STUDIES ON THE NEURO-MUSCULAR PHYSIOLOGY OF A FREE-LIVING PLATYHELMINTH

by

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R E S U M É

The general picture of the physiology of invertebrate neuro-muscular systems as contrasted with the classical vertebrate system is presented and it is clear that one of the groups least investigated is the Phylum Platyhelminthes. An examination of the properties of the myo-neural system of a platyhelminth should be of interest, not only in itself, but also because of a possible relationship with the coelenterates, whose neuro-muscular system has been fairly extensively investigated. The aim of the present work was to determine some of the properties of the myo-neural system of a platyhelminth, and more especially those which would make possible a comparison with the myo-neural systems of other invertebrate phyla, and most particularly with the coelenterates.

A preparation consisting of an entire decerebrate animal was used. The general anatomy of the musculature and basic plan of the nervous system are presented to facilitate an understanding of the experiments described, but a detailed examination of the relationships of nerves and muscles or of the innervation of the latter was not attempted.

The spontaneous activity of such preparations was recorded kymographically and the characteristics of this activity under 'normal' conditions are described, together with observations on the effects on the normal spontaneous activity of treatments with sea waters of different ionic composition and with a number of drugs. The responses of the preparation to controlled electrical stimulation are also reported.

The results are analysed and compared with the results of similar investigations on other invertebrate preparations, in particular the Anthozoa, and an interpretation of these results in terms of platyhelminth-coelenterate relations is discussed.

C O N T E N T S.

1. INTRODUCTION.

One is accustomed to think of neuro-muscular mechanisms in terms of the classical vertebrate skeletal system, which has certain clearly defined characteristics. But recent studies have revealed that the myo-neural systems of the arthropods show characteristics which contrast very strongly with those of the vertebrate system. In the vertebrate skeletal system the development of muscle tension is determined by the amplitude of the stimulus applied to the motor nerve and above fusion frequency, no further recruitment of tension is obtained with increase in frequency (Cooper and Eccles, 1930). On the other hand, in the Decapod Crustacea it has been shown that the development of tension is determined by the frequency of the stimulation applied to the motor nerve and above threshold intensity, the development of tension is independant of the amplitude of the electrical stimulus; the earliest observations of this phenomenon were made by Lucas (1917) and Wiersma (1933). The type of innervation in arthropod systems is spoken of as multiterminal innervation, since it is apparently a general characteristic of such systems that there is a large number of end-plates on each muscle fibre; further, such systems are generally characterised by the fact that each muscle is supplied by only a few axons. In the vertebrate system, on the other hand, with certain exceptions such as frog slow muscle, there is a single junction per muscle fibre, each muscle being supplied by a large number of axons; this type of innervation can thus be termed polyaxonic, and it is convenient to distinguish between multiterminal and polyaxonic systems. Frequency sensitive or multiterminal systems are known in the Decapod Crustacea (Katz, 1949), the Stomatopod Crustacea (Wiersma and Ripley, 1952), in the Insecta (Pringle, 1939; Ripley, 1950; Hoyle, 1955, 1957) and in the Arachnida (Hoyle, 1958).

Among the echinoderms, information is mostly limited to the Holothurians. Du Buy (1936 a) has shown that the magnitude of the response of the pharyngeal retractor muscle of Thyone to an electrical stimulus is dependant on the stimulus strength, while Pople and Ewer (1954) have demonstrated the same characteristics on a preparation of the retractor muscle of Cucumaria. The only report of a frequency sensitive system in the echinoderms is that of Pantin (1935 a), who suggests that a process of facilita-

tion is involved in the neural control of the muscles of sea urchin spines; however, Kinosita (1941) has shown that Pantin's observations could be interpreted as being due to neural delay.

Until recently, most investigations on annelids have centred on the nature of the giant axons of the ventral nerve cord, which have been found to have a number of rather specialised characteristics. Recent observations, however, have revealed a peripheral frequency sensitive mechanism. Thus Wilson (1960 a) has shown that in a preparation of segmental nerves and associated muscles of both a nereid polychaete and a leech, a slow response elicited by stimulation of some of the segmental nerves shows unmistakeable facilitation and is unaffected by the intensity of the stimulation. The same characteristic is shown when the segmental nerves of the earthworm are stimulated (Horridge and Roberts, 1960; Roberts, 1960).

The coelenterate neuro-muscular system has also been found to display the characters of systems with multiterminal innervation ; independance of the response of scyphozoan medusae to the strength of electrical stimulation has been shown (Bullock, 1943), while Pantin (1935 a) has clearly demonstrated the frequency sensitive nature of the anthozoan myo-neural system. However, it has become clear (Batham and Pantin, 1950 a and b; 1954) that the coelenterate nerve net cannot be compared in any simple way with the crustacean peripheral system.

Information on the other invertebrate phyla is very incomplete. The only studies on protochordates are those of Hoyle (1952) who showed that in the monascidian tunicates Phallusia Mammilata and Ascidia aspersa single shocks applied to the whole animal evoked a large response; this response was also found to be independant of the stimulus strength. In the molluscs, Wilson (1960 b) has shown that in a nerve-muscle preparation of the mantle and stellar nerves of octupuses and squids, a slow response elicited by electrical stimulation of the motor nerves shows facilitation with increasing frequency of stimuli, but is unaffected by the intensity of stimulation.

Although the details are by no means clear, a further distinction between multiterminal and polyaxonic systems may lie in the nature of the transmitter substance. In the vertebrate skeletal system, transmission of excitation is mediated by acetylcholine; in the arthropods, particularly in the Insecta, there

is some evidence for the presence of a cholinergic mechanism in the central nervous system (Colhoun, 1958; Mikalonis and Brown, 1941; Twarog and Roeder, 1957) but no evidence for a cholinergic mechanism in the peripheral neuro-muscular system. There is also some evidence for cholinergic transmission in annelids (Fuehner, 1918; Wu, 1939, Bacq and Coppee, 1937) and similarly among the echinoderms (du Buy, 1936b).

Apart from early experiments showing that the brain is responsible for co-ordinating various activities, and one set of pharmacological observations on the liver fluke, nothing is known of the myo-neural system of platyhelminths. If we assume with Pantin (1952) that the coelenterate nervous system represents the most elementary form of metazoan nervous system, it is obviously of considerable interest to investigate the properties of the platyhelminth nervous system, which, while having an apparent net-work, is of a higher level of organisation in the possession of a central co-ordinatory centre - the brain - and large, well-defined nerve trunks. The question of the phylogenetic relations of the platyhelminths and coelenterates has not yet been resolved. While the view which is commonly expressed advocates a close relationship between the two phyla (Grove and Newell, 1957), Pantin (1960) has recently suggested that they are not related at all. A study of a platyhelminth neuro-muscular system might thus be of some assistance in throwing light on this question.

There are clearly two ways in which the properties of the myo-neural system may be studied - either by determining the responses of the animal to controlled electrical stimulation, or by testing the effects of a number of drugs, or solutions of different ionic composition. Similar investigations have been made on the sea anemones in particular. Both these methods have been employed in the present study and, while a comparison with the anemones is of primary interest here, the experiments with ions and drug solutions have been approached on a more general basis, to facilitate comparison with other animal groups.

2. MATERIAL AND METHODS.

1. Animals:

Planocera gilchristi Jacubowa (Jacubowa, 1908) is a marine polyclad living under stones along rocky shores of the Eastern Cape. It is recognised by its oval form and a colour and pattern of pigmentation which distinguishes it from the other species found here.

Collecting:

These animals live just below the low water neap tides level, so that collecting could be undertaken profitably only during the two hours about a low Spring tide. The density of the animal population along the shore fluctuated considerably throughout the year, being maximal for the period September to October, when egg-laying takes place on a far larger scale than throughout the rest of the year, but in general it was seldom possible to collect more than 20 - 30 animals of the required size during a two-hour period. This provided experimental material for a period of from ten days to three weeks, when fresh collections had to be made.

In transporting the animals from the coast, 35 miles away from the laboratory, two precautions had to be observed. One, that the temperature of the water should not be allowed to rise appreciably. This was obviated by placing the animals in plastic containers in an insulated bag; during the Summer months the containers were filled with sea water which had been chilled in the laboratory prior to setting out on a trip. Secondly, over-crowding appears to have a harmful effect which is not simply due to lack of aeration of the water. Since the insulated bag would hold only a limited number of containers, temporary overcrowding of the animals in the containers was often unavoidable. Provided the animals are removed to fresh sea water within two or three hours, overcrowding has no delcterious effects.

Laboratory Maintenance:

This animal proved extremely easy to maintain under laboratory conditions. Animals were kept singly (in the case of large animals of 4.0 to 6.0 cm. in length) or up to two or three only (for smaller specimens) in plastic containers of 500 ml. capacity. Provided the water was changed regularly, no aeration was needed.

The animals were fed every four to six days on small pieces of marine snail and care had to be taken to change the water in the containers within twelve hours after feeding. By following this routine of feeding and changing the sea water, it was found to be possible to keep animals healthy in the laboratory for as long as four months. All the experiments were, however, performed on animals which had been kept in the laboratory for not longer than three weeks.

Size of animals used:

For histological purposes, animals from 0.5 - 2.0 cm. in length were used, but for experimentation, animals of less than 2.5 - 3.0 cm. in length proved too difficult to manipulate, and specimens ranging in length from 3.0 - 6.0 cm. were used. It was found, however, that sexually mature animals in which the genital products had not yet been discharged were unsatisfactory as experimental animals, not only being unreliable in their responses, but also not lasting well as experimental preparations. Once the genital products had been discharged, such animals could be used and since they were commonly the largest specimens, they were valuable as experimental material.

2. Anatomy:

An understanding of the nature of the preparation and of some of the experiments described requires a knowledge of the general plan of the musculature and of the nervous system of *P. gilchristi*.

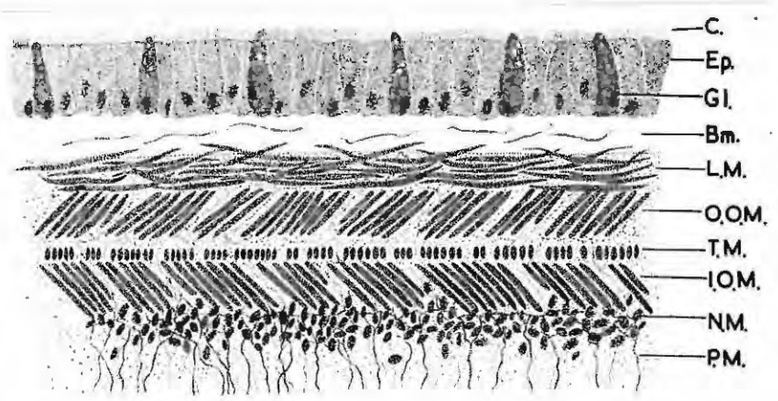
The Musculature:

Lying below the epidermis and rather well-developed basement membrane are a number of muscle layers. These are built up of spindle shaped, smooth muscle fibres, which are arranged in sheets, all the fibres of a sheet being orientated in the same general direction. In addition to the muscle sheets of the body wall, there are four specialised sets of muscles: the musculature of the pharynx, a set of muscles forming a sphincter around the mouth, the muscles associated with the male and female genitalia and the musculature of the lappets of the lateral margins of the body which serve to grip the substratum. The arrangement of these muscles will not be described here, since this study is primarily concerned with those muscles which are involved in movement of the whole animal.

The number and general orientation of the sheets of fibres in the body wall were determined by examining transverse,

Fig. 1 (a) and (b).

(a)



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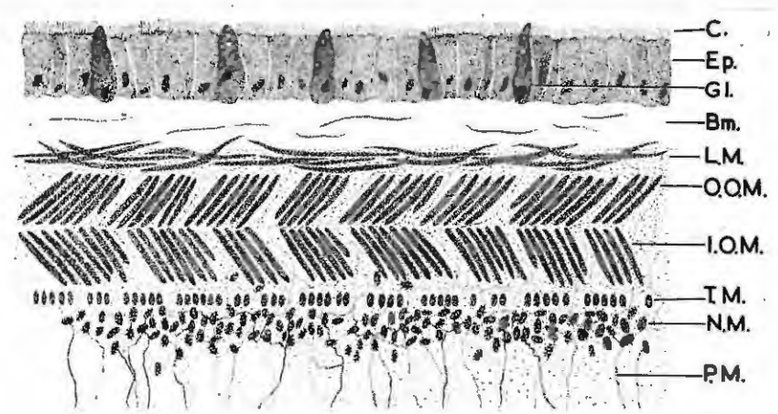


Fig. 1 (a) and (b): Diagrammatic representation of longitudinal sections through the ventral and dorsal body walls respectively.
Bm. = basement membrabe; C. = cilia; Ep. = epidermis; Gl. = gland cells; I.O.M. = inner layer of oblique muscle fibres; L.M. = layer of longitudinal muscle fibres; N.M. = nuclei of muscle cells sunk into the parenchyma; P.M. = parenchymal muscle fibres, embedded in collagen strands; O.O.M. = outer layer of oblique muscle fibres; T.M. = layer of transverse muscle fibres.

longitudinal and oblique sections of the animal. Sections from 5 - 20 μ in thickness were made from animals fixed in sea water Bouin and stained chiefly in Masson's trichrome stain and Heidenhein's iron haematoxylin.

Longitudinal sections through the ventral and dorsal body walls respectively are shown in figs. 1 (a) and (b). It can be seen that there are four layers of muscle fibres in association with each body wall, but whereas the sequence from epidermis to parenchyma is longitudinal, oblique, transverse, oblique in the ventral body wall, it is longitudinal, oblique, oblique, transverse in the dorsal body wall. Further, the muscle sheets of the dorsal body wall are rather more closely packed and less conspicuous than those of the ventral body wall. The latter is particularly true of the longitudinal and transverse layers.

A more satisfactory method for determining the relative extent and relationships of the muscle sheets was found to be as follows: whole, narcotised animals were examined through polarised light under a binocular microscope, making use of the birefringence of the muscle fibres. Where thickness of the animals obscured the picture of the orientation of the muscle layers, animals were fixed in 5% formol in sea water and thin strips of the body wall removed for examination. The general organisation of the muscle sheets of the body was found to be as follows:

The outermost muscle sheet of the ventral body wall - the longitudinal muscles - is not a uniform layer, but displays two conspicuous and distinct parapharyngeal sheets (fig. 2) whose muscle fibres are more densely packed than elsewhere. These sheets run longitudinally one on each side of the pharynx; laterally and posteriorly they merge with the general layer of longitudinal muscles, while in the region just anterior to the pharynx they cross over and merge with the layer of oblique muscles in this region. In the region where the parapharyngeal sheets cross they are overlain by a thin sheet of longitudinal muscle fibres. At the chiasma the muscle fibres of the two parapharyngeal sheets interdigitate.

Below the longitudinal layer, the transverse or circular layer shows some specialisation in that it is rather better developed in the region surrounding the pharynx, though

Fig. 2.

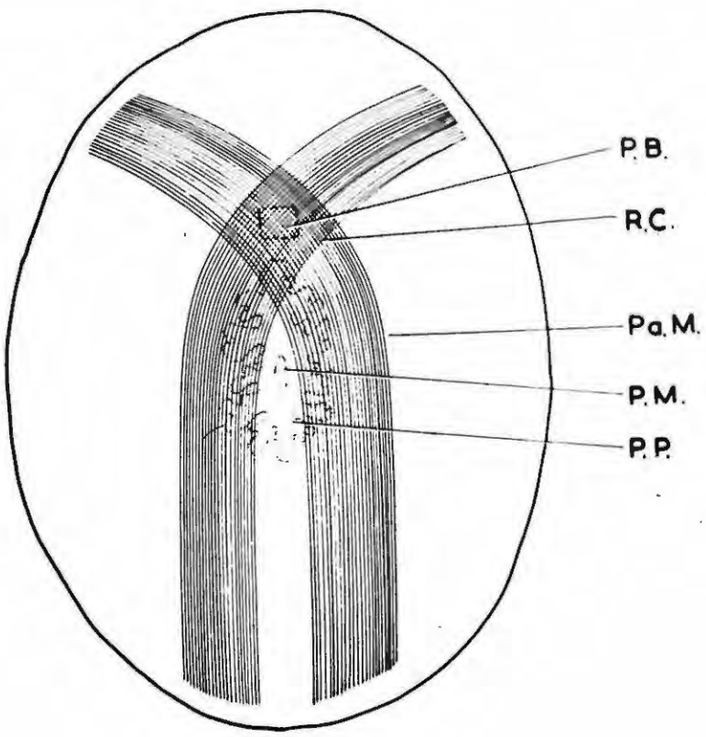


Fig. 2: Diagram showing the position and arrangement of the paraphryngeal muscle sheets.

Pa.M. parapharyngeal muscle sheet;
P.B. the position of the brain;
P.M. the position of the mouth;
P.P. the position of the pharynx.
R.C. region of crossing over of the two parapharyngeal muscle sheets.

Fig. 3.

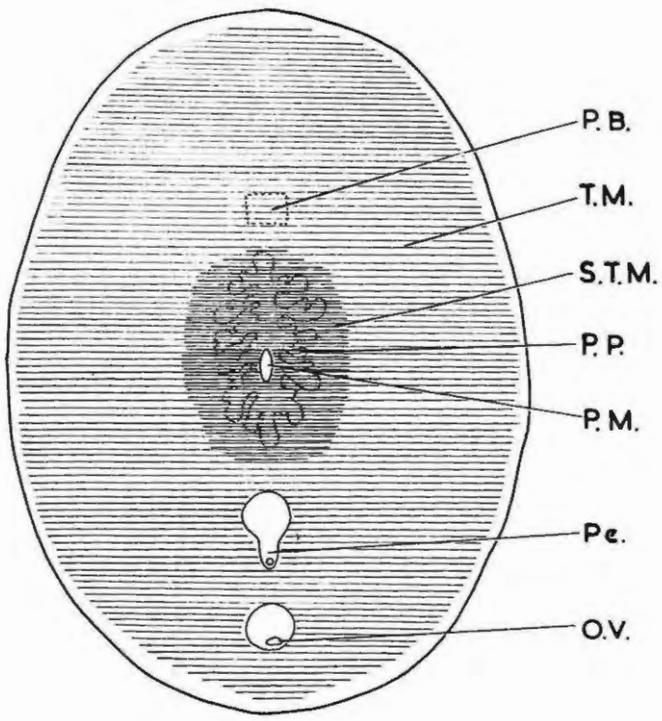


Fig. 3: Diagram of the transverse muscle sheet of the ventral body wall.
Pe. Penis;
O.V. opening of the vagina;
T.M. transverse muscle sheet;
S.T.M. well-developed region of the transverse muscle sheet around the pharynx.
For other annotation see legend to fig. 2.

this is not nearly as marked as in the differentiation of the longitudinal layer into parapharyngeal sheets. (Fig. 3).

The two obliquely running muscle sheets, while showing no differentiation into regions of greater or lesser density of muscle fibres, have a distinct organisation. The fibres of the innermost oblique layer are oriented anteriorly, so that the muscle fibres of the two sides of the body make a chevron pattern with the apex towards the head. The outermost layer is oriented in the opposite direction. In addition, the degree of obliquity with respect to the longitudinal axis of the body changes at the anterior and posterior ends in both oblique layers (figs. 4 and 5).

In the dorsal body wall, the longitudinal and transverse muscle layers show an even distribution and orientation of the muscle fibres. The two oblique layers are organised in the same way as those of the ventral body wall, except that here the degree of obliquity is far more constant from anterior to posterior ends (figs.6 and 7).

Since a discussion of the possible correlation of the arrangement of the musculature with the various movements of which the animal is capable requires a rather lengthy description of these movements, and since it is not directly relevant to the present investigation, this will not be examined in detail here. However, since the movements shown by the preparation of P. gilchristi used in these experiments are almost certainly largely due to the activity of the parapharyngeal muscles, it is of some interest to consider the possible functions of these muscles in the normal activities of the animal. There appear to be two activities in which the parapharyngeal muscle sheets may play an important role:

(i) When removed from the substratum, the animal will usually perform a very rapid curling up of the body, so that the anterior and posterior ends bend ventrally and overlap one another; this protective movement is far more rapid than any of the movements involved in normal locomotion, and it seems reasonable to suggest that it is effected primarily by the large parapharyngeal muscle sheets of the ventral body wall.

(ii) Observations on the ditaxic crawling of the animal over the substratum indicate a possible correlation of this mode of progression with the crossing over of the parapharyngeal sheets in the region just anterior to the pharynx. This movement occurs as follows:

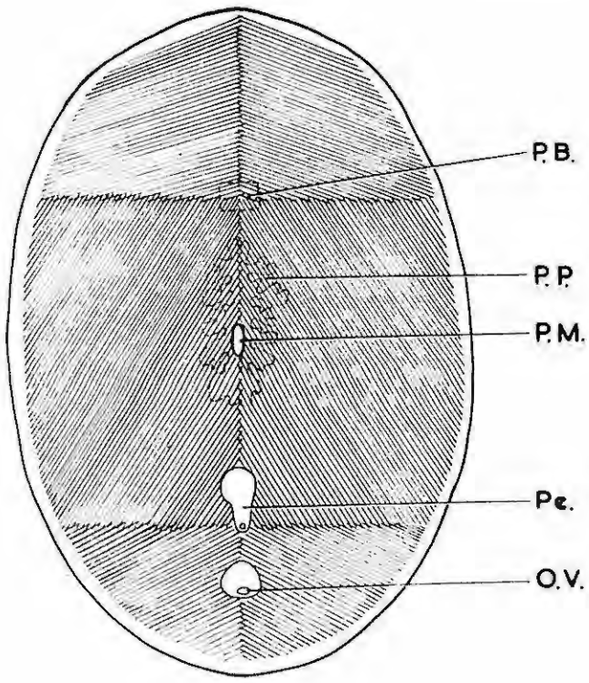


Fig. 4: Diagram showing the arrangement of the inner layer of oblique muscles of the ventral body wall. For annotation see legend to fig. 3.

