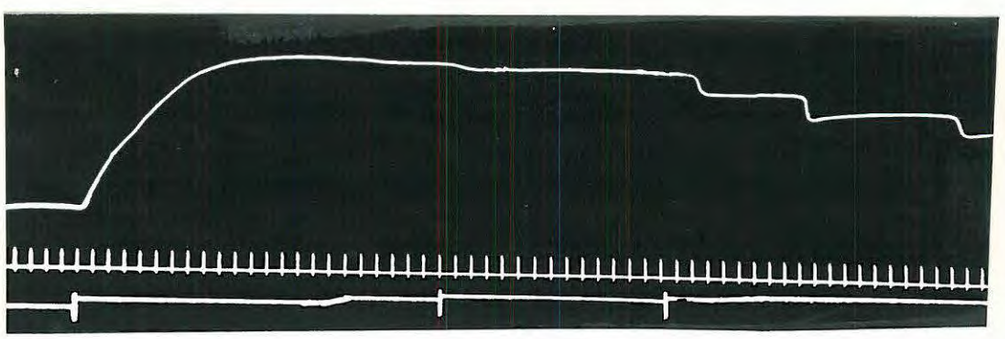

Fig. 76. Parechinus angulosus. Response of a retractor muscle preparation to curare during treatment with acetylcholine. At A acetylcholine to produce a concentration of 1 x 10^{-6}M was added to the bath; at B curare to produce a concentration of 1 x 10^{-6}M and at C curare to produce a concentration of 1 x 10^{-5}M. Time mark : 10 seconds.

in mammalian preparations, as an antagonist to acetylcholine, probably by competitive inhibition. In the lantern retractor muscle preparation curare also antagonises the action of acetylcholine. Thus a preparation brought to contracture by 1×10^{-6}M acetylcholine, while almost unaffected by subsequent treatment with curare at 1×10^{-6}M, starts to make spontaneous contractions when the curare concentration is increased to 1×10^{-5}M (fig. 76). These are sometimes abnormally rapid.

There is a general opinion that the neuro-muscular transmitter to the body musculature of the Echinodermata is cholinergic. Part of the evidence for this view is summarised in the data presented in Table V. It will be seen that in all cases examined these muscles are highly sensitive to acetylcholine and that in the majority of cases this action is potentiated by eserine. Such a view would, on this evidence alone, be acceptable for Parechinus. However it would also be expected that, in this circumstance, curare should act as a blocking agent. In so far as it has been examined the action of curare on echinoderm muscle preparations is highly variable. Thus Wyman and Lutz (1930) report that it inhibits spontaneous activity but does not lower the tone of the cloacal muscles of Cucumaria frondosa, while du Buy (1936) states that, at an unspecified concentration, curare blocked the response of the pharyngeal retractor muscle of Thyone briarius to indirect electrical stimulation, but did not influence the direct action of acetylcholine upon the muscle. In Parechinus, as we have already seen, curare, although it can antagonise the action of acetylcholine, can also enhance the activity of a preparation (fig. 72). Furthermore, in studying the action of eserine upon the longitudinal muscle of Holothuria tubulosa, Bacq (1935) found that this drug not only potentiated the action of acetylcholine, but also that of histamine and tyramine. He emphasises that the action of histamine is distinct from that of acetylcholine and that the potentiating effect of eserine cannot therefore be attributed to a secondary release of acetylcholine.

T A B L E V

Actions of Drugs upon Preparations of Edimoderm Muscles According to Various Authorities.