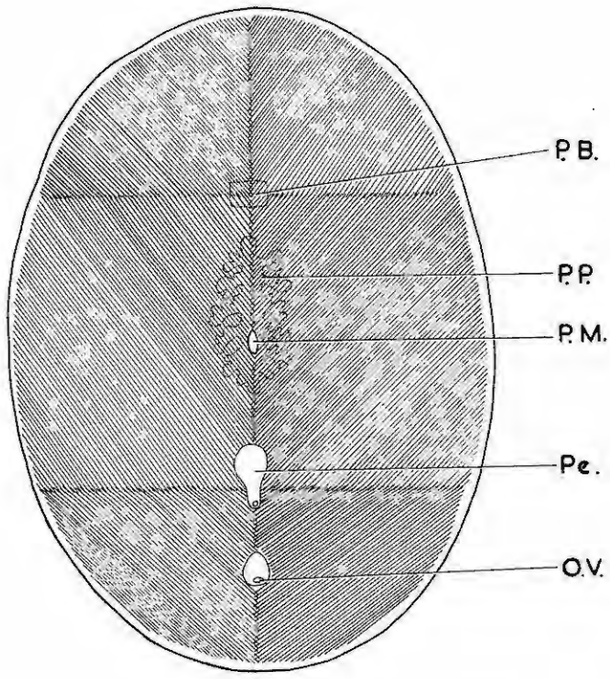


Fig. 5: Diagram showing the arrangement of the outer layer of oblique muscles of the ventral body wall. For annotation see legend to fig. 3.

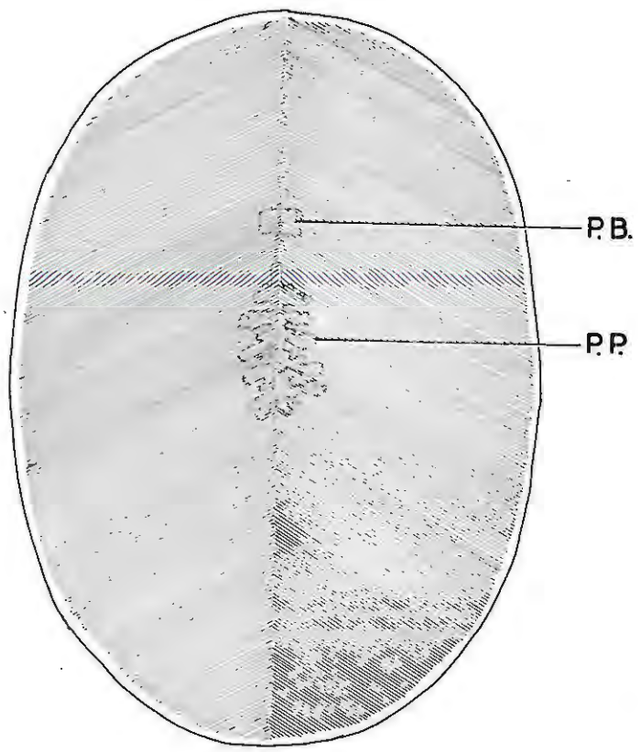


Fig. 6: Diagram showing the arrangement of the inner layer of oblique muscles of the dorsal body wall. For annotation see legend to fig. 9.

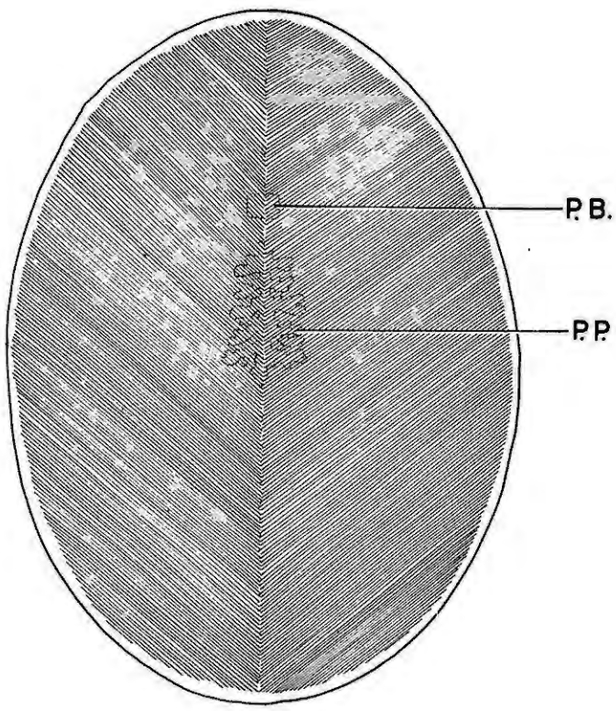


Fig. 7: Diagram showing the arrangement of the outer layer of oblique muscles of the dorsal body wall. For annotation see legend to fig. 9.

Fig. 8.

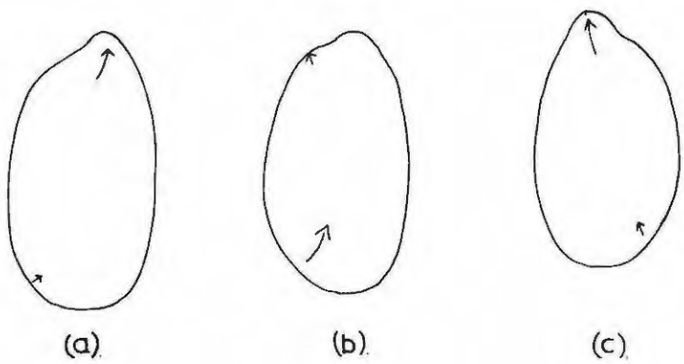


Fig. 8: Diagrammatic representation of the ditaxic crawling movement of Planocera.

the anterior edge of one side of the body extends forwards as shown in fig 8 (a), and the substratum is gripped by the margin of the body in this region. Almost before this phase of the movement is completed, a muscular contraction draws up the posterior end of the body which is diagonally opposite the anterior end which has just extended forwards, (Fig. 8 (b)); the contraction effecting this can be seen to extend diagonally across the body, thus the posterior edge is not drawn directly forwards, but slightly obliquely forwards. The other anterior end then extends forwards and the diagonally opposite posterior end is drawn up, and so on. The arrangement of the parapharyngeal muscle sheets suggests that they might be concerned with pulling up the posterior ends when the animal is crawling.

The Nervous System:

The nervous system of P. gilchristi resembles that of all polyclads in having a brain, immediately anterior to the pharynx, from which issues a number of fairly large nerve trunks. Some of these lead to the ventral and some to the dorsal body wall, where they branch repeatedly just below the innermost layer of muscle fibres, to form ventral and dorsal networks of nerve trunks.

The nature of these nerve trunks was examined in transverse and longitudinal section and, although the precise arrangement could not be ascertained, the trunks can be seen to be composed of large numbers of fine nerve fibres, supported by parallel-running connective tissue fibres. Both the brain and nerve bundles are surrounded by a rather tough connective tissue sheath.

(a) The brain and ventral nerve network:

The arrangement of nerve trunks leaving the brain was studied by dissecting narcotised animals and then staining the surrounding muscles with eosin.

There are seven pairs of rather large nerve trunks which leave the brain and run just below the inner layer of muscle fibres of the ventral body wall (fig. 9). The largest of these are trunks Vl and Vll, which, after leaving the brain, run for some distance before any significant branching takes place.

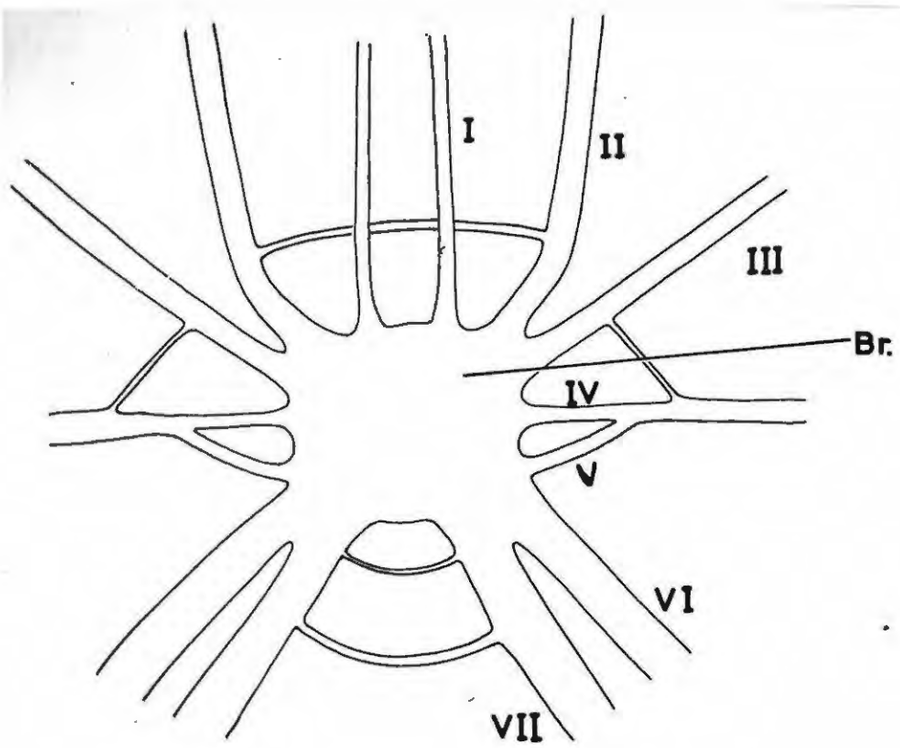


Fig. 9: Diagrammatic representation of the ventral nerve trunks as they issue from the brain. Br.= brain; I - VII = nerve trunks.

Fig. 10.

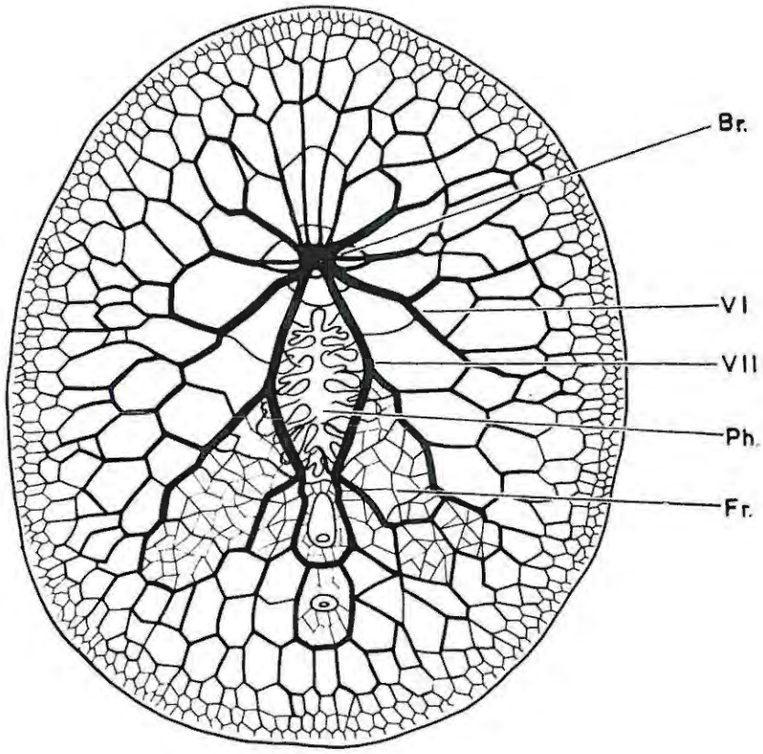


Fig. 10: The ventral nerve network of P. gilchristi. Br. = the brain; Fr.=finer reticulation; Ph.= pharynx; N. VI and N. VII = the main longitudinal nerve trunks of the ventral network.

The nerve trunks all branch repeatedly to form an anastomozing net-work of bundles of nerve fibres, these bundles becoming finer and finer as the periphery of the animal is approached. The arrangement of these nerve bundles can be seen quite clearly by transmitted light and the general plan of this network is illustrated in fig. 10. Vital staining with methylene blue revealed that between the larger bundles of nerve fibres a finer network exists, which again continues to branch into a finer and finer network of nerve fibres.

(b) The dorsal network:

The nerve trunks which issue from the dorsal side of the brain are fewer in number, there being only four pairs; these are much finer than the large trunks of the ventral network. There are, in fact, no nerve trunks in the dorsal network comparable with the large trunks of the ventral network.

Since in the present investigation attention has been directed only to the functional relations of the large ventral nerve trunks and the rest of the ventral network, further detail concerning the dorsal network will not be presented.

It would be satisfying to be able to present a more detailed account of the arrangement of the nerve fibres in the main nerve tracts, and of the innervation of the muscles; several techniques have been used in an attempt to elucidate the fine-structure of the nerve-muscle relationship, but have thus far met with little success. However, for an understanding of the experiments described here, it is considered that this rather limited account should suffice.

3. General Methods:

The general method employed throughout these experiments was to record the activity of the animal mechanically on a smoked kymograph drum. There will be described here only the manner in which such a preparation was set up, the more detailed procedure followed for each set of experiments being described under the relevant sections.

Most of the experiments have been carried out on decerebrate animals, although recordings of activity of entire animals were also made and a number of tests carried out on such animals for comparison. It was found that large animals could be hemisected and each half of the animal used independantly; with smaller animals the slight diminution in size due to regeneration and healing along the cut edge rendered the halves

Fig. 11.

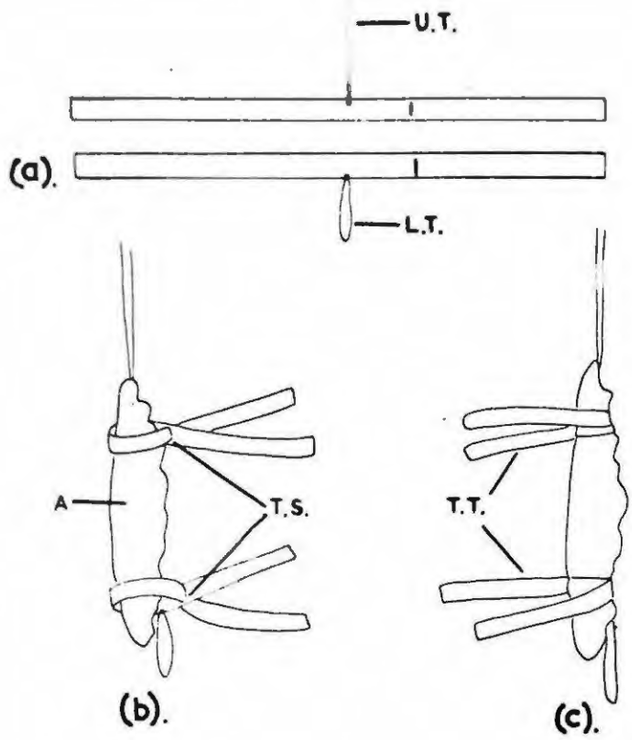


Fig. 11: Diagram showing the way in which an animal was ligatured, using two strips of tape.
A. Animal;
L.T. thread for anchoring lower end of the animal;
U.T. thread for attachment to lever;
T.S. tape passed through slits;
T.T. tape knotted about the animal.

too small to be used. For both these operations - decerebration and hemisection - the procedure was as follows: the animal was narcotised in a solution of equal volumes of sea water and isotonic magnesium chloride for 45 min. The operations were performed under a binocular microscope. Since the position of the brain can be seen clearly by transmitted light, its removal by cutting with a short blade could be performed very neatly. Thereafter, before using the animal in an experiment, a recovery period of at least 24 hr. was allowed and in the case of those which had been both decerebrated and hemisected at least 48 hr.

A problem was initially presented in finding a method whereby the animal could be attached to a lever for recording on a kymograph drum. The simple methods of passing a metal hook through each end of the animal, or securing each end by means of loops of thread proved impossible in this case, since the animal simply tears away from them. In fact, excess pressure applied to any part of the animal results in its splitting into as many fragments as may be necessary to allow escape. In some preliminary experiments it was found that by using nylon wool instead of thread for the ligature, and by further inserting a small strip of foam plastic between the animal and the ligature, a preparation could be set up which would last for a considerable period of time before the animal eventually split away from the ligatures. In fact, fatigue of the preparation often set in some time before the animal eventually tore away. After attempting a number of variations, the simplest method was found to be as follows: two thin and narrow strips of foam plastic, about 3 cm. long, were cut, soaked in sea water, and laid upon two strands of nylon wool, also previously soaked in sea water, and to which threads for attachment to a hook and the lever had been secured. The animal was then laid upon the foam-plastic strips and from there it was a simple step to secure each end. This was done in such a way as to include in the portion between the ligatures the two large parapharyngeal muscle sheets described on p.6. An alternative method was devised and used successfully on large animals: two strips of narrow white tape were cut and a slit made across the tape, one third of the way from one end. The other end was then threaded through this slit and the tape fastened securely with a knot about one end of the animal. This is illustrated in fig 11. The use of tape instead

of wool enabled the pressure of the ligature to be distributed over a wider area of the animal and prevented too tight a constricture in one localised place, thus delaying splitting in the region of the ligature even further. It was not necessary in this case to insert a foam-plastic strip. This method proved very successful, but, as has already been mentioned, it could only be used on rather large animals.

Initially, the animal was first narcotized in a solution of $MgCl_2$ prior to ligaturing as above. In this case it was then necessary to leave the preparation in normal sea water for three to four hours before use, to recover fully from the narcotic. Narcotization was soon found to be unnecessary at this stage, for if the unnarcotised animal is placed with its ventral surface downwards on the foam-plastic, it will cling to this and can be tied up with no difficulty. This latter procedure was preferred since it eliminated the time wasted during the recovery period and thus lengthened the time during which the preparation could be used before it eventually tore away from the ligatures.

Preparations set up in this way could, with care, be made to last as long as 36 hr., but in general, fatigue set in after periods varying from 6 - 18 hr. Even with the use of tape or foam-plastic to distribute the pressure from the ligature more evenly, the animal was liable to tear away very easily if the ligature was made slightly too tight or to slip out if slightly too loose.

Once ligatured, the preparation was attached to a lever which was so counter-weighted that the animal contracted against a light load. This load had to be varied slightly according to the size of the animal and its value was found to be rather critical. This is discussed further on page 15.

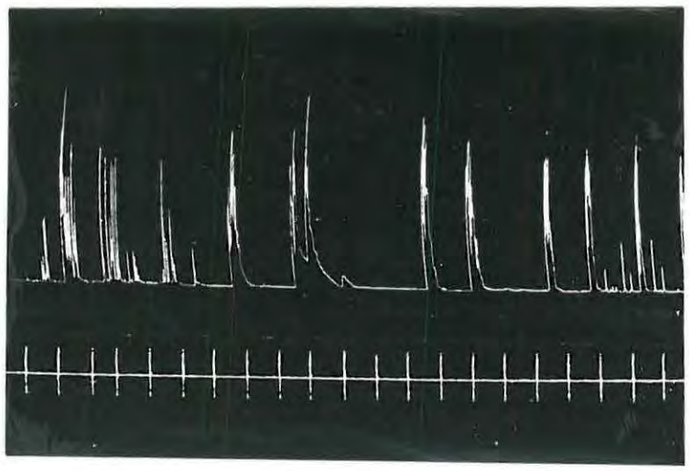
Apparatus:

(a) Recordings: These were all made on smoked paper on a Palmer kymograph, a Palmer signal-marker and time-marker being used to record the time intervals. All contractions are measured as rises above the base-line.

(b) Levers: A number of early recordings were made using a Braun lever but thereafter all recordings were made using Palmer gymbal levers mounted on a rack-work block to allow careful and fine adjustment of the position of the lever.

Fig. 12.

(a)



(b)

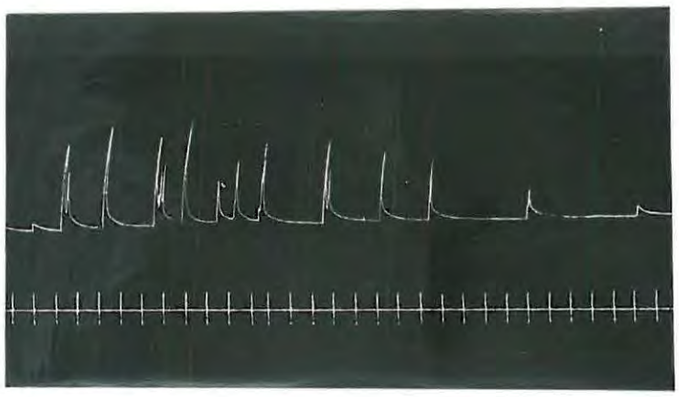


Fig. 12: Records of normal spontaneous activity over a period of several hr.
(a) Activity of an entire animal.
(b) Activity of a decerebrate animal.
Time trace, 30 min.

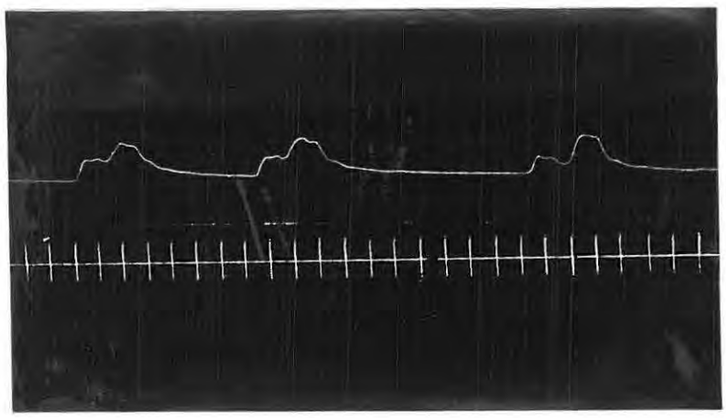
4. Nature of the Preparation:

When P. gilchristi is attached to a lever in the manner described and maintained in fresh aerated sea water, it is found to contract spontaneously and periodically in the absence of any external stimulation. Records of this activity were made for both cerebrate and derebrate animals; figs. 12 (a) and (b) provide examples of mechnical recordings made over a period of several hours. Examination of a large number of recordings revealed no constant difference between the activity of intact and decerebrate animals.

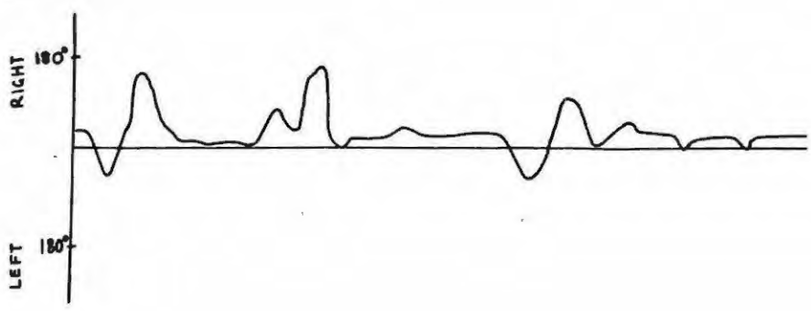
In addition to the longitudinal contractions which were recorded on the kymograph drum, two further types of activity were observed:

(a) A movement of the sides of the body vigorously to and fro, in a manner resembling the swimming movements. These always occurred simultaneously with a longitudinal contraction, although the latter is not necessarily accompanied by such movements. Since there was no apparent way in which these movements could be recorded with any degree of accuracy the matter was taken no further.

(b) A cork-screw twisting of the animal about its longitudinal axis. A simple method used for recording this was as follows: the lower end of the animal was secured in such a way that it could not twist and the upper thread replaced by a thin strand of copper wire; a small silver mirror was attached to the wire connecting the animal to the lever. In this way it was possible to ensure that any twist in the animal would be transmitted to the mirror and not be taken up by the slack of the thread. Using a microscope lamp, light was reflected from the mirror on to an arbitrarily calibrated scale and the position of the reflected spot of light observed and recorded at regular intervals. Longitudinal contractions were recorded simultaneously on a drum and the record of the twisting movement then plotted graphically on the time scale. An example of two such corresponding records is given in figs. 13 (a) and (b). As in this case, a large number of such recordings show a very precise correlation between the two types of movement, but there is also a number in which longitudinal contraction occurred in the absence of any twisting. This can be simply explained as follows: it will be remembered that the two large parapharyngeal sheets of muscle cross over one another at the anterior end; if the muscle on one side only contracts, this



(a)



(b)

Fig. 13: (a) Recording of spontaneous longitudinal contractions. Time trace 30 sec.
(b) Simultaneous recording of corkscrew twisting. 180° indicates approximate degree of twist to left or right.

is likely to cause a twisting to one side or the other, if both contract simultaneously, no twisting would be expected to occur. Longitudinal contractions with a double hump, as in fig. 13(a) were often seen in records from this preparation; these were always accompanied by a twist, usually first to one side and then the other, although sometimes both twists are to the same side of the animal. Since this twisting movement is obviously correlated with longitudinal contraction it was not considered necessary to make separate records of it and in all the experiments performed observations were confined to the longitudinal contractions.

Characteristics of spontaneous longitudinal contractions:

In order to interpret correctly the effects of experimental treatments it is necessary first to present a picture of the activity of a preparation under normal conditions.

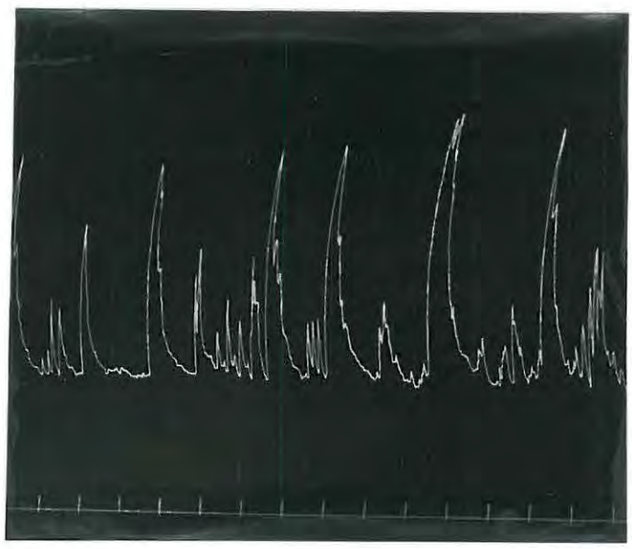
A preparation attached to a lever under a light load begins to stretch and continues to do so for a period of 10 to 40 min. until it reaches a stable basic length; to this it returns after each contraction. This basic length remains constant for the preparation until, after several hours, fatigue sets in and further stretching occurs.

Periodically and quite suddenly a contraction begins, maximal contraction being reached very rapidly. Relaxation follows equally suddenly and, rapid at first, slows down somewhat as the basic length is approached. The duration of the final stages of the relaxation phase is generally briefer in cerebrate than in decerebrate animals; this can be seen by comparing figs 12 (a) and (b). However, some decerebrate preparations were found to relax at the same rate as cerebrate ones.

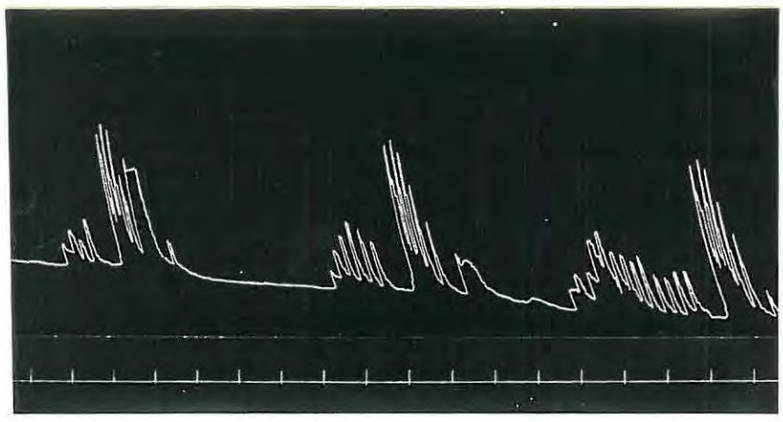
One would not reasonably expect a preparation consisting of a whole animal to exhibit the regularity of activity commonly to be seen in mechnical records of smooth muscle preparations from isolated organs, and indeed a considerable amount of variability is found to occur, not only from individual to individual, but from time to time in the same preparation. In general, the records show an inexact rhythm of major movements, occurring on the average every ten min., but variable from three in ten min. to one every half hr. These major movements alternate with periods of relaxation which may or may

Fig. 14.

(a)



(b)



(c)

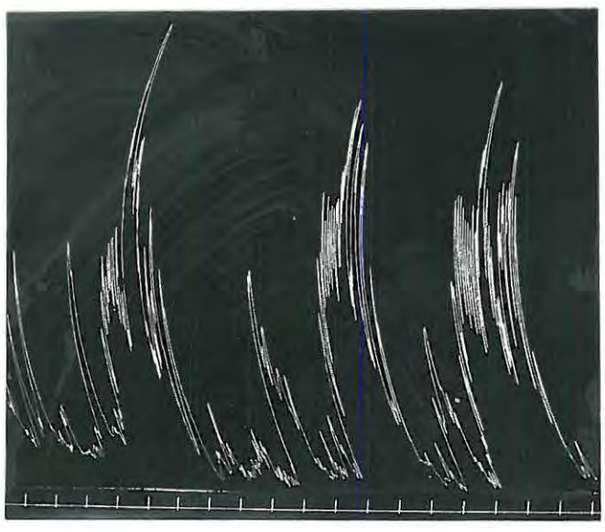


Fig. 14: Examples of variation in records of normal spontaneous contraction. See text for explanation.
Time trace in this and all subsequent records in 10 min. intervals, unless otherwise stated.

not be interrupted by a number of smaller movements; the number of the latter which occurs in the intervening period is highly variable.

The major movements may take the following forms:

(a) Each may be a single large upswing, usually followed by a few small contractions before or during relaxation, as in fig. 14 (a). This is the most usual form of activity.

(b) Each may consist not of a single large contraction, but of an outburst of activity in the form of a rapidly repeated series of contractions, as shown in fig 14 (b).

(c) Occasionally, each large contraction may be followed by the maintainance of a high tone, with clonic movements persisting for as long as 10 to 20 min. This is illustrated in fig 14 (c), and although very rare, it is necessary to take its occurrance into account in assessing critically some of the results to be described. This is discussed further on p18.

The record of activity for an individual may vary from time to time in the following ways:

(i) the above type of major activity may continue for several hours and then, quite abruptly, the large movements disappear for a period, variable in extent, during which only the smaller movements are shown; after a while larger movements may return, although their frequency and size may have changed;

(ii) the frequency with which the large contractions follow one another may also vary in that there may be a gradual increase in the time interval between them as the preparation ages: in a preparatiom in which the time-interval is relatively long a slight mechnical disturbance, such as changing the water around the animal, may increase the frequency, but never very significantly and usually for only a short period;

(iii) the size of the large contractions is perhaps the most variable factor, generally diminishing with age but varying considerably from time to time and being easily influenced by any slight disturbance.

Factors which influence spontaneous activity:

(i) Light: It was found that in the dark an animal will usually contract rather more regularly than in the light, giving a record of type (a) with very little evidence of minor movements. However, this did not form an invariable rule and

while more complicated and irregular patterns could be obtained in the dark, similarly, a regular pattern of type (a) was often shown by an animal in the light. No response could be elicited from the animal by switching on an electric light after it had been in the dark for some time. While it might have been preferable to carry out the experiments in the dark, this was not always possible and the majority were performed in the light.

(ii) Temperature: All the experiments here described were carried out at room temperature which varied from 18° to 26°C at different times of the year. Neither this difference in room temperature nor the daily fluctuations of a few degrees seemed to have any effect on the nature of the activity.

(iii) Tension: As has been mentioned previously, the magnitude of the load against which the animal has to contract was found to be rather critical. When the animal is suspended in such a way that all slack is taken up but no tension applied, either no contractions of any magnitude follow, or else the activity takes the form of contractions which are very irregular with regard to both size and frequency. Thus to elicit a fairly regular pattern of activity a certain amount of tension has to be applied. On the other hand, if this is too great, the animal splits in a few minutes after being attached to a lever. Although the tension has to be varied according to the size of the animal, an initial tension from 0.5 - 0.8 g. was found to be most satisfactory. In general it was found to be better to subject the preparation, for the first five to ten min., to a load that was just greater than optimal for that preparation, so that the animal stretched, and then to replace this by a lighter load. When this was done a rather regular rhythm could be elicited for at least a few hours. The manner in which the optimal magnitude of load for each preparation was judged was as follows: after the initial stretching, the load was lightened; stretching would then normally continue more slowly for another five to ten min., after which a steady basic tension was maintained. If stretching was seen to be continuing at a considerable pace for longer than this, it was generally found to be indicative of too great a load; if the contractions became almost negligible in size after the load had been reduced, a slight increase in load would restore them to a reasonable size.

Fig. 15.

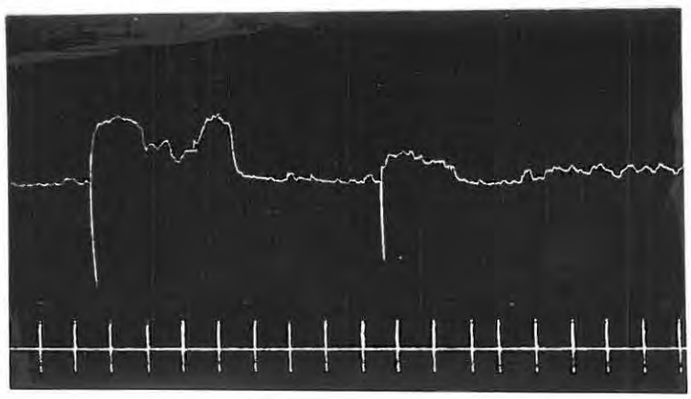


Fig. 15. Response of the preparation to applied stretch. Time trace, 30 sec.

Effect of applied stretch: The effect of the tension on the animal is further emphasised by the fact that when a sudden excess tension is applied and then immediately released, a contraction is elicited, the size of which was found to depend on the extent of the applied stretch. Both cerebrate and decerebrate preparations respond alike. This is illustrated in fig. 15.

5. Interpretation of the Mechnical Record:

It would be satisfying if one could correlate the activity recorded mechanically from a preparation of P.gilchristi with the behaviour of the animal under normal conditions. Certainly the experimentally observed activities do not correspond to normal locomotory movements, for they are of a far slower frequency than the rapidly executed contractions and relaxations shown by the animal when walking or swimming. Indeed the experimental situation, in which the animal is held at a steady tension, is totally abnormal and there is no reason to believe that the responses shown would represent part of the normal behavioural repetoire of Planocera.

This situation differs markedly from activities recorded by other smooth muscle preparations from invertebrates. Thus the rhythmic activity of the isolated extrovert of Arenicola reflects the normal feeding activity of that animal (Wells, 1950), the rhythmical activities of the pharyngeal retractor of Cucumaria may also perhaps reflect a feeding cycle (Pople, personal communication), while the rhythmicity of isolated rings of sea anemones may reflect the peristaltic activity the mid-columns of these animals show during digestion.

Fig. 16.

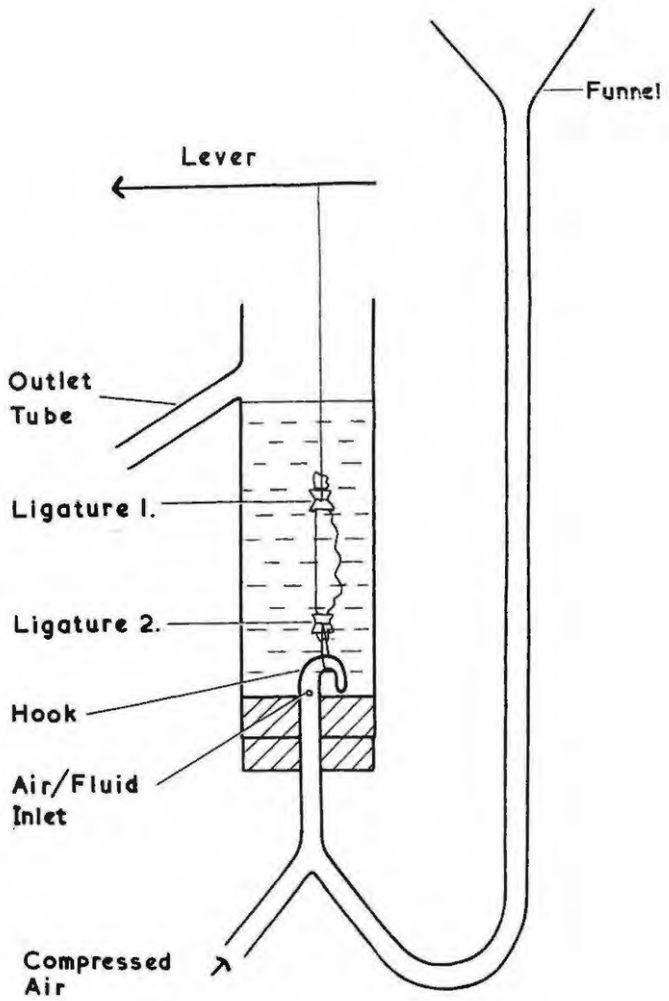


Fig. 16: Diagram of apparatus used throughout the experiments in section 3A.

3. INVESTIGATION.

A. THE EFFECTS OF IONS AND DRUGS.

The effects of changing the ionic proportions of the sea water around the animal and the effects of certain drugs have been studied on the sea anemone, Calliactis parasitica Couch (Ross and Pantin, 1940) and on mid-column and sphincter preparations of Calliactis parasitica and Metridium senile (L.) (Ross, 1960), as well as on a number of other neuromuscular preparations, both vertebrate and invertebrate. An investigation of the effects of similar tests on P.gilchristi has been undertaken mainly with a view to providing some comparative data.

1. Methods:

The same general methods were employed for both the tests involving ions and those using drugs. A preparation was set up in the usual manner and then immersed in sea water in a cylindrical glass vessel, 3 cm. in diameter. This is illustrated in fig. 16. Throughout the experiment air was bubbled slowly through a small hole at the base of the hook. The solution around the animal was changed by stopping the air flow and adding the new solution through a funnel, the over-flow tube allowing the excess liquid to escape. Approximately 200 ml. of new solution was added through the funnel for each experiment, the volume of water contained in the cylinder being approximately 70 ml. This is, admittedly, a rather inexact method. In the case of ions, however, large changes in concentration were being tested so that small errors in the assessment of the exact concentration of the water around the animal were considered to be of little significance. Again, in the case of drugs, a series of concentrations, differed from one another by the power 10 or 5 times the power 10 were tested, so that small errors may be ignored. Finally, it may be pointed out that in a comparative analysis, where different individuals are very likely to have different threshold concentrations for response to any drug or solution, the effect to be observed is a qualitative rather than a quantitative one.

The new solution was left in contact with the animal for varying periods of time. Generally solutions which caused a great increase in activity were washed out within 15 - 30 min. or even less, for prolonged treatment leads to a state of fatigue

from which the animal does not recover. Where there was no apparent effect, the solution was generally washed out only after 1 - 2½ hr. in order to make certain that there was no delayed effect; further, a prolonged test was also made for each solution at what was found to be an effective concentration, to observe any possible waning of the effect. In washing out any solution, 300 to 500 ml. of fresh sea water were used.

2. Validity of the effect:

2.1 Qualitative assessment:

Since the normal spontaneous activity of the animals displays certain irregularities, it is necessary to show that these were not confused with any effect. In the first place, activity of the type (c) described on page 14, in which there is a spontaneous maintainance of tonus at a high level, could be confused with a stimulant effect of any treatment. It has been pointed out that this type of activity occurred very seldom, but any animal which behaved in this manner was discarded and not used in an experiment. In the second place, since there can be a spontaneous slowing down in the frequency of the large contractions or a temporary disappearance of these, this might be confused with an inhibitory effect. The only way in which the latter difficulty could be resolved was by repetition of the test a large number of times. Although the frequency may also be increased slightly, as pointed out on page 14, point (ii), this never exceeded 1.5 times the previous rate. The excitatory effects observed in these tests generally produced a change in frequency which could be distinguished easily from any spontaneous change.

Since the preparations are so variable in their inherent activity, the effects of the treatments can only be determined for each preparation after a fairly long period of preliminary recording. A period of at least one hr. was allowed to elapse after the preparation had been set up, or after normal activity had been resumed after a treatment, before the next test was made. If the activity appeared to be fairly regular, a test solution was then applied; sometimes periods of as long as six hr. had to elapse before the rhythm of activity was sufficiently regular to assume that it would continue thus for a reasonable period of time.

A statistically valid conclusion as to the nature of any effect can only be drawn after a test has been repeated a number of times. In the case of tests involving a change in the ionic proportions of sea water, the following procedure was adopted:

for the excess of any ion tests were made with concentrations ranging from two to ten times the normal concentration of this ion in sea water and the series repeated at least once; when artificial sea water lacking one or more of these ions was tested, each test was repeated at least twice; this was also the case with the tests involving multiple ion effects. In testing the effects of solutions of various drugs, an initial test was made at 1.10^{-3}M, and this was repeated a sufficient number of times to establish the presence or absence of an effect. If an effect was thus demonstrated the testing was repeated at lower concentrations, until the minimal effective concentration was determined. Except in those cases where the supply of a drug was limiting, each test was usually made at least twice at each concentration. Whenever any doubt arose as to the real nature of an effect, the test was repeated until satisfactorily conclusive evidence was obtained.

Criteria of assessment of any effect:

The effect of any treatment was estimated on the basis of the following characteristics:

(i) Tonus: The basic length to which the preparation returned between contractions after treatment was compared with that of the preparation before treatment.

(ii) Frequency: The frequency with which the spontaneous contractions followed one another after treatment was compared with the frequency of contraction in the preliminary record.

There are two further criteria of judgment which have been used by Ross (1960) in his studies of the effects of ions and drugs on sea anemones, neither of which have been employed in the present study. The first is that of an effect of a treatment on the size of the contractions. This has not been used here for the following reasons: firstly, as has been pointed out, the size of the contractions may vary considerably from time to time in the same preparation, and merely changing the sea water around the animal may temporarily increase the size of the contractions; furthermore, as will become evident when the records of many of the tests are examined, the peak of a contraction reached during normal spontaneous activity does not often represent the maximal shortening of which the animal is capable. Thus, while a treatment which causes only a slight rise in tonus will leave the animal sufficient leeway to perform contractions which are larger than normal, a treatment which causes an increase

in tonus to, or almost to, the full extent of shortening will, of necessity, abolish or greatly reduce the size of the contractions at the new level of tone. Finally, whenever an effect on contraction size was observed, and further tests made with the same solution at the same concentration, the effect was never found to be consistent. The second criterion is one which Ross terms a 'direct contraction'. If a preparation is contracting at a constant rate, the time at which the next contraction may be expected can be predicted with a certain amount of accuracy, and if the solution is added immediately after relaxation from a spontaneous contraction has been completed, an immediate contraction on adding the new solution may be presumed to be a direct effect of the treatment. In tests on Planocera an attempt was made to determine the presence or absence or a 'direct contraction', but the irregularity of the spontaneous activity and the rapidity with which the preparation responded to most of the treatments render it very difficult to determine a satisfactory time limit for what is to be considered a direct contraction. Moreover, when an attempt was made to record direct contractions in tests on Planocera so much variability was found for a single treatment that this criterion is considered to be rather meaningless.

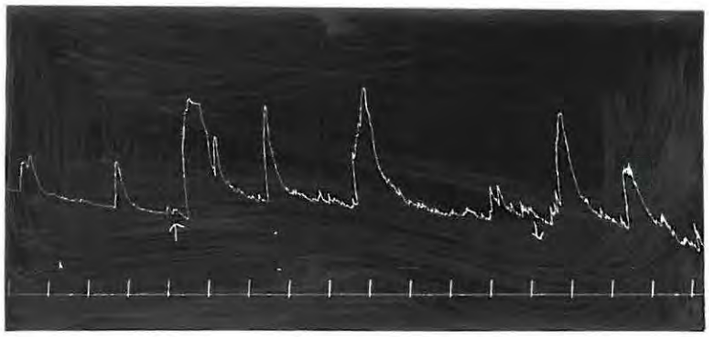
2.2. Quantitative assessment:

As a result of considerable quantitative variability in the responses from individual to individual, it is difficult to make precise quantitative measurements, particularly on the basis of so few tests at each concentration of either ion or drug. It must be remembered that the results here presented are intended to serve primarily as a basis for a comparative study, and as such do not require so much a quantitative as a qualitative basis. In spite of this, it is considered necessary to present as much quantitative data as possible, even though these cannot be considered as anything but very inexact.

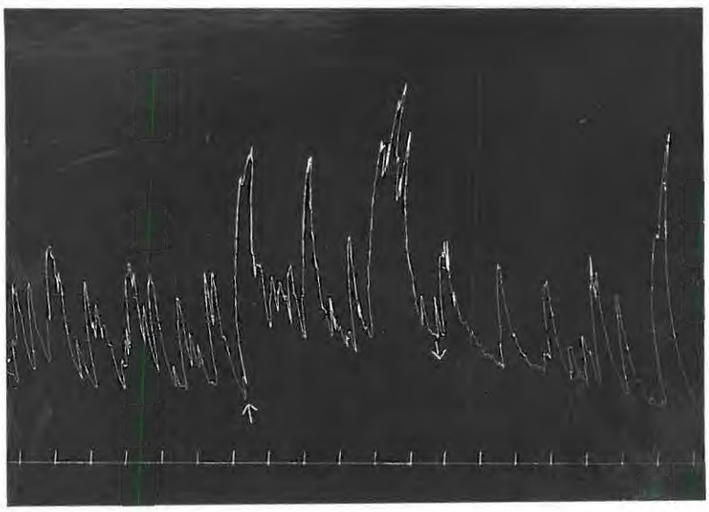
The following arbitrary scheme of quantitative representation of the effects has been used in tables I and III to VII, in which the results of treatments with ions and drugs have been summarised. In these tables '0' indicates no effect; an increase in tone or activity is indicated by a number of '+' signs, graded as follows: the effect on tone was rated according to the magnitude of the increase compared with the average size of the spontaneous contractions in the preliminary record: an increase amounting to less than 50% of the contraction size was rated '+'; 50-75% as '++'; 75 - 100% as '+++'; and more

Fig. 17.