Organism	Preparation	Experiment	Acetylcholine	Curare	ACh + curare Antagonism	Eserine	ACh + eserine	Atropine	Nicotine	Adrenaline	Authority
Parechinus angulosus	Lantern retractor	Direct action of drugs	Contracture 1×10^{-5}M	Enhanced activity 1×10^{-6}M		Enhanced activity 1×10^{-6}M		No effect 1×10^{-4}M	Enhanced activity 5×10^{-5}M	Depresses activity 1×10^{-5}M	Present thesis
Echinus esculentus	Lantern retractor	Direct action of drugs	Contracture 1×10^{-5}				Contracture 1×10^{-9}			?Contraction	Bacq, 1937
Holothuria tubulosa	Longitudinal muscle	Direct action of drugs	Contracture 1×10^{-10}				Sensitises			Contraction	Bacq, 1935
Holothuria stellata	Longitudinal muscle	Effect of drug response to on elec. stim.				Potentiates		Blocks			Riesser, 1933
Stichopus regalis	Longitudinal muscle	Effect of drug on response to elec. stim.				Sometimes depresses					Riesser, 1933
Stichopus regalis	Longitudinal muscle	Direct action of drug	Contracture 1×10^{-7}		Antagonises 1×10^{-4}		Sensitises 1×10^{-9}		Contraction 1×10^{-7}	No effect 1×10^{-3}	Bacq, 1939
Stichopus californicus	Longitudinal muscle	Direct action of drugs	Contracture 1×10^{-6}			Enhances tone and activity 4×10^{-6}		Decreases tone 5×10^{-5}	Enhances tone 5×10^{-7}	Enhances tone and activity 1×10^{-5}	Iriye and Dill 1940
Cucumaria frondosa	Cloacal muscle	Direct action of drugs		Inhibited activity but not tone 5×10^{-5}		Enhances tone and activity 5×10^{-5}		No clear effect 1×10^{-3}	Enhances tone decreases activity 3×10^{-5}	Depresses activity 1×10^{-5}	Wyman and Lutz, 1930
Thyone briarens	Pharyngeal retractor	Various	Contracture 1×10^{-9}	Blocks response indirect to elec. stim. but not to ACh.						Relaxes muscle 1×10^{-4}	du Buy, 1936

There is therefore reason to believe that the action of both curare and eserine upon echinoderm preparations is not fully comparable to their action on mammalian preparations. This must be borne in mind in considering the interpretation of the effects of drugs upon the lantern retractor muscle preparation. Such an interpretation, in terms of the hypothesis already developed, is attempted below.

It is suggested that the action of both adrenaline and of nor-adrenaline is a generalised hyperpolarisation of the muscle membrane so as to block all nervous impulses. Such a hyperpolarising action of adrenaline upon smooth muscle is known from the work of Bülbring (1957) upon the taenia coli of the guinea pig. Similarly it seems likely that the powerful contracture produced by acetylcholine is due to a generalised depolarisation of the muscle membrane. Again a parallel may be drawn with the depolarizing action of this drug upon the taenia coli (Bülbring, 1957). On this view the antagonistic actions of adrenaline and acetylcholine lie at the level of the muscle membrane and a balance may be struck between the activity of the two drugs so as to permit the muscle to display spontaneous activity when both are present.

It is next desirable to consider the effect of eserine. This drug becomes effective only gradually and cannot readily be washed out from the preparation. When an attempt is made to wash it out the behaviour of the preparation changes (fig. 70), suggesting that it is more firmly attached to certain acceptors than others. After first application the preparation displays rhythmic activity which is far more rapid than that normally shown by these preparations. In terms of our present hypothesis this must be attributed to more frequent activity of the I cyton and suggests that the drug is acting at the synapse between the recurrent branch of the S cyton and the I cyton. Frequent discharge of the I cyton not only produces a rapid rhythmic activity, but prevents the full development of the tone which

can be achieved in the presence of eserine. This, which depends upon a blockade of the cholinesterase at the myoneural junctions, is not displayed until the preparation has been washed and the I cyton is no longer abnormally active.

This interpretation meets with an immediate difficulty. In assigning to eserine a stimulatory role on the I cyton, we imply that this junction is cholinergic and it would therefore be expected that acetylcholine should also increase the frequency of rhythmical contractions by its stimulatory effect on the I cyton. This is not in fact the case (fig. 68). However it has already been emphasised that in echinoderm preparations eserine is known to have non-specific actions as far as cholinesterase is concerned and it is concluded that its stimulatory action on the I cyton is such a case.

It is next neccessary to consider the action of curare. Here it must be emphasised that the potency of drugs with a curare-like action varies greatly from species to species among the mammalia. Thus for example, considering the responses of tibialis anticus, the cat is far less sensitive to curare than the rat, while with decamethonium the cat is vastly more sensitive than the rat. Clearly the precise character of the acceptor site for these drugs varies from species to species. Furthermore there is good evidence that decamethonium may effect a block in two distinct ways. It may act both as a competitive inhibitor and as a depolarizing agent. Again the relative importance of these two actions varies with species. Where the depolarizing action of the drug is dominant, it will act transiently as a potentiating agent until its depolarizing action dominates (Zaimis, 1953).

Chemically curare and decamethonium have much in common and it is therefore possible to visualise a situation in which curare also displays two modes of action which may further be independent in their expression if there should occur two very distinct types of acceptor protein. It has been shown that

curare does indeed antagonise the action of acetylycholine. This action can be attributed to competitive inhibition at the muscle membranes, allowing rhythmic activity of the preparation to be displayed in the presence of acetylcholine (fig. 76). This peripheral action of curare is normally masked by its excitatory effect when the drug is added alone (fig. 72). If, however, the concentration of curare is increased sufficiently it will act as an inhibitor of the rhythmic activity which it stimulates at lower concentrations (fig. 77).

The activating effect of curare upon the preparation may be attributed to its second action, that of a depolarizing agent. It is suggested that this depolarizing action of curare is upon the S cyton. Here normally the action of the GI cyton is inhibitory and hyperpolarizing. This hyperpolarization is antagonised by curare so that the S cyton becomes more active. Alternatively the action of curare can here also be regarded as one of competition with the transmitter substance released by the G.I. cyton. But in either case the transmitter substance cannot be acetylcholine as rhythmical activity, which has been attributed in part to the action of the S cyton, continues in the presence of acetylcholine. Were the transmitter from the G.I. cyton acetylcholine we would expect an inhibition of such activity.

In this way it is possible to explain the action of drugs related to cholinergic systems upon the preparation. The actions of atropine and nicotine are partly explicable on the assumption that the myoneural junction is nicotinic in character like vertebrate striated muscle, but it is probable that nicotine acts also by a direct stimulation of the cells of the motor complex in the same fashion as it acts upon the cells of sympathetic and parasympathetic ganglia. The action of G.A.B.A. is probable one of antagonism to cholinergic transmission; certainly it has such a role in Strongylocentrotus (Florey and McLennan 1959).

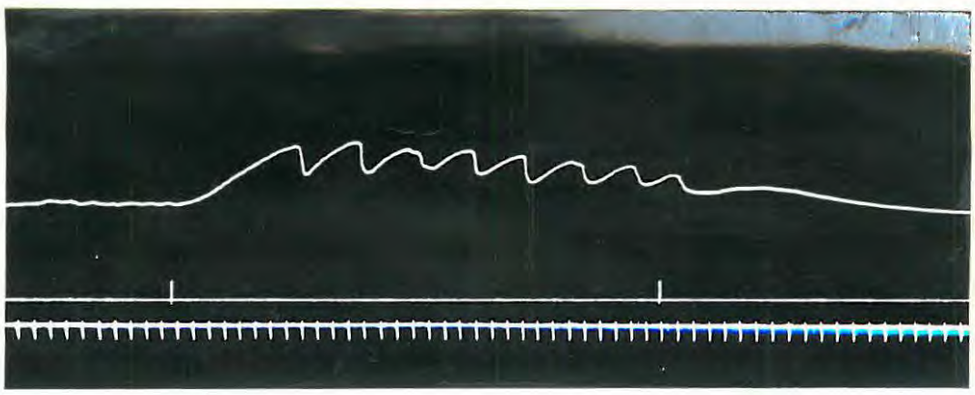

Fig. 77. Parechinus angulosus. Response of a retractor muscle preparation to curare. At the first signal curare was added to a concentration of 1 x 10^{-5}M and at the second sufficient to increase the concentration to 1 x 10^{-4}M. Time mark : 30 seconds.