(a)



(b)



(c)

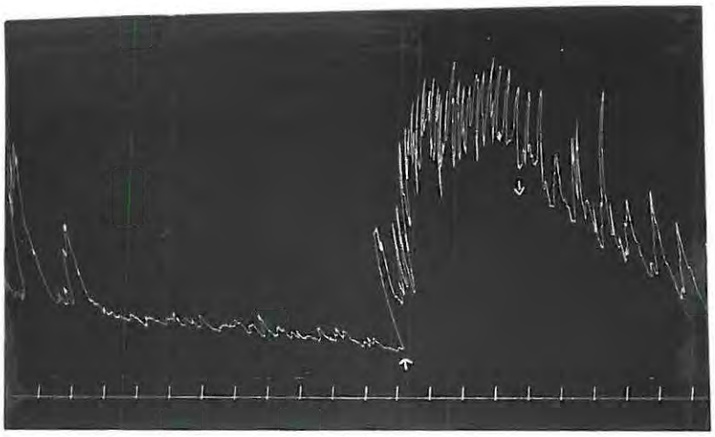


Fig. 17: The action of excess Ca^{++}.
(a) Ca^{++} x3; (b) Ca^{++} x6; (c) Ca^{++} x8.
In this, and all subsequent records, the first arrow, reading from left, to right, indicates addition of the new solution; the second arrow indicates washing out with fresh sea water.

than 100% as '++++'. The effect on rate of activity was rated as follows: an increase of less than twice the normal frequency was rated as '+'; 2-4 times as '++'; 4-8 times as '+++'; and more than 8 times as '++++'. In the same way, depressant effects were rated as '-', '- -', '- - -' and '- - - -', using the same scheme of grading. Complete abolition of activity as a result of a treatment is indicated by 'Ab'. In most cases, in the tables, the average effect for all the tests performed at each concentration has been presented; in those cases in which extreme variability was found, this is indicated by presenting the alternative effects thus: 0/+, et cetera.

3. The Action of Ions.

The ionic environment of the preparation was altered in two ways: (i) in the case of testing the effect of an excess of any ion, a definite amount of an isotonic solution of the ionic salt was added to a definite volume of sea water; (ii) to test the effect of the absence of any ion, natural sea water was replaced by artificial sea water lacking that particular ion, using the data compiled by Pantin (1946). Tables I and III are tabulations of the effects for single and multiple ion changes respectively. The following is a description of the effects of such treatments.

3.1 Single Ion Effects. (Table I).

CALCIUM: (Fig. 17 (a), (b) and (c); fig. 19).

When a preparation is exposed to an excess of eight or ten times the normal concentration of Ca^{++} [1] in sea water there is an immediate and maintained increase in tonus which may continue to increase for five to ten min; this is usually accompanied by a fairly marked increase in the frequency of contraction, as can be seen in the figure.

For an increase in Ca^{++} concentration of less than eight fold, the effects are very variable. At a concentration of Ca^{++}x6 there is always a maintained increase in tone, but the effect on frequency is variable, being slightly increased in some preparations and unaltered in others.

At concentrations of Ca^{++}x4 or x5 the variability is even more marked. In four out of six cases there was an increase in tone which was, however, not very great, and in the remaining

[1] Hereafter all ions will be represented in this form.

TABLE I.

Single Ion Effects.

		Tone.	Rate.
K^+	x 2	+/ + +	0/+
	x 4	+ + +	+ + +
	x 6	+ + + +	+ + +
Ca^{++}	x 2	0	0
	x 4	0/+ +	0/+ +
	x 6	+ +	+ +
	x 8	+ + +	+ + +
Mg^{++}	x 2	- - -	- - / Ab.
	x 4	- - - -	Ab.
	x 6	- - - -	Ab.
K^+ -free		+/+ + + then 0/-	0/+ + + +, then 0/-
Ca^{++}-free		- - -	- - - -
Mg^{++} -free		+ + +	+ +
Pure NaCl		+ + + +	+ + + +
NaCl-free (replaced by sucrose)		- - -	- -

Fig. 18:

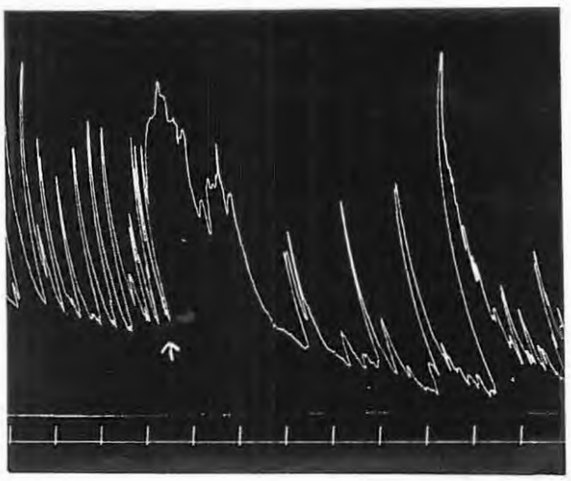


Fig. 18: The effect of artificial sea water.

two, no effect at all. In two of the four cases the effect appeared to wane after 20 min., and the preparation returned to the original baseline. In the remaining two cases there was a clear increase in the rate of contraction. At a concentration of Ca^{++}x3 there was a slight effect on tone in one of three trials only, while at Ca^{++}x2 there is no effect at all.

Before the effects of the absence of any ion can be assessed, it is necessary first to test the effects of artificial sea water which contains all the major constituent ions in the correct proportion. When the normal sea water around an animal is replaced by artificial sea water there is always an initial rise in tonus which is maintained, without increase in frequency of contraction for a period of 10 - 15 min. when a return to the original basic length ensues. (Fig.18). In three out of six cases the basic length in artificial sea water was slightly lower than in normal sea water, but remained constant. After relaxation from the initial increase in tonus there may follow a short period of relative inactivity, lasting for five to ten min., after which activity is resumed. In some cases there seems to be little effect on the activity, but in general, artificial sea water seems to render the activity very irregular, with respect to both size and form of the contractions and to the interval between successive contractions.[1]

These effects of artificial sea water become clearer when they are compared with the result of replacing the sea water around a preparation with fresh but normal sea water. As has already been mentioned, in a preparation in which the frequency of activity has slowed down somewhat this may have the effect of very slightly increasing the rate of contraction; apart from this, changing the sea water is completely without effect and activity continues unaltered after the change.

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[1] Although Pantin (1946) advises that sodium bromide may be omitted from artificial sea water, it is considered that the effects just described may be attributed to the lack of this salt from the artificial sea water used here.

Fig. 19:

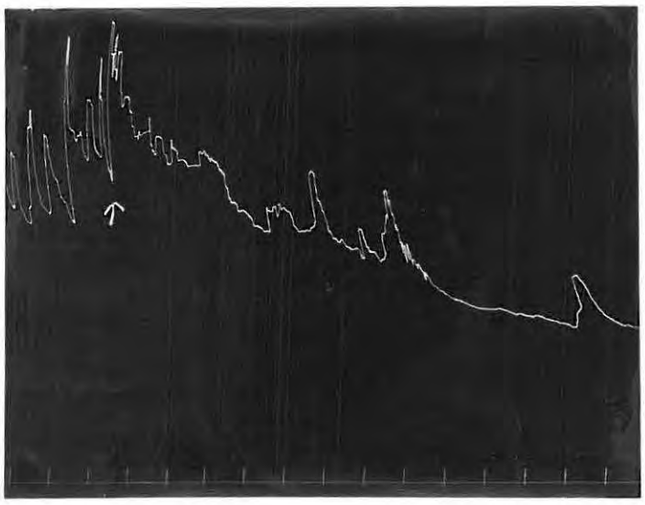


Fig. 19: The action of Ca^{++}-free artificial sea water.

The effect of Ca^{++} deficit was tested in two ways: by first replacing the normal sea water with artificial sea water containing all the constituent ions in their correct proportions, and then, after the preparation has accommodated itself to the artificial sea water, replacing this with artificial sea water lacking Ca^{++}; in other tests the change from normal sea water to sea water lacking Ca^{++} was made directly. In both cases, Ca^{++}-free artificial sea water caused at first a temporary rise in tone: the latter effect was, however, much reduced in a preparation which was first allowed to accommodate to artificial sea water. This initial response is followed by a slow but steady decline in tone and a fall in the rate of contraction. Activity does not, however, appear to be completely abolished in every case, and even after an exposure to water lacking Ca^{++} for two to three hr. there may be an occasional contraction. Washing out usually resulted in a very rapid recovery of activity, even after a lengthy exposure to Ca^{++}-free conditions; recovery of tone was more prolonged. In two of six tests the relative fall in tone appeared very slight and activity continued at a reduced rate.

These experiments were repeated on both intact and decerebrate animals which were free and not set up as a preparation. When a cerebrate animal is exposed to a concentration of Ca^{++}x10, it begins swimming vigorously to and fro until, after about 20 min., the movements become so rapid that co-ordinated locomotion is impossible. A decerebrate animal performs writhing and twitching movements which increase in frequency. When a cerebrate animal is placed in Ca^{++}-free artificial sea water, there is an initial excitation in the form of outbursts of swimming, lasting from 10 min. to as long as an hour; as in the mechanical record, this period was reduced by first allowing the animal to accommodate to artificial sea water. After this initial excitation, the animal remains motionless and gradually relaxes tension. However, even after a period of up to six hours, the animal will usually give a slight response by way of a contraction to a mechanical stimulus. As for the preparation, recovery is rapid and there is a return of slight activity and tone almost immediately the animal is replaced in normal sea water.

Altogether the effects of Ca^{++} are not striking; they may be summarised as follows: Ca^{++}-excess is only markedly excitatory at a very high concentration; Ca^{++}-lack has at first a slight excitatory effect which can be distinguished from the effect of

Fig. 20.

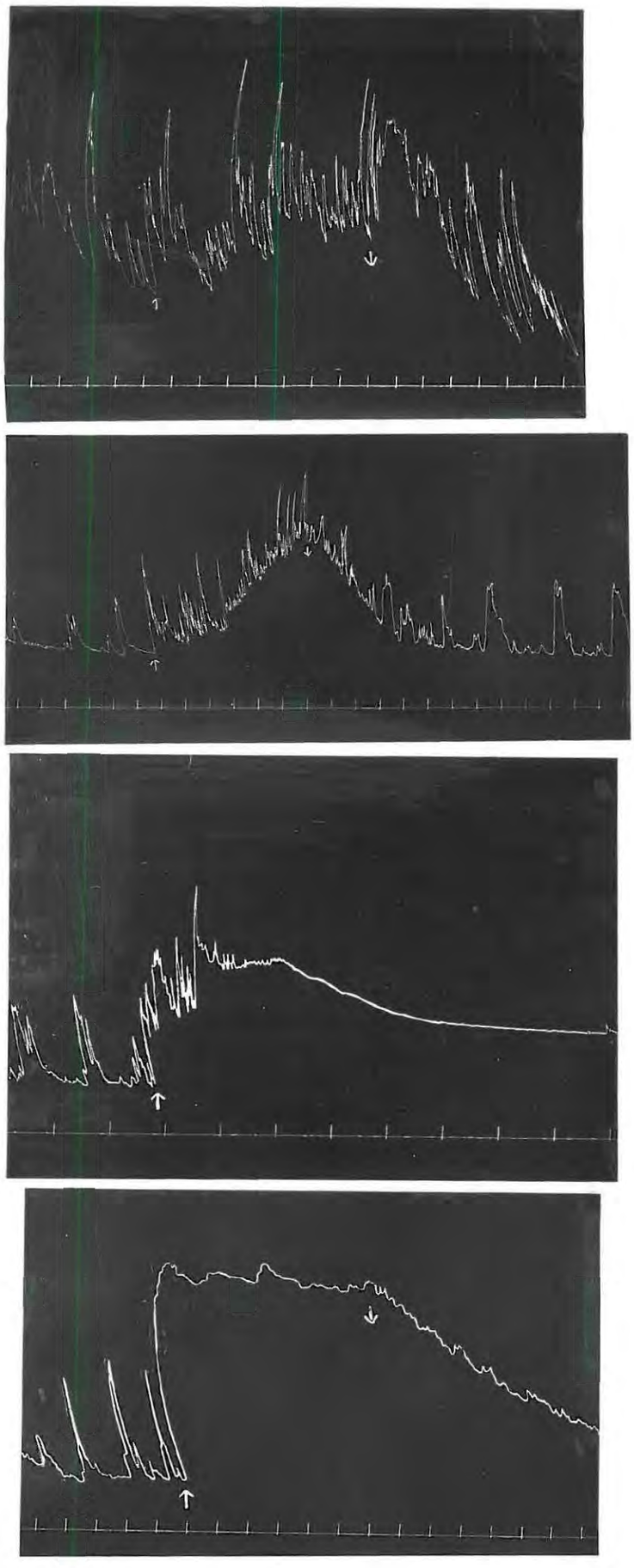


Fig.20: The action of excess K^+.
(a) K^+x2; (b) K^+x4; (c) K^+x4, not washed out; (d) K^+x6.
Time trace of (c), 30 min.

artificial sea water. Thereafter, there is a definite depressant action which is, however, slow to act and does not lead to complete abolition of activity or response to mechanical stimulation.

POTASSIUM: (Figs. 20 (a) - (d); 21(a) and (b)).

The effects of excess K^+ are qualitatively very similar to those of Ca^{++}-excess, but are far more pronounced at all concentrations. Thus even at K^+x2 there is a gradual rise in tone. At concentrations of K^+x3 and above, there is an immediate rise in tone. This may continue to increase for the first five or ten minutes, and is maintained as long as the preparation is in contact with the solution. There is, as well, a marked increase in the frequency of contraction at the new level of tone. All these effects are more marked, the greater the concentration of K^+, until, at concentrations of K^+x6 and above, the increase in tone may be so great that contractions cannot be superimposed and spontaneous activity appears to be abolished. Unlike the responses to excess Ca^{++}, these effects are constant for each concentration of K^+.

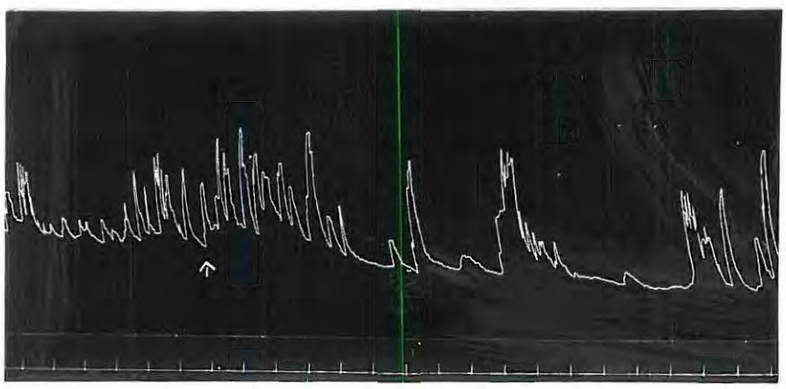
After washing out with normal sea water, there is a rapid return to normal tone and activity rate. Fig 20 (c) shows the effect of prolonged exposure to an excess of K^+x4. As can be seen, there is an eventual decline in the very high induced tone, but it never returns to the original base-line, while activity at this level of tone appears to be abolished; it seems reasonable to interpret the latter effect as a result of extreme fatigue rather than a depressant effect due to long exposure to excess K^+.

Compared with the effects of Ca^{++}-excess, those of K^+-excess are rather dramatic; a glance at the figures shows that the effect of a concentration of K^+x4 may be roughly compared quantitatively with those of a concentration of $Ca^{++}x8$.

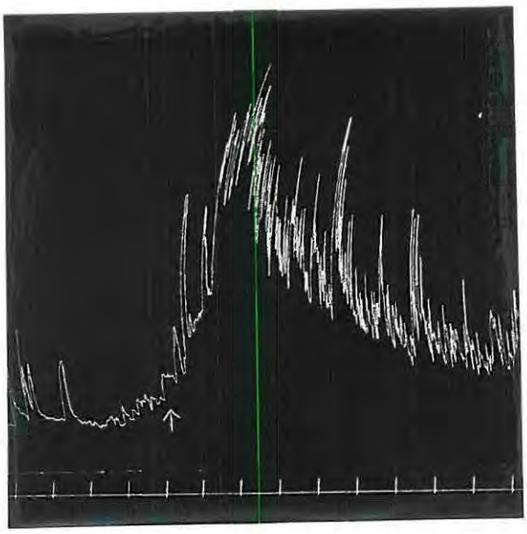
Although the response to excess K^+ is very similar to that of excess Ca^{++}, the effect of K^+-free sea water is not the same as that of Ca^{++}-free sea water. The response to K^+ deficit is rather variable from preparation to preparation, but in no case did the preparation show a simple reversal of the effect of excess K^+.

There was always an initial temporary rise in tone, and this occurred irrespective of whether the preparation had first been allowed to accomodate to artificial sea water or whether the change was made directly. In some cases this was only slight,

Fig. 21.



(a)



(b)

Fig. 21 (a) and (b): The action of K^+-free artificial sea water.

Figs. 22 and 23.

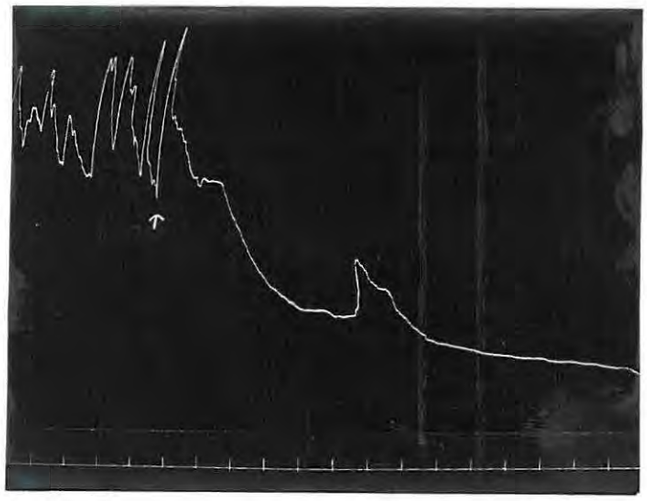
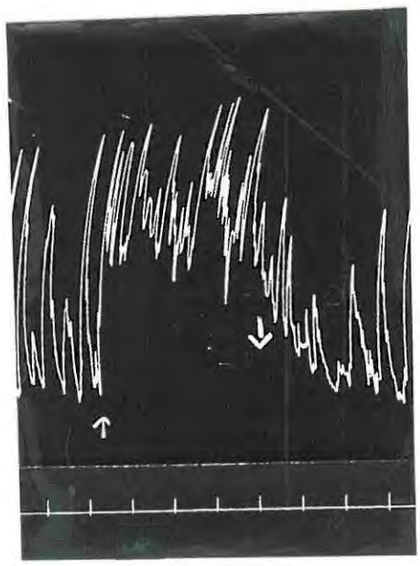


Fig. 22: The action of an excess of Mg^{++} x3.

(a)



(b)

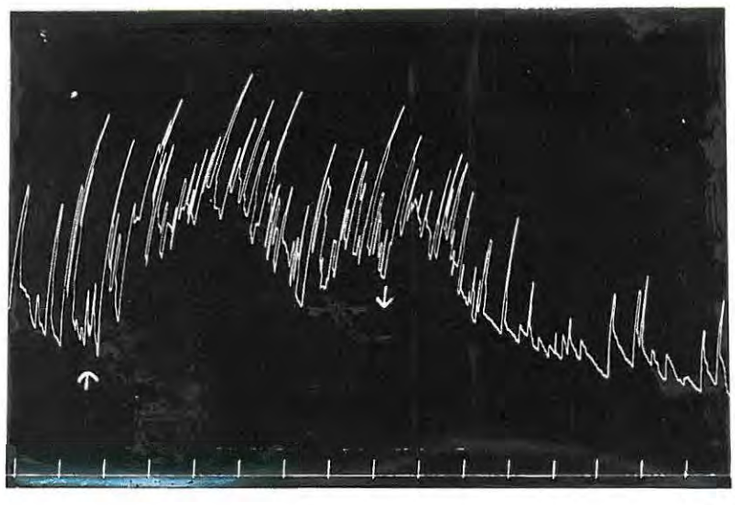


Fig. 23. (a) and (b): The action of Mg^{++}-free artificial sea water.

and the tone fell back to normal within 10 - 30 min; in two of four such cases this was followed by a slight and rather insignificant fall in tone and activity rate. In two further tests, there was not only a very marked rise in tone, but also a pronounced increase in activity rate as the tone fell back to normal; in one trial this continued for as long as two hr. (fig. 20 (b)). This variability renders the effects of K^+- lack difficult to assess with any clarity; there is a general disturbance of normal activity, but in no case was there any sign of a depressant action due to lack of K^+. A number of tests with a concentration of K^+x 0.5 gave the same results as K^+-free sea water, except that no significant initial excitation was shown in response to these tests.

Thus the overall picture is that K^+ are highly excitatory, but that, all other ions being present, they do not appear to be essential for activity and in fact, apart from an initial stimulatory effect, their absence from the environment has surprisingly little effect on activity.

Tests made on whole animals verified the data obtained from the mechanical record of activity in that K^+-excess had the effect of inducing continuous swimming which, at concentrations of K^+x6 rapidly passed into a state of complete tetanus, the margins of the body merely twitching slightly in an uncoordinated manner, but the animal was quite incapable of normal locomotion. Exposure to a medium lacking K^+ first had the effect of causing the lateral margins of the animal to curl ventrally, and then induced a prolonged outburst of swimming. After exposure to this medium for several hr. the animals showed no signs of either depression or stimulation of normal activity, nor inhibition or enhancement of responses to mechanical stimulation or feeding.

MAGNESIUM: (Fig.22; fig. 23 (a) and (b)).

Since $MgCl_2$ has been used as a narcotic on these animals, the depressant action of excess Mg^{++} was to be expected. Even at a concentration of twice the normal there is a rapid fall off in tone; the rate of fall in tone at this concentration is variable from preparation to preparation, but at higher concentrations it is constant and very rapid. The rate of activity, too, declines at a concentration of Mg^{++}x2, but is not always completely abolished at this concentration; at higher concentrations all activity is eventually abolished, the rate of abolition

Fig. 24.

(a)

(b)

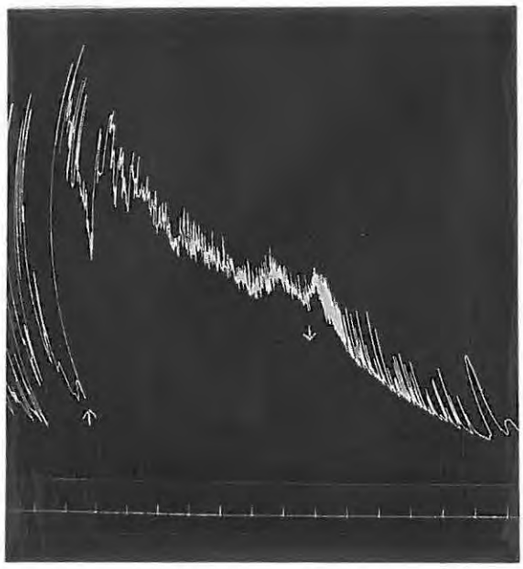


Fig. 24: (a) The response to artificial sea water in which the NaCl has been replaced by isotonic sucrose.
(b) The action of pure isotonic NaCl.

increasing with increasing concentration of Mg^{++}. Recovery from exposure to a concentration of Mg^{++}x6 for one hr. is slow, at least 30 min., but usually much longer being required before any spontaneous activity again becomes apparent.

Treatment with Mg^{++}-free artificial sea water produces a fairly marked increase in tonus, which may reach a peak immediately (fig. 23 (a)) or gradually (fig. 23 b.). The increase in tone is never so great that activity cannot be superimposed at the new level of tone. In addition there is a variable increase in the frequency of contration, but this is never more marked than for K^{+}x4 or Ca^{++}x8.

SODIUM: (Fig. 24 (a) and (b)).

While increasing the concentration of any of the three cations, Ca^{++}, Mg^{++}, or K^{+} by adding the chloride salt to sea water will alter the relative proportions of chloride ion to other anions in sea water, the magnitude of the change is small and it was considered that it could be neglected. This view seems further justified in so far as it later appeared that no specific effect could be attributed to the sulphate and bicarbonate ions. Addition of chloride solutions of particular cations will also affect the chloride/cation balance, but the magnitude of the effect is so small that it, too, has been neglected. Similarly, ommission of the chloride of a particular cation from artificial sea water has a very small effect on the chloride balance.

When, however, it is desired to test the effect of reducing the sodium ion concentration, the problem is more serious as ommission of the sodium chloride from artificial sea water leaves a solution in which the relative proportions of chloride to other anions is vastly decreased. This can be met by replacing sodium chloride by choline chloride. Unfortunately none of this reagent was available and recourse had to be made to the expedient of replacing sodium chloride by an equivalent volume of isotonic sucrose. This is unsatisfactory as the effect observed in such solutions may be due to sodium lack, anion unbalance, or both. For this reason, these experiments are not fully comparable with those already described. Similarly, experiments described below, in which the responses of the preparation of a solution of sodium chloride alone are observed, are not strictly equivalent to such experiments as increasing the concentration of other cations in an otherwise almost balanced solution. Nevertheless, as will become clear, the results of the experiments to be described are significant.

When a preparation is exposed to a solution in which the sodium chloride has been replaced by an equal volume of isotonic sucrose, but in which all the other components of artificial sea water are present in the correct proportions, there is a drop in tone and a slowing down of activity. The activity is, however, never completely suppressed and recovery, on return to normal sea water, is extremely rapid, there being a very sudden return to normal tone and activity. This is thus very different from the effect of Mg^{++}-excess which, also causing a decrease in tone, supresses activity and has a long recovery period.

Exposure to pure NaCl has the most marked of all the excitatory effects witnessed in these tests: there is an immediate rise in tonus, which may fall off slightly within the first halfhour, but never returns to normal as long as the preparation remains in the solution. The most striking effect, however, is on the frequency of activity, as can be seen in fig. 24 (b). This, too, continues as long as the preparation is exposed to pure NaCl. Washing out results in a rapid return to normal tone and activity, provided the preparation has not been exposed to NaCl for longer that approximately an hour; a longer period usually resulted in fatigue from which the preparation did not recover. These effects will be discussed further presently.

SULPHATE AND BICARBONATE IONS:

Excesses of both these ions up to concentrations which were eight times the normal were tested, but with no effect on the preparation other than a slight disturbance of the regularity of the rhythm.

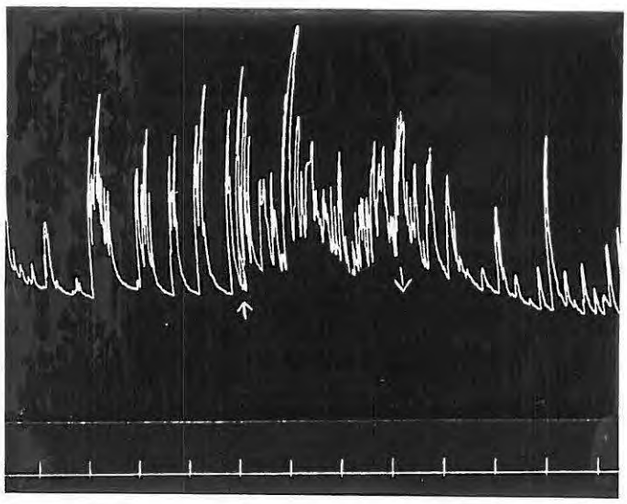
Similarly, artifical sea waters lacking one or other of these ions were tested. In neither case could any specific action, distinguishable from the usual effects of artificial sea water, be recognised. The effects of bicarbonate free sea water in particular were confusing;intwo out of four tests there was a suggestion of slightly greater initial excitation than is normally evidenced when a preparation is exposed to artificial sea water, but if there is an effect, it has thus far not been possible to distinguish it from the effects of artificial sea water which contains all the constituent ions.

3.2) Ionic Balance:

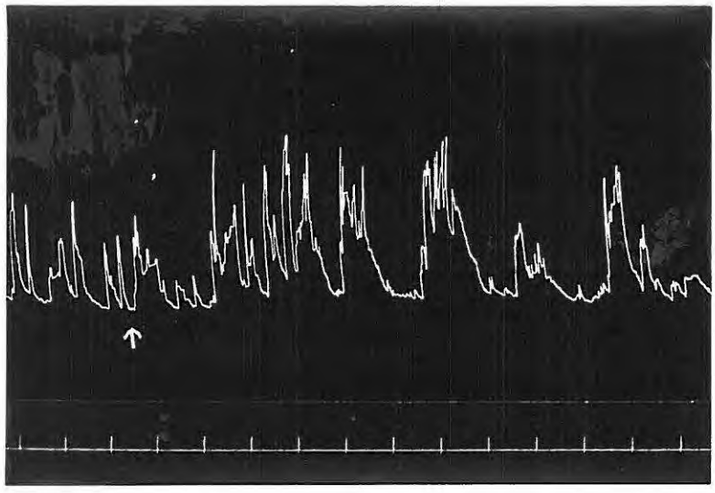
On the basis of these tests it would appear that calcium, potassium, magnesium and sodium all exert definite effects on the animal. There remains, however, the possibility that in

Fig. 25.

(a)



(b)



(c)

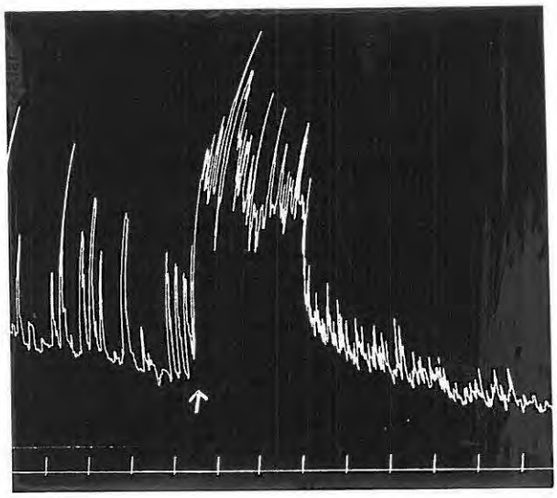


Fig. 25: The response to excess Ca^{++} when the Mg^{++} is adjusted to the correct concentration for artificial sea water.
(a) Ca^{++} x4; (b) Ca^{++} x5; (c) Ca^{++} x8.

any of the tests applied, the effect attributed to an excess or lack of any particular ion may simply be due to a corresponding slight decrease or rise in concentration of another ion, not with respect to the ion whose effects are being tested, but with respect to the solution as a whole. This may be explained more clearly as follows: when an excess of any ion is added to a given amount of sea water, the total volume is increased, so that the concentration of each of the other ions in solution is effectively decreased. Table II shows the effect of increasing the concentration of a single ion on the concentrations of the other ions in the sea water. It can be seen that the effect of adding excess K^+ is the smallest, yet a concentration of K^+x6 involves a change in concentration of the other ions of some eight per cent. The effect of adding Mg^{++} is the greatest, involving a 30 per cent change for a concentration of Mg^{++}x4. Thus it may be argued that the effect observed in any of the tests involving the excess of any single ion may be due to the corresponding reduction in concentration of another ion or ions.

This may be checked as follows: in adding an excess of, for example, Ca^{++} or K^+ to a definite volume of sea water, the amount of Mg^{++}, which should be present in that volume for the concentration of Mg^{++} to be normal, can be calculated and the correction made by adding the required amount of isotonic $MgCl_2$.

The results of such experiments are described below, and, as will be seen, they emphasise the importance of the concentration of Mg^{++} relative to the other cations.

CALCIUM: (Fig. 25 (a) - (c)).

Since the effects of Ca^{++} excess and Mg^{++} deficit are alike in causing hyperactivity, while excess Ca^{++} and K^+ deficit do not have opposing effects, it is obvious that if the excitatory effect of Ca^{++} is an indirect effect, this is likely to be due to a deficit in the Mg^{++} concentration. When compensation is made for the Mg^{++} concentration, the effect of excess Ca^{++} is diminished, but not abolished altogether. At a concentration of Ca^{++}x8 there is still a marked rise in tone and activity rate, but in more than half the tests both these effects waned after a period varying from 10 to 20 min. and there was a return to the normal base-line and contraction frequency before the solution was washed out with normal sea water. At lower concentrations, because of the great variability in the magnitude of the response, even when the Mg^{++} concentration was not adjusted, it was difficult to assess the effect very accurately; the increase in tone appeared to

TABLE II.

The effects of excess of one ion on the concentrations of other ions.

Treatment.	Decrease in concentration of other ions expressed as a percentage of normal concentration.
K^+ x 6	8.28%
Ca^{++} x 8	16.38%
Mg^{++} x 2	12.72%
Mg^{++} x 4	30.42%

Figs. 26 and 27.

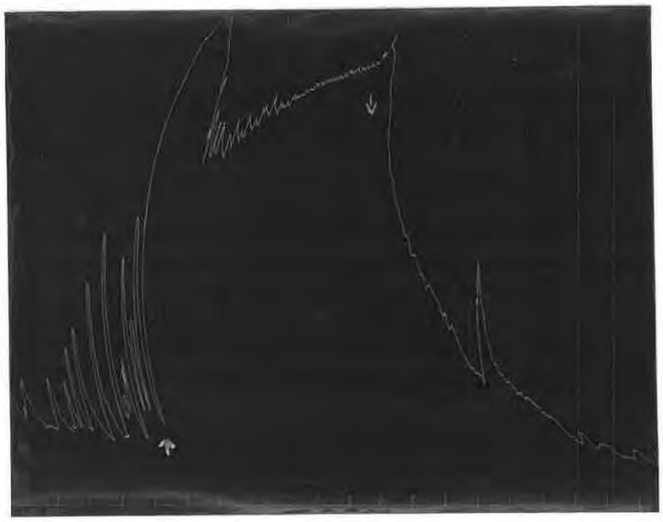


Fig. 26. The action of six times normal K^+ when the Mg^{++} concentration is adjusted.

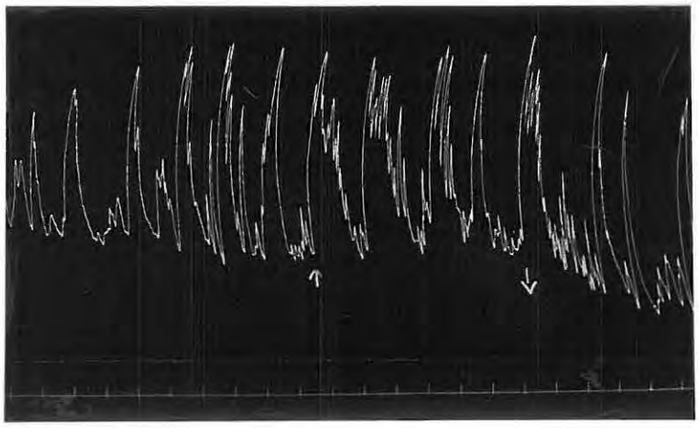


Fig. 27: The action of a solution of NaCl containing the correct amount of $MgCl_2$ for artificial sea water.

be reduced and while in some cases increased tone and activity rate were maintained, in more than half the tests there was no effect at all on tone or activity, even for Ca^{++}x5.

Making compensation for the deficit in K^+ concentration, appeared to have no effect on the response to excess Ca^{++}, and the variability was no more marked than for the unadjusted solution.

POTASSIUM: (Fig. 26.).

Since the effect of a concentration of K^+x6 is more marked than that of Mg^{++}-free sea water, and since such an increase in the K^+ concentration causes only an 8% reduction in the Mg^{++} concentration, no marked reduction in the effect of excess K^+ was to be expected; in fact, adjustment of the Mg^{++} concentration had no effect at all on the response to excess K^+.

SODIUM: (Fig. 27.).

When the preparation is exposed to a solution of NaCl with the correct amount of Mg^{++} for that volume, the dramatic effect of pure NaCl is completely abolished. There is no increase in tone, but there is some evidence of an excitatory effect either in the form of an increase in frequency or as outbursts of activity; this slight hyperactivity is, however, in no way comparable with the effect of pure NaCl.

It thus appears that, in the presence of a normal concentration of Mg^{++}, sodium chloride has only a very small excitatory effect, and the striking response shown in pure sodium chloride solution (fig.24b) is to be regarded as an expression rather of Mg^{++}-lack than of a major excitatory action of Na^+ itself. That the abolition of the excitatory effect of pure NaCl is due to the addition of Mg^{++} and not simply to a dilution of the NaCl is shown as follows: a solution of NaCl and sucrose, where the sucrose replaces in volume all the missing components of sea water, was tested and the effect found to be identical with that of pure NaCl alone.

The difference between the behaviour of an animal in pure NaCl and in NaCl with Mg^{++} was also observed by placing free animals in such solutions. In pure NaCl the animal initially curled up with the anterior and posterior ends turned ventrally and overlapping one another; thereafter the animal showed signs of extreme hyperactivity and continued to writhe and twist in a series of rapidly repeated and unco-ordinated contractions for as long as it was left in the solution. In NaCl plus Mg^{++} the animal did not curl up, nor was there any sign of increased

tonus, but the animal continued to crawl round and round the container. This differs from the excitatory effects witnessed in the case of either K^+ or Ca^{++} excess, in that the latter always induced continuous swimming activity, until the increased tonus became so great that co-ordinated locomotion became impossible.

MAGNESIUM:

Since the action of excess Mg^{++} is more marked that that of Ca^{++}-free artificial sea water, and since K^+ deficit appeared to have little effect, it is not surprising that adjustment of either the Ca^{++} or the K^+ concentration, or the concentrations of both ions simultaneously did not diminish the effectiveness of Mg^{++} in depressing the activity and lowering the tone of the preparation.

Experiments with ion lack, using artificial sea waters corrected for the effect of the absence of one ion by adding isotonic NaCl showed no differences from those already reported with unadjusted mixtures.

In review it may be said, therefore, that whilst K^+, Ca^{++} and Na^{++} all have an excitatory effect, that of K^+ is by far the most outstanding. Lack of K^+, on the other hand, does not appear to have any depressant effect. Absence of Ca^{++} produces at first a slight excitation and then a definite, although rather slow depressant effect, while NaCl deficit results in a fairly marked fall in tone, with a slight fall off in activity. It is the Mg^{++} ions that have the most clear-cut opposing actions with excess and lack, the former being highly inhibitory and the latter producing hyperactivity. The importance of the inhibitory action of Mg^{++} is markedly emphasized by the difference in response to pure NaCl and to an NaCl solution containing the correct amount of Mg^{++} found in normal sea water. Indeed, the normal low level of tone cannot be achieved simply by the absence of the two excitatory ions Ca^{++} and K^+; it positively required Mg^{++} as evidenced by the marked tonus displayed in pure NaCl solutions.

3.3. Multiple Ion Effects. Table III.

It has been shown that changes in the ionic environment of the animal may have profound effects on its behaviour. Clearly the correct ionic environment for normal functioning may depend upon the absolute concentration of each ion species in sea water, or upon the relative concentrations of certain ions which exert antagonistic actions upon the myo-neural system. Thus, for example, in Calliactis Ross (1960) finds that any increase in Mg^{++} concentration above that found in normal sea water has a

TABLE III.

Multiple Ion Effects.

	Tone	Rate
K^+ x 2 Ca^{++} x 4	+ + +	+ +
K^+ x 2 Ca^{++}-free	0	0
K^+ x 2 Mg^{++} x 2	- -	- -/Ab.
K^+ x 4 Mg^{++} x 2	+ +, then -	0/+, then-
K^+ x 6 Mg^{++} x 2	+	0/+
Ca^{++} x 2 Mg^{++} x 2	0/- -	0/- - -
Ca^{++} x 4 Mg^{++} x 2	0/+, then 0	0
Ca^{++} x 6 Mg^{++} x 2	0/+, then 0	0
Ca^{++}-free Mg^{++}-free	+/+ + +	0/+ +
K^+ -free Ca^{++}-free Mg^{++}-free	+ +	+ +
Mg^{++} x 2 Ca^{++} x 2 K^+ x 2	0	0
Mg^{++} x 4 Ca^{++} x 4 K^+ x 4	0/+	0

Figs. 28 and 29.

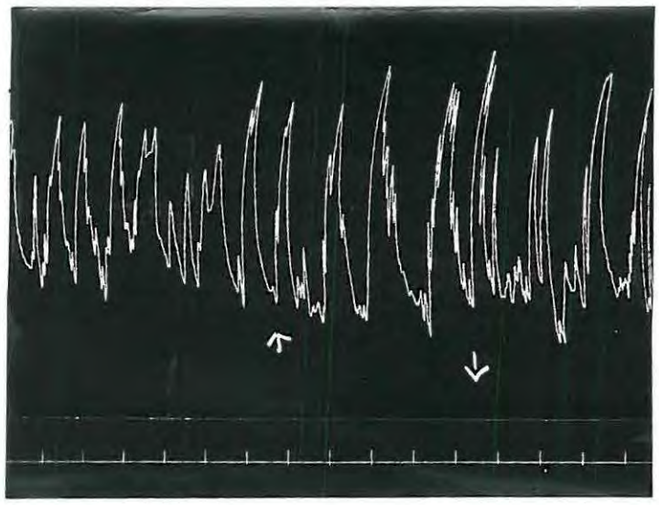
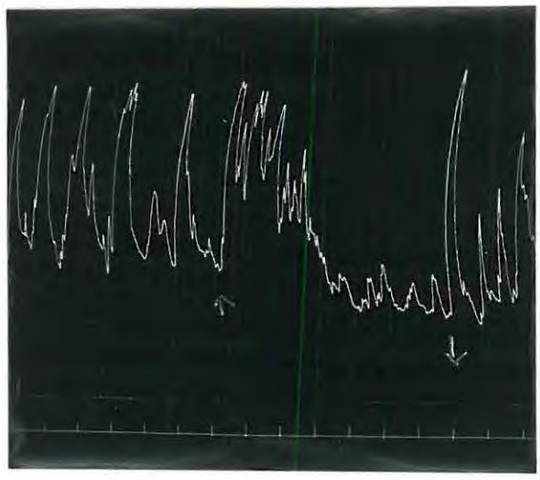


Fig. 28: The action of Ca^{++}-free artificial sea water containing twice the normal K^{+}.

(a)



(b)

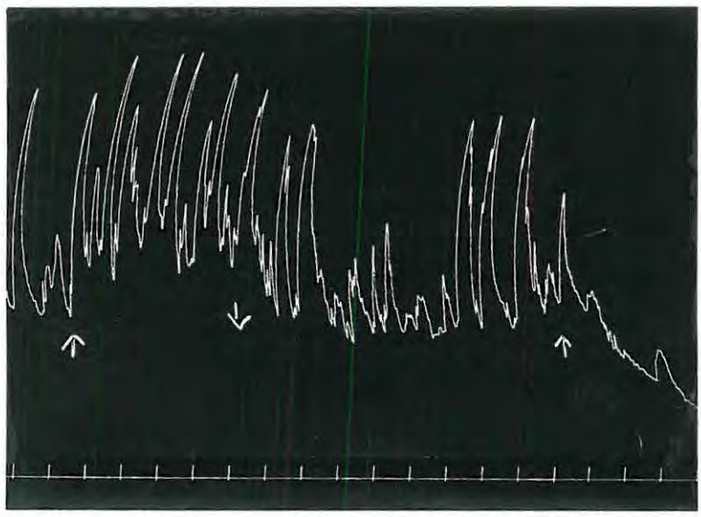


Fig. 29: The interaction of K^{+} and Mg^{++}.
(a) K^{+}x4, Mg^{++}x2; (b) K^{+}x6, Mg^{++}x2; the third arrow indicates addition of Mg^{++}x2.

depressant action which cannot be off-set by increasing the concentration of other cations, while in many other tissues which display spontaneous activity it is known that limited changes in the concentration of one ion species can be balanced by alterations in the concentration of some other ion or ions. Thus in the tortoise heart the concentrations of Ca^{++} and K^{+} may be increased or decreased three fold without affecting the activity of the heart, provided the relative proportions of the two ions remains the same (Clarke, 1927).

The following tests, in which the concentration of two or three ions was changed simultaneously, were designed to investigate the nature of the interaction between different ions.

Interaction of Ca^{++} and K^{+}: (Fig. 28).

The simultaneous raising of the Ca^{++} and K^{+} concentrations seemed to have a simple additive effect, but since the action for each ion alone varies in magnitude, it is difficult to assess the effect with any degree of accuracy.