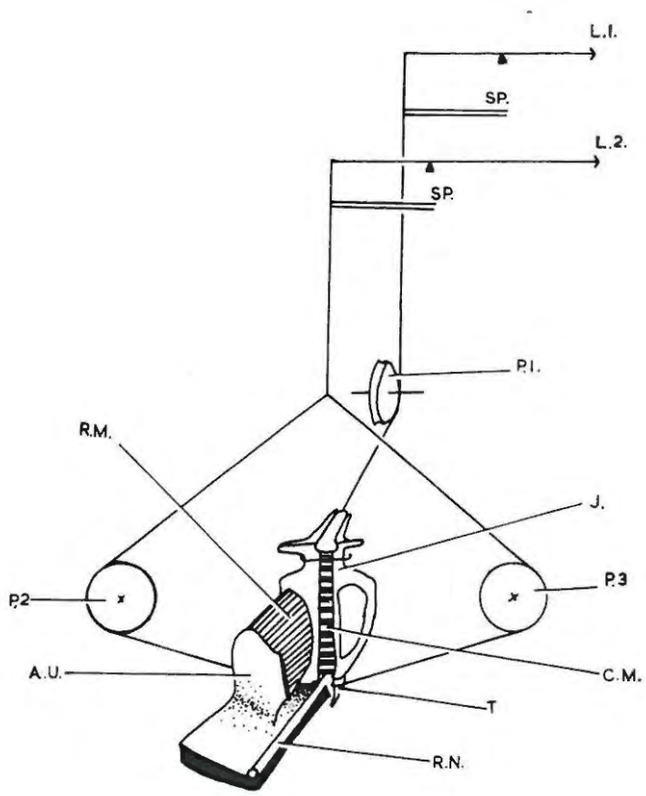

Fig. 78. Experimental arrangement for studying the activity of the retractor and comminator muscles at the same time. The retractor muscle of one side has been omitted to show the attachment of a recording thread to a tooth. A.U. auricle; C.M. comminator muscle; J. jaw; L 1 & 2. recording levers; P 1, 2 & 3 pulleys; R.M. retractor muscle; R.N. radial nerve; SP. spring; T. tooth.

While it has been possible to accomodate the results obtained from the study of drug action within the compass of the hypothesis first elaborated, it has been neccessary to urge special considerations to explain the action of both eserine and curare. This suggests that the initial assumption of the existance of a motor complex may be incorrect. Such a structure has not been demonstrated histologically and the possibility has to be considered that the spontaneous activity, the starting point for the elaboration of subsequent ideas, may be myogenic, not neurogenic, in origin.

6. An alternative hypothesis.

There are two structures which display myogenic activity and which have been the subject of close study during recent years, namely the rabbit heart and the taenia coli muscle of the guinea pig. They differ in their characteristic responses to acetylcholine and adrenaline. To the heart adrenaline is stimulatory, to the taenia coli inhibitory; acetylcholine is typically inhibitory to the heart, but stimulates the activity of the taenia coli. Both these latter actions can, however, in certain conditions, be reversed.

In considering the mechanism of the myogenic heart Burn, (Burn and Vane, 1949; Bülbring and Burn, 1949; Burn and Kottegoda, 1953) has produced evidence which suggests that the rhythm is determined by a cyclical synthesis and breakdown of acetylcholine. The further details of this mechanism have yet to be elucidated, but since it is possible to demonstrate acetylcholine in the perfusate from a isolated rabbit heart (Briscoe and Burn, 1954), it would appear that this material is liberated from the muscle cells during the course of their activity. In studies upon the taenia coli Bülbring (1957) has shown that the tension developed by the muscle during its spontaneous myogenic activity is correlated both with the frequency of development of small spikes and the level of polarisation of the muscle membrane. Bülbring compares the sponteneous activity of the taenia coli with that of a

spontaneously active receptor cell. This analogy is, however, of little immediate value since the mechanism of generation of spontaneous activity in receptor units is unknown.

In considering a possible myogenic interpreptation of the results obtained with Parechinus, ideas suggested by both of these studies have been used. As an initial postulate it is suggested that within the muscle cells lies a system synthesising acetylcholine and that this is transported outwards to specialised receptor sites where it produces a local depolorisation of the muscle membrane. For clarity in subsequent discussion these specialised receptor sites, which are considered to control rhythmical activity and not background tone, will be called "rhythm sites." As more acetylcholine is liberated or possibly as it is liberated more rapidly the area of depolarisation around the rhythm sites spreads and the tonus developed by the muscle increases. But at a certain level of depolarisation the membrane at the rhythm sites becomes permeable to a hyperpolarising agent which resets the electrotonic level of the membrane, and the muscle relaxes. This hyperpolarisation renders the rhythm site membrane impermeable to the hyperpolarising agent which is now fairly rapidly destroyed and depolarisation by acetylcholine around the rhythm sites starts again. The endogenous acetylcholine and hyperpolarising agents are the counterparts of the S and I cytons of the previous hypothesis. Such a hypothestical mechanism would provide the basis for a rhythmic activity but this normally occurs against a basic tone which may be regarded as a reflection of a slow general liberation of acetylcholine distant from the rhythm sites. The action of exogenous acetylcholine is then to effect a general depolarisation of the muscle and, except at low dosages (fig. 68), this conceals the inherent activity of the muscle. Curare added to a muscle brought to full contracture by acetylcholine antagonises this generalised depolarisation and the inherent activity of the muscle may once more be seen.

A rather similar interpretation can be offered of the

activity of curare alone. Partial blockage of the general receptor sites leads to a greater liberation of acetylcholine which causes a general rise in tone, while at the rhythm sites partial blockage by curare causes the formation of acetylcholine to continue until there is a level of depolarisation adequate to permit the release of the hyperpolarising agent and a consequent fall in tension. All that has been achieved is a new level of activity.

With eserine the position is different. At the rhythm sites the blockage of cholinesterase causes a more rapid build up of acetylcholine and a more rapid depolarisation so that the hyperpolarising agent is liberated sooner and the rhythm of activity established is more rapid. The eserine will also act generally so that the tone of the muscle will rise. On washing, the eserine is more readily removed from the rhythm sites, the rate of liberation of hyperpolarising agent now falls and the tone of the muscle rises still more.

Adrenaline presumably acts, as on the taenia coli, by causing a generalised hyperpolarisation which can be antagonised by acetylcholine both generally and at the rhythm sites to allow rhythmic activity to be established.

It is now necessary to see whether the effects of light stimulation may also be explained in terms of a myogenic hypothesis. Any effect of light must be attributed to the presence of photosensitive substances which, in the first hypothesis, were located in the neurones of the motor complex and here must lie in the muscle itself.

We have seen that white light contains two elements, one of which is excitatory : this excitation we may attribute to a depolarising action of some photosensitive substance. The other effect is inhibitory and may be regarded as due to a hyperpolarising action of a second photosensitive material. This latter is particularly sensitive to some wavelengths in a mercury lamp and produces a general hyperpolarisation with an accompanying

the nerve ring beneath the comminator muscle when these were severed. One side of the preparation was screened from the other by a red filter and one muscle then stimulated by light. No response was obtained from the other muscle.

A further limited number of experiments were conducted in which all five pairs of lantern retractor muscles were recorded at the same time, using the technique already described (fig. 26). The muscles were excited to spontaneous activity by the addition of a low concentration of curare. Examination of the records shows that the muscles appear to contract independently and there is no evidence of coordination of their activity in these conditions.

Attempts to produce reflex retraction of the jaws were unsuccessful but during the course of these experiments one surprising result was obtained. A rapid jaw closing reflex may readily be shown if one of the pharyngeal lips between the jaws is touched. Whichever lip is stimulated all the jaws are brought together at once. It seemed reasonable to assume that this action is coordinated by way of the circumoral nerve ring. However if the ring is cut in two places on opposite sides of the animal and the preparation allowed to rest for a period of two hours or more, this coordinated closing of the jaw is still displayed if one pharyngeal lip is stimulated. This experiment makes it clear that some inter-radial reflex coordinatory pathways, other than the circumoral nerve ring, exist in the lantern. These possibly lie in the pharynx which is abundantly innervated. The probability is that stimulation of a pharyngeal lip causes impulses to be propagated over the whole pharyngeal network. Nerves from the pharynx carry this information by way of the circumoral nerve ring to the comminator muscles within the limits of a radius : as a result, section of the nerve ring does not destroy this coordinated snapping of the jaws.

Reciprocal inhibition between the longitudinal muscles of the body wall and the pharyngeal retractor muscle of Cucumaria

loss of tone (fig. 52).