An increase of $K^{+}x2$ was found to abolish the effect of Ca^{++}-lack in two out of three trials, there being no effect on tone or activity in these two trials. At higher concentrations of K^{+} the effect of Ca^{++} deficit was over shadowed by the excitatory effect of K^{+}.

Interaction of Mg^{++} with K^{+}: (Fig. 29 (a) and (b)).

A series of different combinations of Mg^{++} and K^{+} was tested as follows: at a concentration of $Mg^{++}x2$, $K^{+}x2$ there is a slow fall-off in tone and a delayed depressant effect on activity; but, although delayed, both the effect on tone and that on activity were essentially the same as the effects of $Mg^{++}x2$ alone. A concentration of $K^{+}x4$, $Mg^{++}x2$ produces at first a sharp rise in tone; a state of high tone is maintained for 15 to 20 min. after which there is a sudden fall-off in tone to a level slightly lower than before the treatment. In one case, during the initial period of high tone, increased frequency was observed; once the tone had fallen again the level of activity was, in all cases, slightly reduced. Thus at this concentration of K^{+} and Mg^{++} the two effects practically balance one another. At a concentration of $K^{+}x6$, $Mg^{++}x2$ there is usually an effect which is roughly equivalent to $K^{+}x2$.

Thus if excess Mg^{++} is added together with sufficient K^{+} to bring the Mg^{++}/K^{+} ratio back to that found in sea water, the effect of Mg^{++} dominates. A new balance when the Mg^{++} is double is achieved when the concentration of K^{+} is multiplied by

Figs.30 and 31.

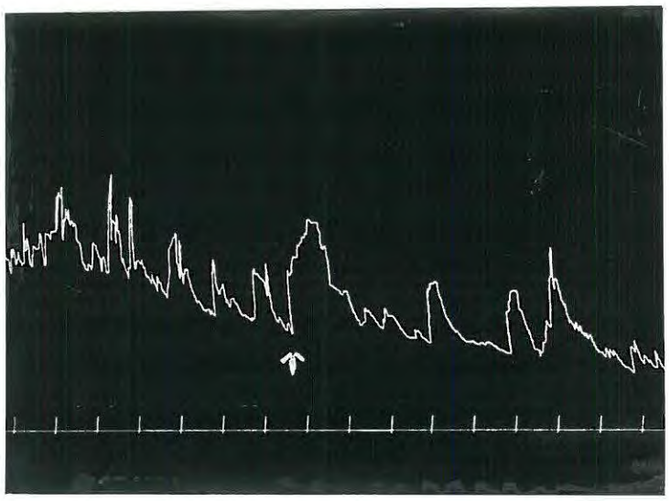


Fig. 30: The interaction of Ca^{++}x4 and Mg^{++}x2.

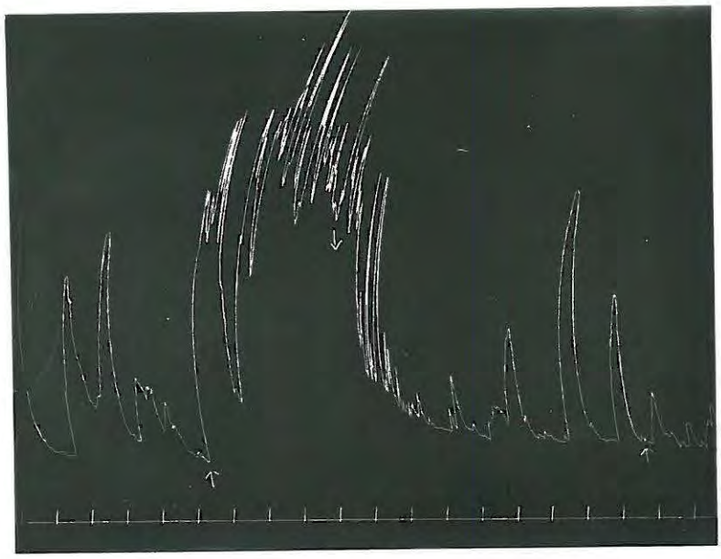


Fig. 31: The effect of artificial sea water lacking both Ca^{++} and Mg^{++}.

four or six. Clearly normal activity is not determined simply by the ratio of K^+/Mg^{++} found in sea water.

Interaction of Ca^{++} and Mg^{++} : (Figs. 30 and 31).

As for K^+, the interaction of an excess of Mg^{++} with an excess of Ca^{++} was tested by exposing the preparation to a series of combinations of different concentrations of the two ions. For Ca^{++}x2, Mg^{++}x2 there was, in three of five trials, a slow depressant effect, somewhat less marked than for Mg^{++}x2 alone. In the other two trials the usual effects of Mg^{++}x2 were not witnessed, and no effect on tone or activity was observed. For concentrations of Ca^{++} of x4 to x8 the effect of Mg^{++}x2 was abolished but only very slight signs of hyperactivity were observed in some cases.

When the preparation was exposed to artificial sea water lacking both Ca^{++} and Mg^{++} the effect was essentially that of Mg^{++}-lack; this response was marked in some of the tests, and quantitatively similar to the effect of Mg^{++}-lack, while in others it seemed to be considerably reduced, giving rise to only an ill-defined period of hyperactivity.

The fact that in some trials the effect of excess Mg^{++} was abolished by bringing the Mg^{++}/Ca^{++} ratio back to that of normal sea water, together with the fact that Mg^{++}x2 can only be balanced by an excess of K^+x4 or x6, raises the possibility that the "activity" of these ions is directly proportional to their valency, but clearly a wide range of concentrations would have to be tested to substantiate such an hypothesis. However, in some trials, with a normal ratio of Mg^{++}/Ca^{++}, the Mg^{++} effect dominated. In view of this it was considered possible that the significant balance in sea water may not be that of Ca^{++}/Mg^{++} or of K^+/Mg^{++}, but of $K^+ + Ca^{++}/Mg^{++}$. Four tests in which the K^+ and Ca^{++} concentrations were raised by the same relative amounts as the Mg^{++} concentration showed that while activity became irregular, the depressant action of Mg^{++} was abolished. This was not, however, tested for concentrations of Mg^{++} greater than four times the normal.

In the course of these experiments two further effects emerged. The first concerns the action of the anions of sea water, It was earlier reported that no effects could be detected by adding an excess of either sulphate or bicarbonate ions to normal sea water, or by omitting either of these ions from an artificial sea water solution. If, however, the artificial sea water lacking K^+, Ca^{++} and Mg^{++} - that is, containing Na^+ as the only cation, but with the anion composition of artificial sea water - is compared with that of isotonic sodium chloride, the latter is found to be far more

Fig. 32.

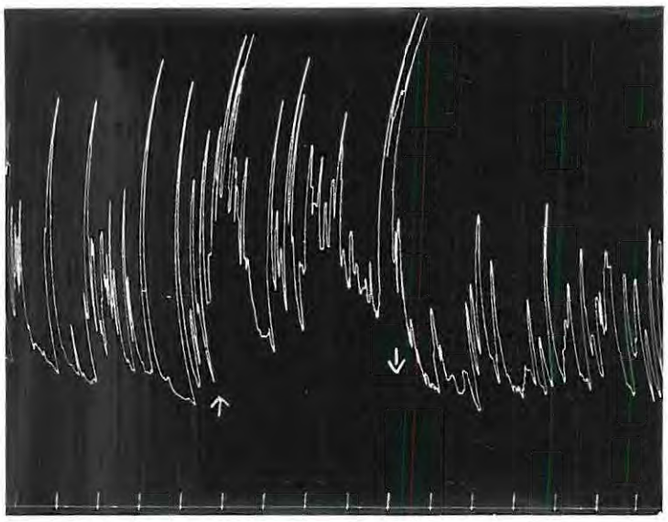


Fig. 32: The action of artificial sea water lacking Ca^{++}, Mg^{++} and K^{+}.

excitatory in its effects than the former. (cf. fig. 32 with fig. 24 (b)). Further, the irregularity of activity of a preparation in which the Mg^{++}, Ca^{++} and K^{+} concentrations are increased, but the ratio between these ions maintained at that of normal sea water, would indicate that the other anions and sodium are not without importance in maintaining the normal activity of the animal. The second concerns the excitatory effect of artificial sea water lacking both Ca^{++} and Mg^{++} compared with that lacking Ca^{++}, Mg^{++} and K^{+}. While it might be expected that an artificial sea water containing Na^{+} alone would be less excitatory than one containing Na^{+} and K^{+}, this is not found to be the case. Indeed, the latter is, if anything, less excitatory, suggesting some antagonism between the excitatory actions of K^{+} and Na^{+}.

It is thus clear that, with the exception of Mg^{++}-lack, the effects of changing the concentration of any one ion can be balanced by simultaneously changing the concentration of another ion. In other words, the conditions for normal functioning of the neuro-muscular apparatus of the animal are maintained by a rather delicate balance between the different ionic components of sea water. The roles of the different ions may be summarised as follows: K^{+} are excitatory, but are not essential for contraction; Ca^{++} are not markedly excitatory, but are essential for the maintainance of tone and normal activity in the presence of the normal amount of Mg^{++}; Mg^{++} are essential for relaxation - the hyperactivity caused by their absence cannot be overcome by simultaneous absence of any other ion. The role of Na^{+} is rather less clear, but the presence of either Na^{+} or Cl^{-} would appear to be necessary for the maintainance of tone.

4. The Action of Drugs.

The drugs used in these experiments were selected either because they are known or believed to act as synaptic transmitters or as blocking agents at the neuro-muscular junctions in other animals, or else because, although their mode of action is un known, they have been found to have effects on the neuromuscular systems of other invertebrates, and thus provide material for comparison. The number of drugs available was very limited; however, those that have been used in these tests are representative of the different groups of drug-types used in experiments on other invertebrates. They will be treated in four groups in the account

which follows:

4.1 Acetylcholine and drugs which act on cholinergic junctions;

4.2 Sympathomimetic amines and their antagonists;

4.3 Indolalkylamines and their antagonists;

4.4. Some miscellaneous substances.

Since solution of many of these drugs altered the pH of the sea water, it is important first to note the effect of a change of pH of normal sea water. This was done by adding small quantities of hydrochloric acid or hydroxyl ions in the form of sodium hydroxide to sea water and no effect on the activity of the animal was found over a range from pH 5 to pH 9; above or below this range the change caused hyperactivity although no increase in tone. In spite of this apparent tolerance of a rather wide range of pH values, wherever it was possible to do so without altering the solubility of the drug, the solutions were adjusted to bring the pH back to that of normal sea water. In no case was any test made with a solution of a pH outside the above range.

The results obtained are summarised in tables IV - VII, and are reported below. [1]

4. 1 ACETYLCHOLINE AND DRUGS ACTING AT CHOLINERGIC JUNCTIONS:

Table IV.

It is well known that cholinesterase is extractable from the tissues of representatives of nearly all the major invertebrate phyla tested. (Prosser, 1946, Table III). Large quantities of cholinesterase have been found in the bodies of flatworms Dendrocoelum and Planaria (Bullock and Nachmansohn, 1942) and in Procerodes (Bacq, 1937). However,the relevance of such information to the nature of the transmitter-substance is extremely suspect, for the following reasons: firstly, what little information we have as regards the effects of acetylcholine itself on the neuromuscular systems of invertebrates does not correlate well with the relative amounts of cholinesterase extractable from the tissues of the same animals; secondly, the cholinesterase need not necessarily be associated with a cholinergic mechanism of synaptic transmission: thus it is known that a cholinesterase is associated with the mechanism of active transport

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[1] The form in which each drug was used and the manufacturers of the drug are given in Appendix. I.

TABLE IV.

The effects of drugs in group 1.

Treatment		Tone.	Rate.
Acetylcholine	1.10^{-3}	0	0
Acetylcholine	1.10^{-4}	0	0
Eserine	1.10^{-4}		
Escrine	1.10^{-4}	0	0
	1.10^{-3}	0/++++	++++, then- - or Ab.
Curare	1.10^{-4}	0	0
	1.10^{-3}	0/+	0

Figs. 33 and 34.

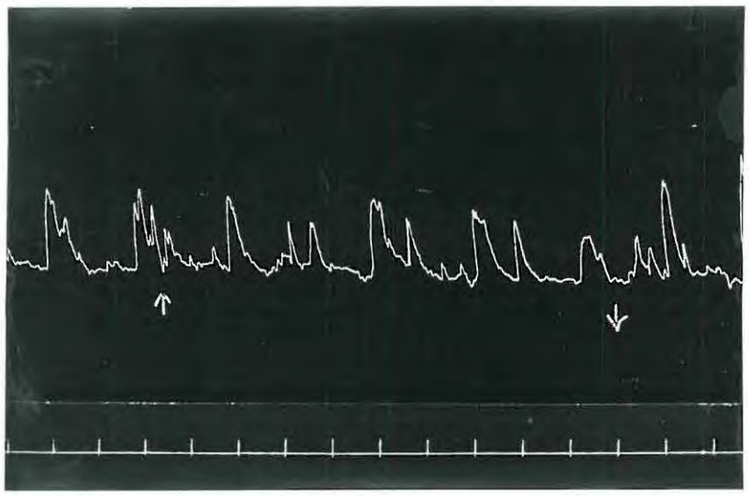
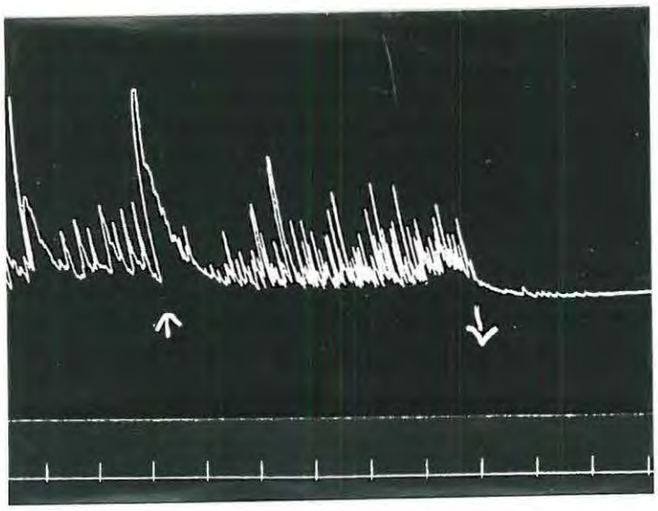


Fig. 33. The action of acetylcholine at 5.10^{-4}M.

(a)



(b)

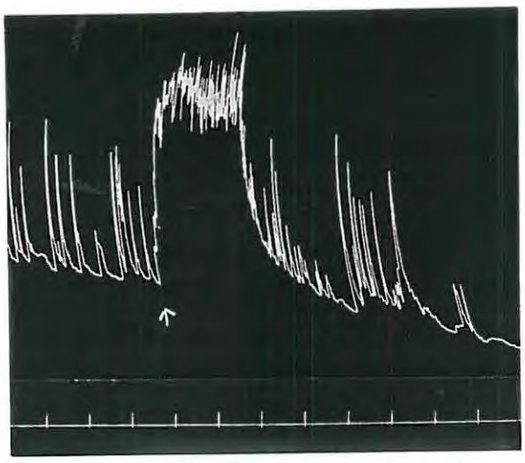


Fig. 34: The action of eserine: (a) at 1.10^{-3}M; (b) at 2.10^{-3}M.

of Na^+ (Koch, 1954), and it is likely that this may be of fairly general occurrence; further, there is evidence that ciliary mechanisms are associated with a cholinergic process (Burn and Day, 1958; Milton, 1959), and any cholinesterase associated with cilia would be present in extracts from the whole animal or even certain parts of the animal. The only reliable method of determining the presence or absence of a cholinergic mechanism is to observe the physiological effects either of acetylcholine itself, curare (known to block cholinergic junctions) or eserine (known to potentiate the effects of cholinergic transmission due to its blocking action on cholinesterase. With this in mind, the responses of P. gilchristi to acetylcholine, curare and eserine have been tested.

Acetylcholine and Eserine: (Figs. 33; 34 (a) and (b)).

A number of invertebrate preparations have been shown to be sensitive to fairly low concentrations of acetylcholine and also to eserine, which prolongs the effects of nervous stimulation (Bacq, 1947).

In Planocera acetylcholine itself has no effect on the preparation, even at a concentration of 1.10^{-3}M. Eserine on the other hand, has a very marked effect at a concentration of 1.10^{-3}M. The rate of activity is very greatly increased, while the effect on tone is variable, two tests showing a marked enhancement of tone, while in a further two there was no effect at all on tone. In those tests which showed enhanced tone, this fell rapidly after a period of 10 - 15 min., as did also the rate of activity, while in one further test, all activity was abolished after the eserine solution had been washed out. It is known that in vertebrates the effect of eserine is to block the action of cholinesterase, thus prolonging the life of acetylcholine liberated at the nerve endings, which, after an initial excitation, produces a permanent state of depolarization and acetylcholine block. It might appear that the above effect of eserine is, in fact, a result of acetylcholine block, after an initial excitation, but the fact that there is no effect at 1.10^{-4}M eserine would indicate that this should be regarded as being due to some adverse physiological action of the eserine at so high a concentration. Further, simultaneous treatment with acetylcholine and eserine both at concentrations of 1.10^{-4}M is without effect. The latter result together with the complete absence of any response to acetylcholine itself renders it further likely that the initial excitatory effect

Fig. 35.

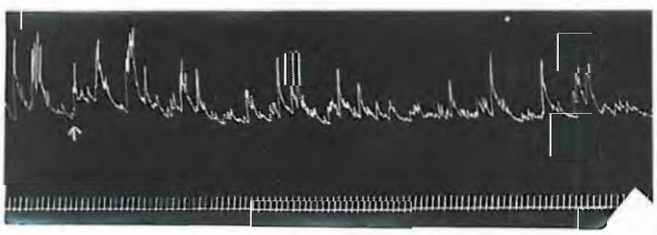


Fig. 35: The action of curare at 5.10^{-4}M.
Tiem trace, 5 min.

of eserine at a very high concentration is purely one of sensory stimulation; that is, the drug is an irritant. It would clearly be desirable to carry the investigation of this effect further by testing the response of the animal to other anti-cholinesterases, but thus far no other drug of this nature has been available.

Curare: (Fig.35).

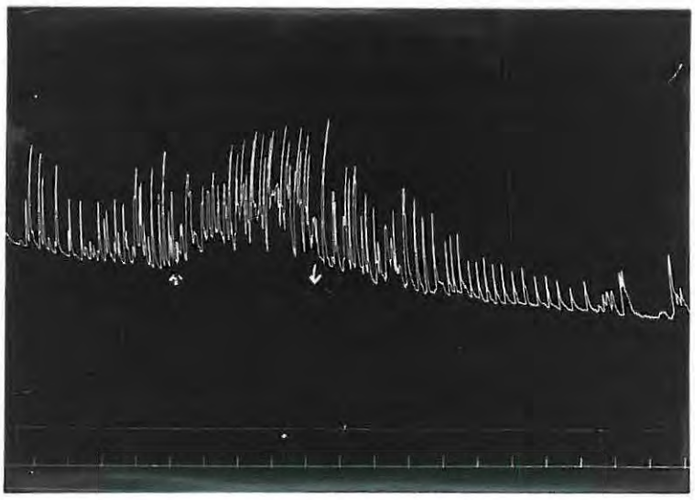
This drug, known to block cholinergic junctions in vertebrates, has no effect on the activity of a preparation of P. gilchristi, nor is there any blocking of normal locomotory patterns in the free, intact animal. At a concentration of 1.10^{-3}M there is sometimes a slight indication of hyperactivity and general disturbance of the pattern of activity; the latter can probably be regarded merely as a result of an irritant action of so high a concentration. Even after exposure of many hours to such a concentration there is no loss of tone nor any sign of diminishing activity, while the response to mechanical stimulation is likewise never abolished.

An interesting observation was made on free animals. In normal sea water, a very characteristic feeding response can be elicited from both cerebrate and decerebrate animals when food extract is liberated close to the animal from the end of a pipette. When such animals are exposed to a solution of curare at 1.10^{-4}M, this response is completely abolished after a period of approximately 10 to 15 min. The fact that decerebrate animals will show the response indicates that the block is peripheral and not central. Further, the fact that locomotory activity and response to mechanical stimulation remain normal in curarised animals indicates that this is a very specific block of some sensory transmission.

These results lead very clearly to two conclusions; firstly, apart from the apparently rather anomalous effect of eserine at high concentrations, there is no evidence at all for a general system of cholinergic transmission in this animal; secondly, there appears to be a peripheral, sensory cholinergic link. The latter may, in part, account for the large amounts of cholinesterase extractable from the body of the three triclads mentioned above.

Fig. 36.

(a)



(b)

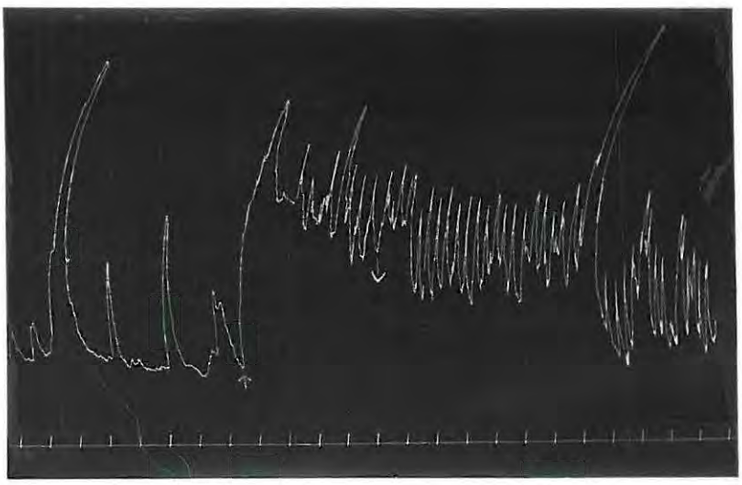


Fig. 36: The action of adrenaline.
(a) 1.10^{-5}M; (b) 5.10^{-4}M.

4.2 SYMPATHOMIMETIC AMINES AND THEIR ANTAGONISTS: Table V.

The effects of adrenaline and nor-adrenaline on the sympathetic nervous system of vertebrates and the role of nor-adrenaline as a sympathetic transmitter substance are well known; in addition, a number of other catechol amines, such as tyramine, act in a similar way.

The presence of adrenaline and nor-adrenaline in the nerve cord of annelids and insects has been demonstrated (Gaskell, 1914; Östlund, 1954), but experiments with other invertebrates were inconclusive. Another unidentified catechol amine ("catechol - 4") has been extracted from Noctiluca, Metridium and Mytilus, as well as from some insects which appear to contain considerable quantities of catechol amines (Östlund, 1954). Further, a definite response to adrenaline has been demonstrated in a number of invertebrate preparations (review by Bacq, 1947; see also Ross, 1960). The role of such substances in the myo-neural systems of invertebrate animals is thus of considerable interest.