A short light pulse produces an abnormally rapid depolarisation which may, in certain preparations, cause release of the hyperpolarising factor at the rhythm sites. This release occurs independently of any release of acetylcholine and so the hyperpolarising action is clearly displayed. It is this which can be seen in the post-stimulatory relaxation in fig. 49.

A long light pulse produces at first a rapid depolarisation whose effect may be partially antagonised by the release of hyperpolarising factor, but there follows a slowly increasing depolarisation and, after a critical level is achieved, release of hyperpolarising factor occurs as in normal spontaneous activity. On the cessation of illumination if this has been prolonged, the membrane rapidly repolarises to produce a sharp "off" relaxation (fig. 51).

It is thus possible to account for the effects of photostimulation by assuming the presence of only two photosensitive substances, one excitatory, one inhibitory. It is interesting to note in this connection that in Aplysia two photosensitive pigments have been shown to occur in certain neurones, one excitatory in action, the other inhibitory (Arvanitaki and Chalazonitis quoted by Kennedy, 1960).

The assumption that the lantern retractor muscle is myogenic offers the attraction of a certain economy of hypothesis, but this is insufficient to exclude the view that the activity of the muscle is myogenic in origin. As a final criterion the results of some preliminary experiments upon multiple muscle preparations may now be considered.

7. Coordination and interaction between muscles of the lantern.

If a coordinatory system exists it might be expected that factors affecting one lantern retractor muscle would influence others. This point was first investigated using a double muscle preparation. Two neighbouring retractor muscles from either side of a radius were dissected free, care being taken not to injure

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has been demonstrated by Pople and Ewer (1958). If the present preparation has similar characteristics to that of Cucumaria it should be possible to demonstrate such effects. The lantern retractor muscle may be considered as having two antagonists, the lantern protractors and the comminator muscles. The former are very slender, the latter more powerful but are short and deeply placed between the jaws.

In a series of experiments the lantern protractor muscle was freed at its pyramidal end from the lantern but left attached to the perignathic girdle. Stretches applied to the protractor muscle in no way modified the responses of the retractor muscle of the same sector.

The possible interactions between the comminator and retractor muscles were studied with the experimental arrangement shown in fig. 78. Using a double retractor muscle preparation, a thread was attached to the aboral pole of the jaws to provide a record of the movement of the retractor muscles. Two further threads were attached to the two teeth. These ran out laterally, and, passing across two pulleys, were joined to act on a single auxotonic lever. In this way the jaws were pulled apart by the tension of the lever and the movements of the comminator muscles could be recorded. The comminator muscle was stimulated by pulses of 10msec. duration at a frequency of 2/sec. The result of such an experiment is shown in fig. 79. It can be seen that stimulation of the comminator muscle results in a fall in tone of the retractor muscle after stimulation has ceased. The reverse experiment using electrical stimulation of the retractor muscle proved to be unsatisfactory, as the movement of the jaws by the retractor muscle acted back onto the lever recording from the comminator muscles. No evidence of loss of tone of the comminator muscles during stimulation of the retractor muscle was obtained, but such an event might have been obscured by the retractor artifact. The results do decisively demonstrate the presence of a reflex inhibitory action upon the lantern retractor muscle.