Adrenaline: (Fig. 36 (a) and (b)).

Adrenaline has a marked excitatory effect on this preparation and acts at concentrations which, while high compared with the effective concentrations for vertebrate and some other invertebrate preparations, are low compared with the concentrations at which other drugs affect this preparation. Thus at a concentration of 5.10^{-6}M there is a slight increase in tone, while at 1.10^{-5}M the response is quite distinct: at this concentration there is a gradual rise in tone. At still higher concentrations, however, there is an immediate and maintained increase in tone, and a clear increase in the rate of activity, this latter rising to three to six times the normal rate at concentrations of 1.10^{-4} or 1.10^{-3}M.

Nor-adrenaline: (Fig.37).

Like adrenaline, this drug has an excitatory effect on the preparation. It is, however, not effective at concentrations below 5.10^{-5}M. At higher concentrations the effect on tone is usually the same as that for adrenaline at comparable molar concentrations, but the effect on rate of activity is very variable. In order to compare the effects of the two drugs more effectively, eliminating the possibility of individual variations from preparation to preparation, a number of tests were performed

+TABLE V.

The effects of drugs in group 2.

Treatment.		Tone.	Rate.
Adrenaline	1.10^{-6}	0	0
	5.10^{-6}	+	0/+
	1.10^{-5}	++	++
	1.10^{-4}	+++	+++
Nor-adrenaline	1.10^{-5}	0	0
	5.10^{-5}	+	0
	1.10^{-4}	+++	++
Tyramine	1.10^{-4}	0/+	0
	1.10^{-3}	+	0
3-Hydroxy-tyramine	5.10^{-4}	+	0
	1.10^{-3}	++	0
Ergotamine(1)	1.10^{-4}	0	0
Yohimbine	1.10^{-4}	0	0
	1.10^{-3}	+++	++++
	1.10^{-3} (2)	0	0

1 The effect of Ergotamine on the response to Adrenaline.

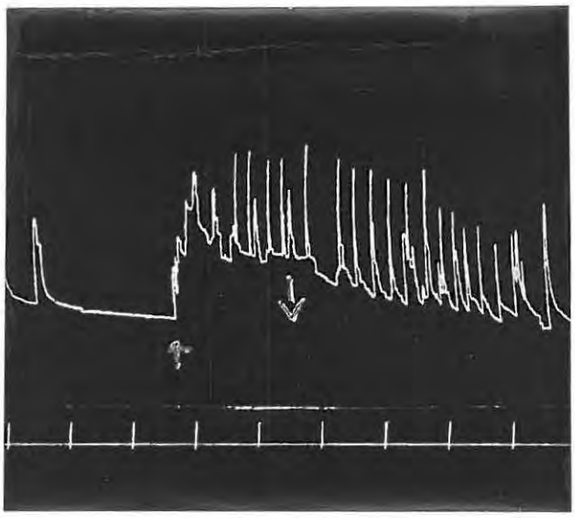
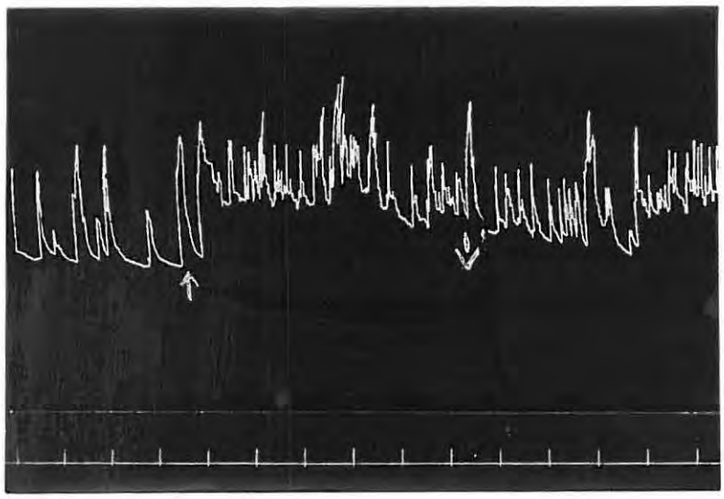


Fig. 37: The action of nor-adrenaline at 1.10^{-4}M.

Fig. 38.

(a)



(b)

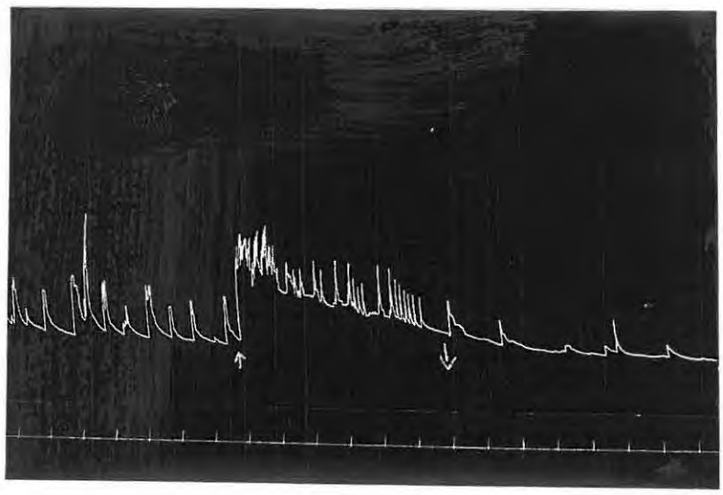


Fig. 38: The action of adrenaline and nor-adrenaline on the same preparation.
(a) Adrenaline at 1.10^{-4}M;
(b) Nor-adrenaline at 1.10^{-4}M.

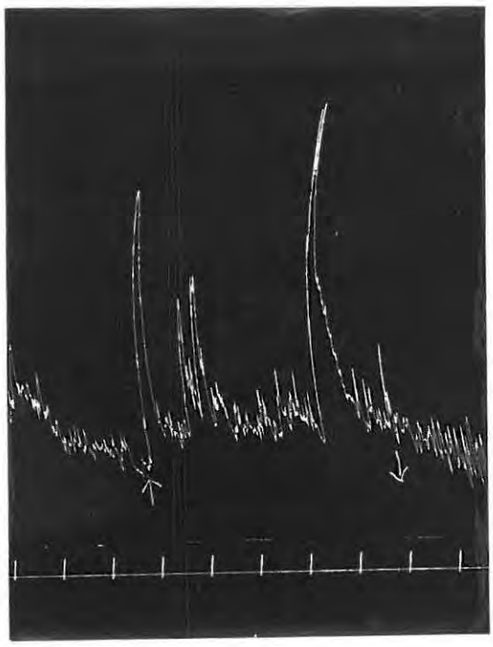
in which the two drugs were tested successively on the same preparation and at the same molar concentration, (fig.38 (a) and (b)). The order in which the drugs were tested was reversed in different tests and at least one and a half hours allowed to elapse after the initial test was made before the next was applied. Using this method, it was found that at concentrations lower than 1.10^{-4}M the effect of nor-adrenaline on tone is weaker than that of adrenaline, while nor-adrenaline has no effect on the rate of activity at these concentrations. Tests at concentrations of 1.10^{-4} or 1.10^{-3}M usually showed a comparable increase in tone, while only half the tests showed a comparable effect on the rate of activity: while the effect of adrenaline is relatively constant for the same molar concentrations, that of nor-adrenaline is not, being clear-cut in some tests and negligible in others. Further, the effect of nor-adrenaline may wane after approximately 20 min., before the solution has been washed out. It is clear that the effect of nor-adrenaline is less marked than that of adrenaline, and the variability in the magnitude of the response may be related to its weaker action.

Tyramine: (Fig. 39 (a) and (b)).

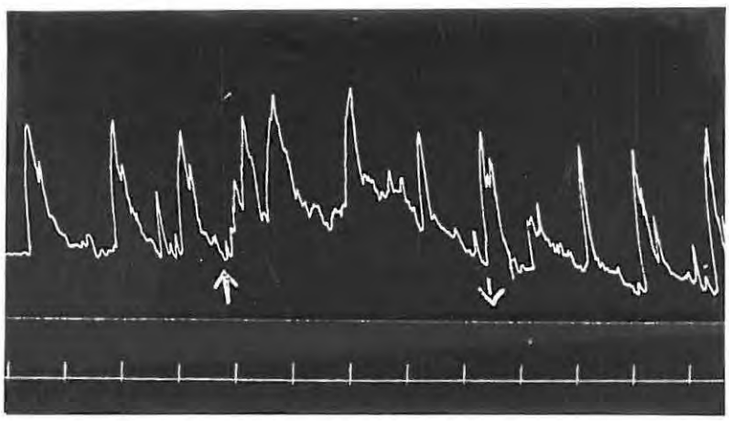
Tyramine has been found to have an adrenomimetic effect on a number of vertebrate preparations. Thus Fange and Östlund (1954) demonstrated such an action on the hearts of a number of marine vertebrates as well as on that of a cephalopod (Eledone cirrosa), the effective concentration in all cases being, however, much higher than for adrenaline. Similarly, Castelnuova (1952) demonstrated a very variable excitatory effect of tyramine on the amphibian heart.

Tyramine, at a concentration of 1.10^{-3}M, was found to have only a slight effect, if any at all, on a preparation of P.gilchristi in five of six tests; a very slight effect on tone was observed, but this was not usually maintained for more than 20 min., while there was no effect at all on activity rate in these tests. In a sixth test, there was a fairly marked rise in tone and an increase in activity rate; however, this could not be repeated, and would appear to be atypical of the response of Planocera to tyramine. At lower concentrations there was no effect at all on the preparation.

Fig. 39.



(a)

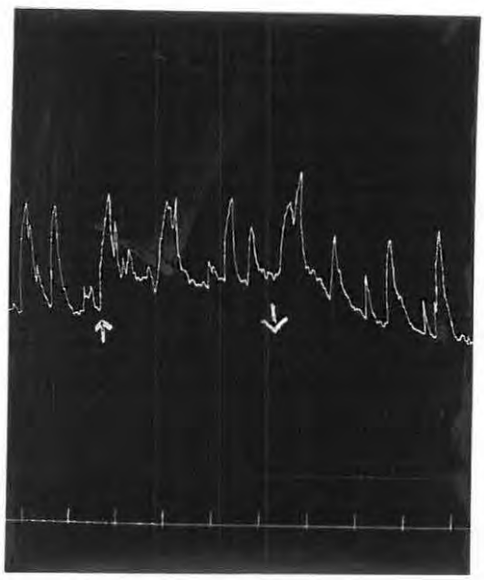


(b)

Fig. 39: The action of tyramine:
(a) at 1.10^{-4}M; (b) at 1.10^{-3}M.

Fig. 40.

(a)



(b)

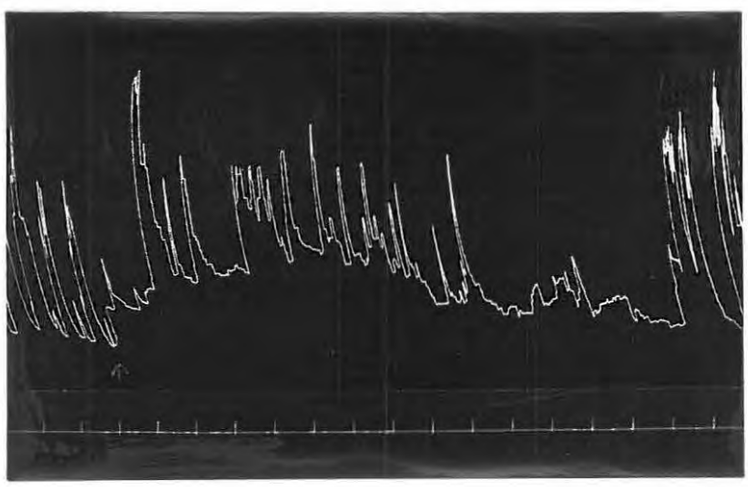


Fig. 40: The action of 3-hydroxytyramine:
(a) at 5.10^{-4}M; (b) at 1.10^{-3}M. In (b) the solution was not washed out.

3-Hydroxytyramine: (Fig. 40 (a) and (b)).

In comparing the effects of different drugs it is of interest to note any structural similarities or differences between them; adrenaline and nor-adrenaline are both 1:2 hydroxy-compounds, while in the carbon chain the methyl group of adrenaline is missing in nor-adrenaline (Fig. 41). It thus seemed possible that the similarity in their effects could be related to the identical position and number of the hydroxyl groupings in the two compounds. In this respect it is of interest to compare their effects with the rather negligible effect of tyramine, the 1-hydroxy-analogue of nor-adrenaline. It thus seemed desirable to test the action of the 3-hydroxy-analogue of tyramine, namely 3-hydroxytyramine.

Like tyramine, the effects of this drug are not at all marked: at concentrations of 5.10^{-4}M or 1.10^{-3}M it causes a slight increase in tone, which may, however, wane after about 30 min. The effect on rate of contraction is, if anything, to produce a slowing down; the latter effect is, however, so slight that the possibility cannot be excluded that such a change in rate of contraction may not have occurred spontaneously. Certainly there is no enhancement of the contraction rate, while activity seems to have become rather irregular at 1.10^{-4}M concentrations of the drug.

Antagonists of Sympathomimetic Amines:

Certain substances have been found to act as blocking agents at adrenergic junctions in vertebrates. There are a number of groups of such substances, which include some ergot and indole alkaloids. From each of these two groups, one substance, known to be effective in vertebrates, namely ergotamine and yohimbine respectively, have been tested on P. gilchristi.

These substances do not dissolve readily in sea water, even when the pH is adjusted to that of distilled water. Quantities of each drug which amounted to concentrations of 1.10^{-5}M to 1.10^{-3}M were made up and used, but in no case was solution complete and it is impossible to estimate the exact concentrations. In the description which follows, the concentrations referred to are those which would have resulted had all the substance dissolved.

Ergotamine:

This was found to have no noticeable effect on the preparation at any of the concentrations tested, and addition

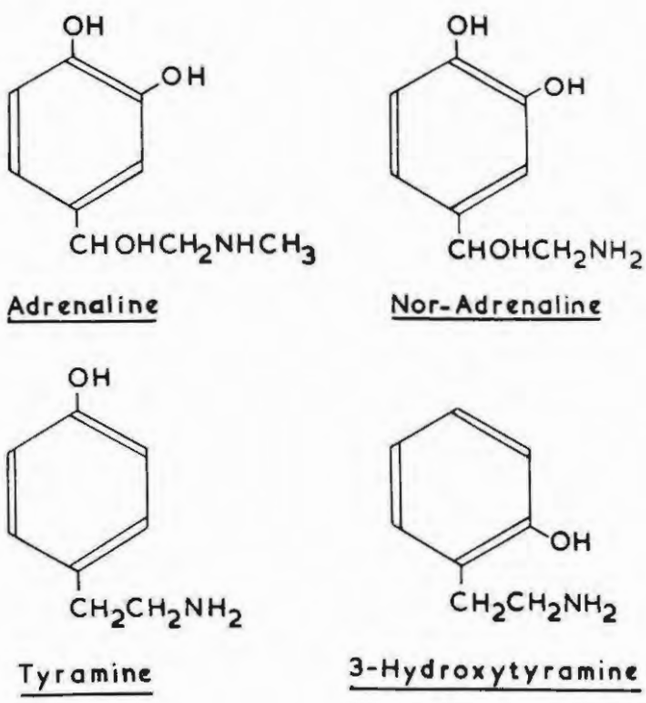


Fig. 41: Diagram showing the structural relationship between the three compounds - adrenaline, nor-adrenaline, tyramine and 3-hydroxytyramine.

of adrenaline after exposure to ergotamine for a period of one to two hours elicited the usual response.

Yohimbine:

At a concentration of 1.10^{-5}M yohimbine had no effect; at 1.10^{-4}M there was no effect in three cases, but in a fourth there was, at first, an apparent excitatory effect which took the form of an increase in the rate of activity, but not tone; after washing out, activity was abolished and the tone fell; immediate addition of a solution of adrenaline failed to elicit a response. Even in a solution of 1.10^{-3}M adrenaline, this preparation remained inactive for two hr. In five tests at 1.10^{-3}M there were clear signs of hyperactivity in the form of increased tone and activity rate; washing out with normal sea water resulted in a rapid return to the normal base-line and activity rate, while washing out with adrenaline resulted in a typical maintainance of tone and activity rate, for as long as the preparation was exposed to the adrenaline solution.

The general tenor of these observations is to suggest that this drug can act as an irritant. This is not due to the presence of particles of the drug in suspension, for tests with clear solutions prepared by decanting, gave, at 1.10^{-3}M similar results to those obtained with a suspension. The only observation which runs contrary to this interpretation was that made by a suspension at 1.10^{-4}M. Here the drug appeared to act in a depressant manner and to inhibit the normal response to adrenaline. In this case, however, the preparation failed to recover from the drug treatment, even after four hr., suggesting some physiological abnormality. To conclude, on the basis of this single observation, that yohimbine can exert an antagonistic effect to some adrenergic type of activity is certainly not warranted.

4. 3. INDOLALKYLAMINES AND THEIR ANTAGONISTS: Table VI.

Interest has recently been aroused in this class of substances by the discovery of the powerful neuro-muscular action and wide-spread occurrence of 5-hydroxytryptamine (5-HT) (Page, 1954; 1958). It has been identified in extracts from the tissues of a number of invertebrates (Erspamer, 1948; Welch, 1953) and experiments have led to the suggestion of a possible transmitter role of 5-HT in some invertebrates

TABLE VI.

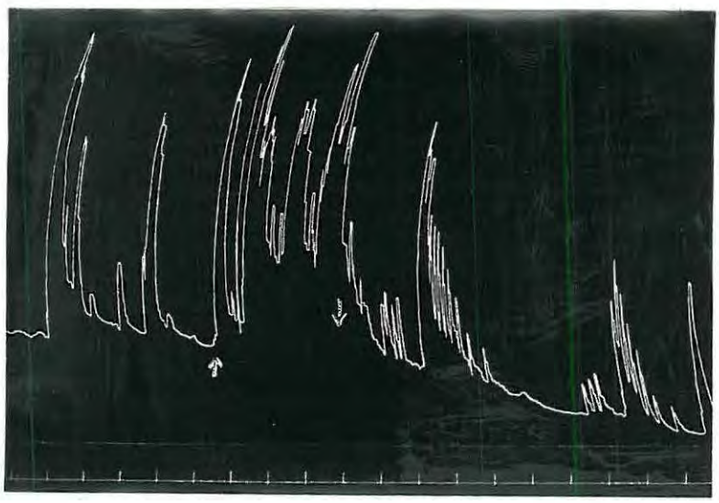
The effects of drugs in group 3.

Treatment.		Tone.	Rate.
5 - H T	1.10^{-5}	+	0
	5.10^{-5}	+	0
	1.10^{-4}	++	0/+
	1.10^{-3}	++	0/+
Tryptamine	1.10^{-4}	0	0
	1.10^{-3}	+++	++++
L S D - 25	1.10^{-6}	0	0
	1.10^{-5}	++	0
	1.10^{-4}	++	+
	1.10^{-3}	++	++
B O L	1.10^{-5}	0	0
	1.10^{-4}	0	0
	1.10^{-3}		
Harmine	1.10^{-4}	+, then -	+, then -
Yohimbine[1]	1.10^{-3}	0	0

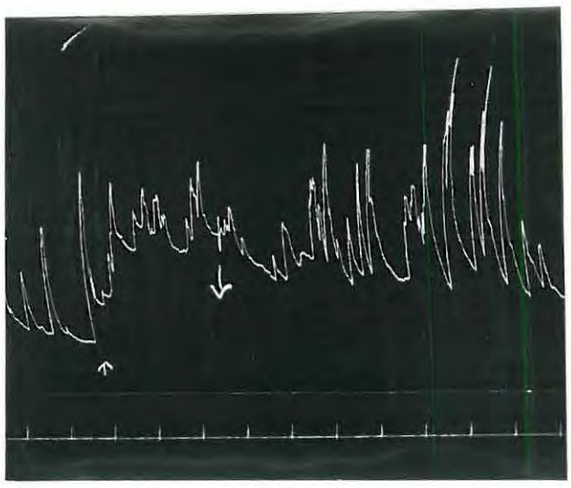
(1) The effect of yohimbine on the response to 5-HT.

Fig. 42.

(a)



(b)



(c)

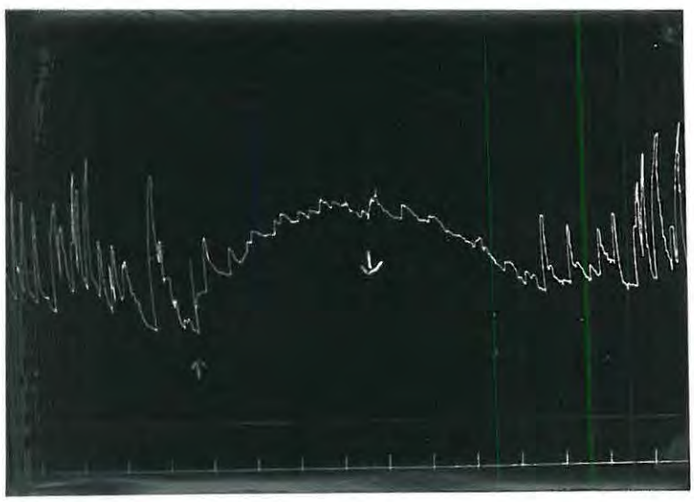


Fig. 42: The action of 5-hydroxytryptamine:
(a) at 5.10^{-5}M; (b) at 1.10^{-4}M;
(c) at 1.10^{-3}M.

(Erspamer, 1954; Welsh, 1956). Further, a powerful action of 5-HT on a preparation of the liver fluke, Fasciola hepatica, has been demonstrated (Mansour, 1957).

5-Hydroxytryptamine: (Fig. 42 (a) - (c)).

The effects of 5-HT are very variable. A response in the form of a fairly marked increase of tone was obtained in two of four tests at a concentration of 1.10^{-5}M 5-HT, and in one of these there was a temporary increase in the rate of activity, lasting for about 15 min. But, although a response may be obtained at a concentration of 1.10^{-5}M, the effect of a 1.10^{-4}M solution is still rather variable, there being a clear increase in tone in three of five tests at this concentration; in one of the remaining two tests there was an initial increase in tone which waned after approximately 15 min., while in the last there was only a negligible effect on tone. At 1.10^{-3}M the effect on tone is more constant, but no more pronounced than at 1.10^{-5} or 1.10^{-4}M, while no clear effect on activity rate could be seen. [1]

Tryptamine: (Fig. 43 (a) and (b)).