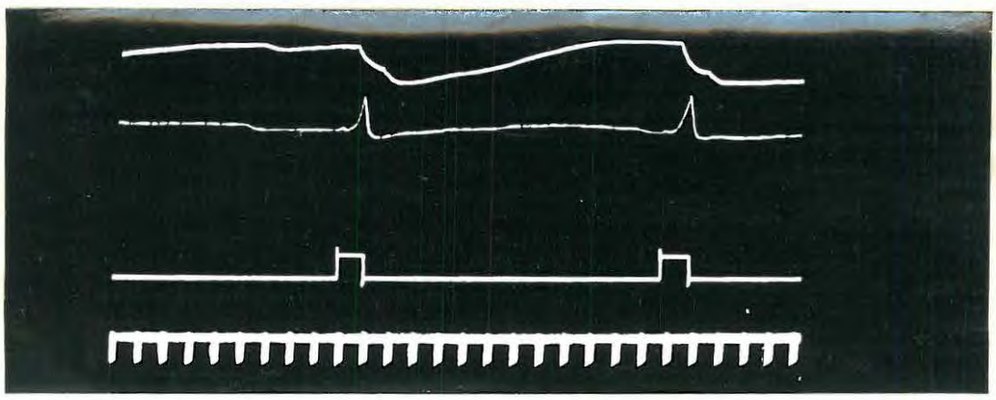


Fig. 79. Parechinus angulosus. Simultaneous records of movements of the retractor muscle, upper trace, and the comminator muscle, lower trace. Signal indicates time of stimulation of the comminator muscle. Time mark : 30 seconds.
Note: It is highly likely that the check in the fall of tone of the retractor muscle shown in these records is due to mechanical interference by the contraction of the comminator muscle. Since these records were taken with gimbal levers accurate registration is not achieved.

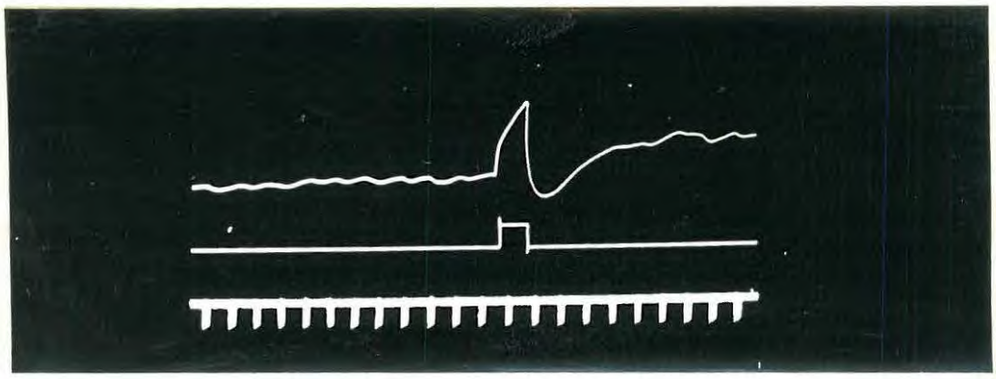


Fig. 80. Parechinus angulosus. Response of a directly stimulated retractor muscle to square wave pulses of 10 msec. duration and a frequency of 2/sec. Duration of stimulation, 30 seconds. Time mark: 30 seconds.

A Final Discussion.

Two opposing interpretations have been offered of the mechanism underlying the effects observed in these preparations of the lantern retractor muscle. The one, the neurogenic hypothesis, involves two unsatisfactory postulates. Firstly there is the assumption of the existence of a motor complex which has not been successfully demonstrated. Secondly the hypothesis involves possibly as many as four distinct photosensitive materials. The majority of effects observed are readily explained by the hypothesis, which is, however, so flexible as to make critical experiment difficult.

The second hypothesis, the myogenic hypothesis, is in certain features unsatisfactory. It is clear from experiments with the comminator-retractor muscle preparation that the tone of the retractor muscle can be inhibited and this observation compels us to postulate either the presence of inhibitory fibres acting directly upon the muscle, or, more elaborately, excitatory fibres which maintain the tone of the muscle and are inhibited either at their origin, or at the myoneural junctions. Furthermore the myogenic hypothesis can account neither for the fact that, with D.C. stimulation, an alteration in the direction of current flow changes the response of the muscle (fig. 61), nor for the twitch like responses at the make and break of a D.C. stimulus. A pure myogenic hypothesis can therefore be excluded.

The twitch-like responses to D.C. stimulation can only be interpreted on the assumption that the muscle is supplied with excitatory nerves and we have seen that there is some evidence for the presence of inhibitory nerves as well. More direct demonstration of these ideas comes from the response of the lantern retractor muscle to square wave stimulation (fig. 80). This type of response is almost certainly due to stimulation of nerve fibres and the close similarity between this and the response to photostimulation is compelling evidence that at

least many of the phenomena we have considered are of neurogenic origin. Attention is particularly directed to the post stimulatory inhibition. This is almost certainly the same event as the collapse of tone observed at "break" following D.C. stimulation in figs. 62B and 63A and parallels not only the maintained inhibition after stimulation of the comminator muscle (fig. 79), but also the loss of tone following a brief light pulse (fig. 49).

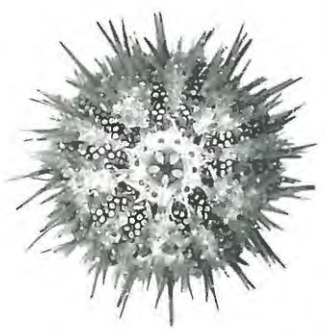
Photostimulation of both excitatory and inhibitory events has been found in Diadema. Millott and Yoshida (1960 a) have demonstrated the existence of a complex control over the spines of this urchin which shows a response to shadows. If the activity of a single spine is studied, it is quiescent in the light, but on removal of the photostimulus the spine beats regularly for about one minute. This effect is interpreted in terms of the double action of light. The light is believed, on the one hand, to cause a build up of excitation and on the other to inhibit this excitation, which cannot be expressed until the light is extinguished. The origin of the rhythmical beating of the spines has not been considered by these authors.

The parallel with events in Parechinus is clear, but here there are excitatory neurones which respond quickly and directly to light stimulation. There is reason to believe that these may fairly rapidly adapt to light and, as is suggested by the results shown in fig. 45, only fairly slowly recover. The "slow" response of the muscle may therefore be a direct response to light equivalent to the inhibited building up of excitation postulated by Millott and Yoshida. Further the rhythmic activity which characterises many preparations or follows particular treatments may indeed be myogenic in origin, as is perhaps the beating of the spines of Diadema when the shadow reflex is evoked.

The lantern retractor, on this view, resembles a vertebrate heart. There is, in the intact animal, a myogenic rhythm which may be inhibited or may be overruled by a fast

contraction. In the experiments using a five muscle preparation the rhythm of the individual muscles was asynchronous, but this may well be an experimental artifact arising from unequal tensions upon the retractor muscles and comminator muscles and the unsatisfactory device of stirring the preparation to activity by curare. We have seen that the comminator muscles are indeed coordinated in their activity; it may be that an initial contraction by them brings the activity of the ten retractor muscles into phase to contribute, by their inherent activity, to the characteristic regular jaw movements which are shown by Parechinus.

In this preliminary study of the lantern retractor muscle many different types of stimulation - light, direct current, drugs and square waves - have been used at different times. The behaviour of the preparations is highly variable and all too frequently phenomena produced by different modalities of stimulation have been regarded as equivalent. In further study it will be essential to demonstrate that the correlations which have been here assumed are indeed valid, and especially to see whether post-stimulatory inhibitions to light and electricity have or have not any relation to the phenomena of relaxation during spontaneous activity. The former are almost unquestionably neurogenic, the latter may perhaps be myogenic in origin.



SUMMARY.

1. The anatomy of the ossicles and muscles of the jaw apparatus of Parechinus angulosus is described.

2. The histology of the musculature is reviewed and attention is drawn to certain structural features not previously noted.

3. Previous views of the mode of operation of Aristotle's Lantern are discussed and criticised in the light of the present findings.

4. An account is given of the histology of the nervous system which suggests that the hyponeural nervous system is either more elaborate in Parechinus than in other species studied or that previous descriptions are incomplete.

5. When stimulated by long light pulses the retractor muscle characteristically responds with an initial quick contraction followed by a slower contraction. If the light stimulus is very prolonged the muscle may be stimulated to rhythmic activity.

6. The effect of variations in intensity and duration of photostimulation are described as well as the behaviour of the muscle to short light pulses and superimposed light stimuli.

7. Evidence is presented that photostimulation is both excitatory and inhibitory in action and a provisional hypothesis to explain the observations elaborated.

8. The action of direct current stimulation upon the muscle is described. The response is influenced by the direction of flow of the current and possibly also by its intensity.

9. The behaviour of the muscle in response to drugs known to affect cholinergic systems is described and attention is particularly drawn to the excitatory effects of curare and the complexity of the response to eserine.

10. An examination of the behaviour of the retractor muscle when its antagonists are stimulated provides evidence for reciprocal inhibition.

11. It is concluded that while many aspects of the complex responses shown by the retractor muscle are neurogenic in origin, the possibility that the rhythmic activity is myogenic cannot be excluded.

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