Like 5-HT this substance has an excitatory effect, but while the minimal effective concentration is much higher, being 5.10^{-4}M, the response to a 1.10^{-3}M solution is far more marked that that to 5-HT at any concentration. There is a marked and maintained rise in tone and a very vigorous increase in the rate of activity. Even after washing out, although the tone usually returns rapidly to the normal base-line, the increased rate of activity continues, sometimes for an hour or more.

Lysergic acid diethylamide: (Fig. 44 (a) and (b)).

Lysergic acid diethylamide (LSD-25) has been shown to antagonise the effect of 5-HT very effectively in mammalian preparations (review by Page, 1958), while in a number of invertebrate preparations it has an effect which is similar to the

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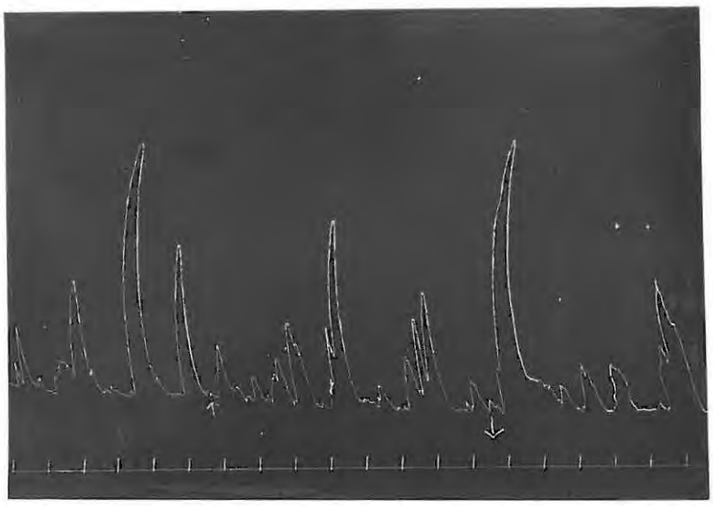
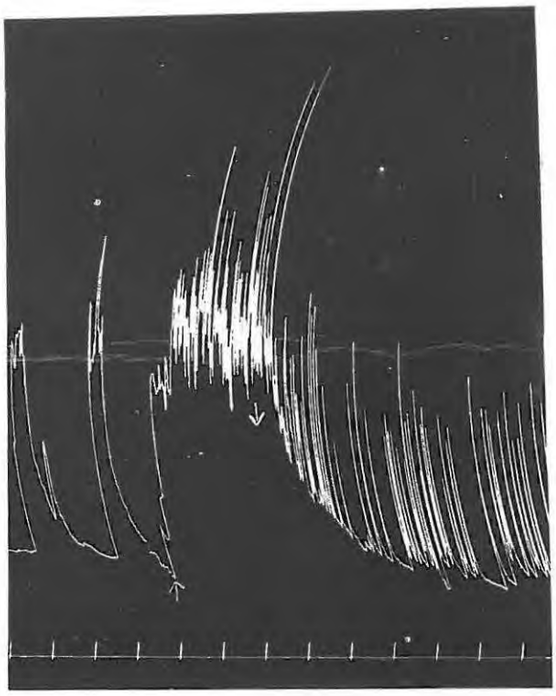
[1] Since this substance was used in the form of the creatinine sulphate, it would clearly be desirable to perform a control experiment using creatinine sulphate alone, but thus far such an experiment still has to be done.

Fig. 43.



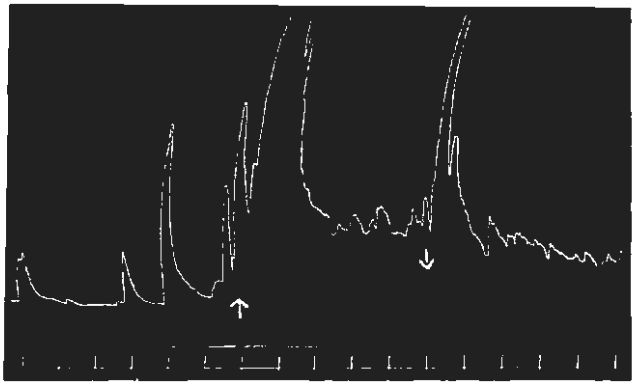
(a)



(b)

Fig. 43: The action of tryptamine:
(a) at 1.10^{-4}M; (b) at 1.10^{-3}M.

(a)



(b)

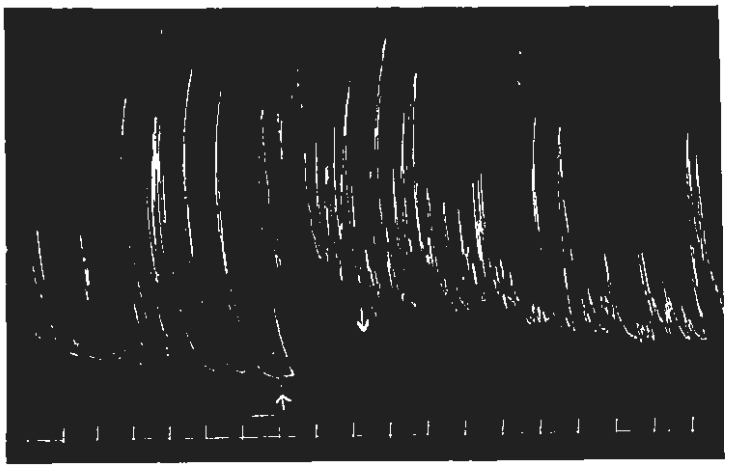


Fig. 44: The action of LSD-25.
(a) at 1.10^{-5}M; (b) at 1.10^{-4}M.

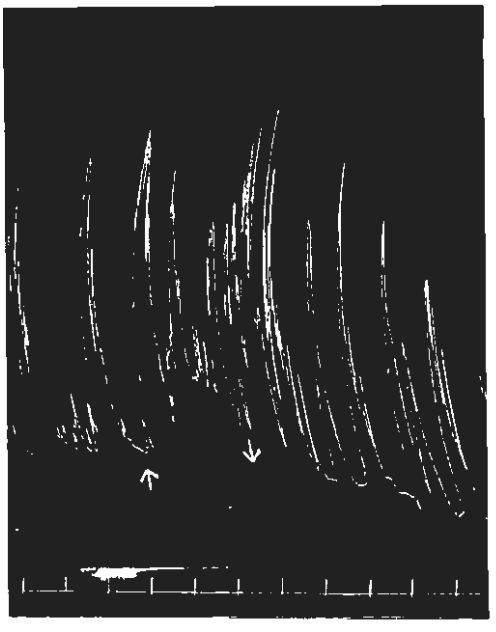


Fig. 45: The action of BOL at 1.10^{-4}M.

action of 5-HT (Shaw and Woolley, 1956; Welsh and McCoy, 1957); similar effects have been demonstrated on the liver fluke in particular (Mansour, 1957). Since the tryptamine structure is present in the molecule of LSD-25, clearly it is possible that the drug may act either as an antagonist or as an analogue of 5-HT.

Planocera resembles the other invertebrate preparations tested in that the effect of LSD-25 is, like that of 5-HT, an excitatory one. The minimal effective concentration is 1.10^{-5}M, when there is an increase in tone, but no evidence of increased rate of activity. At higher concentrations there is, as well, a slight increase in the rate of activity, but this is never comparable with the great effect of tryptamine at a concentration of 1.10^{-3}M.

Antagonists of 5-HT:

Three compounds, all of which contain the indole structure and which may be regarded as complex analogues of 5-HT, have been found to depress activity in the intact liver fluke and to antagonise the effects of 5-HT or LSD-25 (Mansour, 1957); these compounds are bromolysergic acid (BOL), harmine and yohimbine. Further, BOL has been found to be a more effective antagonist than LSD-25 on mammalian preparations (Page, 1958) and also antagonises the action of 5-HT on the Venus heart (Welsh and McCoy, 1957). It was thus considered to be of interest to examine their action on preparations of P.gilchristi

Bromolysergic acid: (Fig. 45).

BOL was tested repeatedly at concentrations of 1.10^{-5}, 1.10^{-4} and 1.10^{-3}M, but with no clear-cut depressant effect. Since experiments on the liver fluke have shown that a decerebrate preparation is unaffected by BOL, these tests were carried out on both intact and decerebrate preparations, but with the same results. In two tests at 1.10^{-4}M and in all tests at 1.10^{-3}M there was an initial stimulatory effect, followed by a slight decrease in tone and a very slight slowing down of activity after the drug had been washed out. This is not very easy to distinguish from the changes in activity which may occur in normal sea water, but would appear to be indicative of a slight depressant action. Three tests were performed in which 5-HT was

Fig. 46.

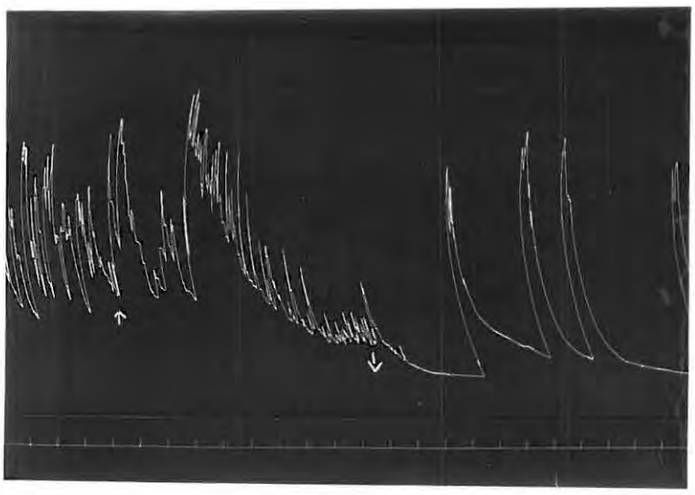


Fig. 46: The action of harmine at 1.10^{-4}M.

added to the environment of the preparation after exposure to BOL for 30 to 60 min; in two cases the effect appeared normal, but in the third the addition of 5-HT had no effect on the preparation; however, the variability in the action of 5-HT itself renders it impossible to base any clear conclusion on this/one experiment. In one test in which tryptamine, at a concentration of 1.10^{-3}M, was used instead of 5-HT, the effect of tryptamine was unaltered by a 1.10^{-3}M solution of BOL. It is possible that the relative amounts of BOL and either tryptamine or 5-HT are very critical and that the depressant action will only be revealed if these are finely adjusted, but it is obvious that a very large number of tests would be necessary to determine this. On the basis of these present results, however, BOL does not appear to have any clear-cut depressant action.

Harmine: (Fig. 46).

Harmine was very difficult to dissolve in sea water; the solutions used were made up with an amount equivalent to a 1.10^{-4}M solution of the drug, but complete solution was not obtained, so that the actual concentration was less than 1.10^{-4}M, but could not be assessed any more accurately. In only two of four tests did harmine have any effect, and where this occurred, it only became apparent after 5 to 30 min. In these two tests there was an initial excitation in the form of a rise in tone and activity rate, but the tone fell rapidly to a level below the normal base-line; after washing out, there was a slowing down of the rate of activity. It would thus appear that harmine has a variable and not very vigorous depressant action. The fact that slowing down of activity only follows washing out of the drug solution may be due to a masking of the depressant action by the excitatory effect of harmine; the latter may possibly be due to some irritant effect, particularly since the solutions used were not decanted and contained small particles of the drug in fine suspension.

In spite of this depressant action of the drug itself, there appeared to be no modification of the response to 1.10^{-4}M 5-HT or to 1.10^{-3}M tryptamine, added after the preparation had been exposed to a solution of harmine for approximately two hr.

Fig. 47.

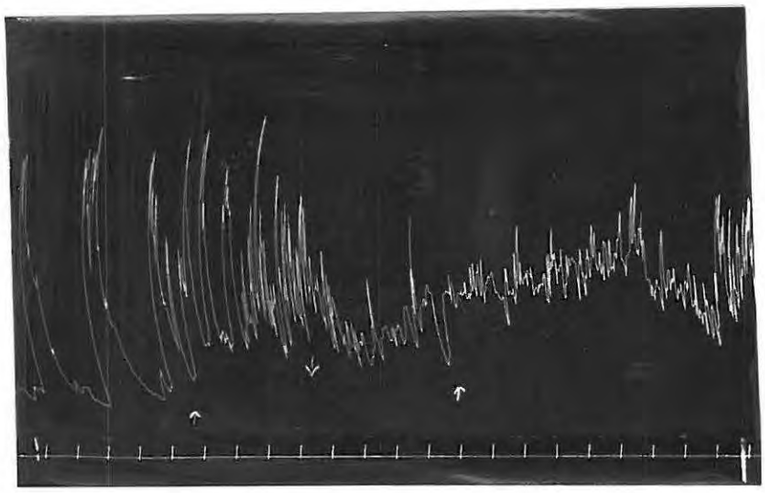


Fig. 47: The action of yohimbine at 1.10^{-3}M, indicated by the first two arrows; the action of 5-HT at 1.10^{-3}M after treatment with yohimbine, indicated by the third arrow.

Yohimbine: (Fig. 47).

The effects of yohimbine itself on a preparation have already been described. Two tests were performed in which, in the first, 5-HT at 1.10^{-3}M and in the second, tryptamine at 1.10^{-3}M were added after the preparation had been exposed to a 1.10^{-3}M solution of yohimbine for one to two hr. Again no difference between these responses and those obtained in the absence of yohimbine could be detected.

Thus, while slight depressant effects due to these three substances can be detected, they are weak; in no case was there any marked sign of a depressant action, or any evidence for the antagonistic action to the effects of 5-HT and tryptamine reported for the liver fluke.

4. 4) MISCELLANEOUS SUBSTANCES: Table VII.

The following tests have been performed with a number of unrelated substances, chosen because of known effects on other invertebrate preparations.

Gamma-aminobutyric acid: (Fig. 48 (a) - (c)).

The widespread occurrence of this drug and its inhibitory action on a variety of preparations, both vertebrate and invertebrate, has recently aroused much interest (Elliot and Jasper, 1959).

Its effect on P. gilchristi is, however, not at all well-defined. At concentrations below 1.10^{-4}M there is no effect at all, even after long exposure of the preparation to such a solution. Even at concentrations of 1.10^{-4} and 1.10^{-3}M the response is very variable, there being in some cases no apparent effect. However, in 6 out of 14 tests at these concentrations, there was definite evidence of a depressant action; at 1.10^{-4}M this takes the form of a slowing down of the contraction rate, but the contractions are never supressed altogether. At 1.10^{-3}M there is usually an initial period of excitation, expressed as an increase in tonus and rate of activity; the increased tone is, however, not maintained. After washing out, one of two things followed: either there was a return to normal activity rate, or there was complete supression of activity, although no drop in tone. This could possibly be explained as follows: GABA at 1.10^{-4}M has a slight depressant action, but a higher concentration, 1.10^{-3}M, is necessary for this to be made really effective; at such a high concentration, however, this compound has an excitatory effect as well. Once the GABA has been replaced by

TABLE VII.

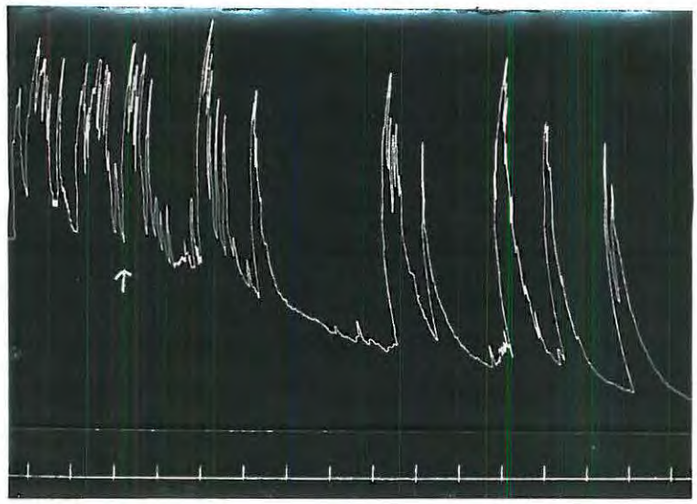
The effects of drugs in group 4.

Treatment.		Tone.	Rate.
G A B A	1.10^{-5}	0	0
	1.10^{-4}	0/-	0/-
	1.10^{-3}	-/++, then -	- -/++, then -(1) or Ab.
Picrotoxin	1.10^{-4}	0	+
	1.10^{-3}	++	+++
Glutamic acid	1.10^{-3}	+	0
Cadmium chloride	1.10^{-4}	0	0
	1.10^{-3}	0	0/Ab.
Cysteine	1.10^{-3}	0	0
	1.10^{-2}	0	0
Strychnine	1.10^{-4}	+, then -(2)	+++
	5.10^{-4}	- -	+++
	1.10^{-3}	+++	+++

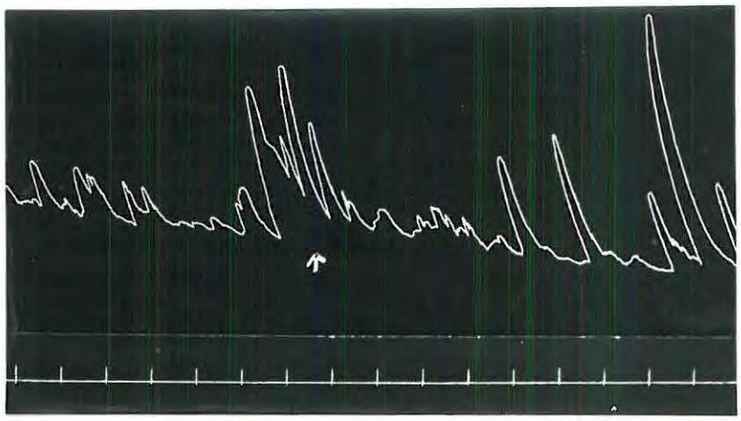
(1) The negative effect after washing out.

(2) Ditto.

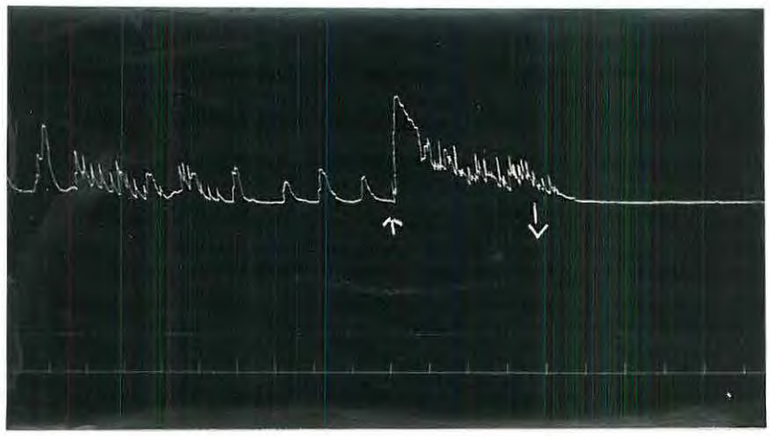
Fig. 48.



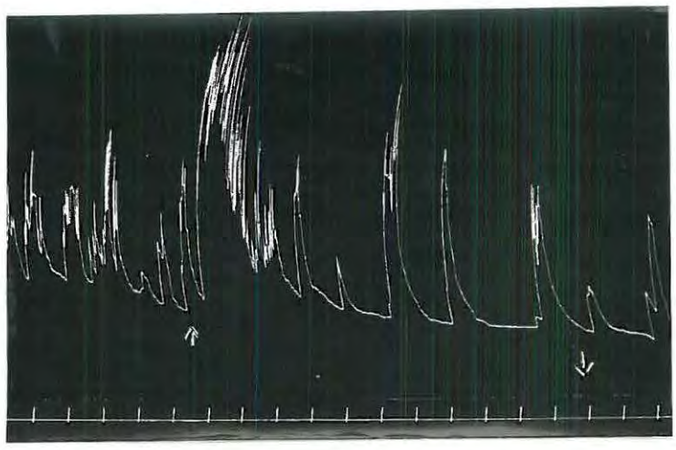
(a)



(b)



(c)

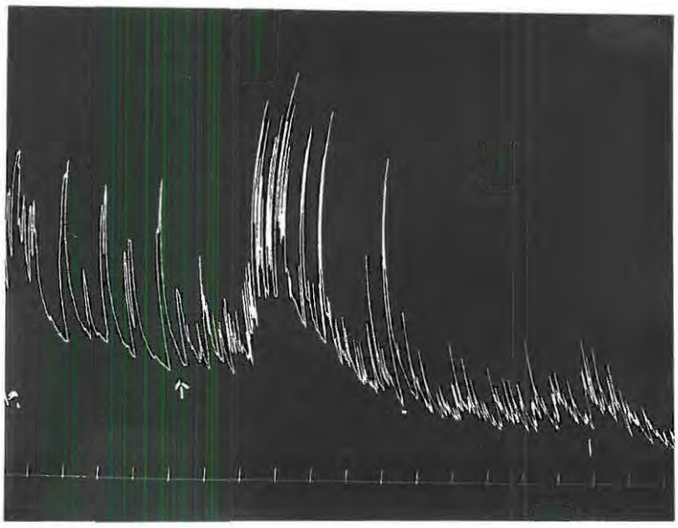


(d)

Fig.48 (a) - (d): The action of GABA at 1.10^{-3}M.

Fig. 49.

(a)



(b)

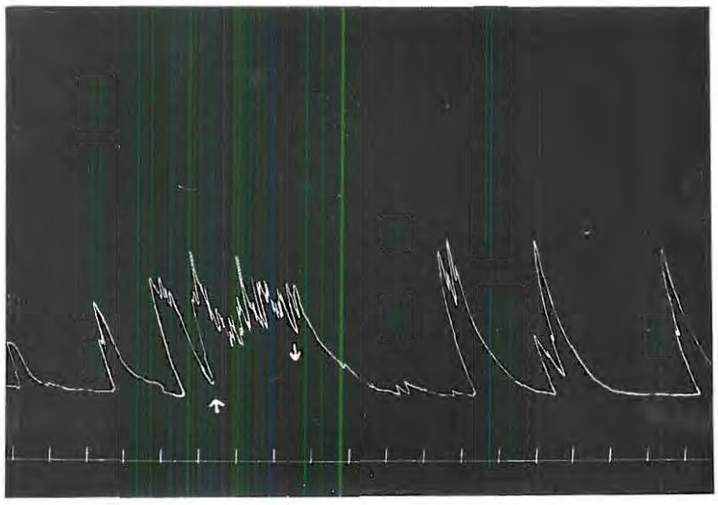


Fig. 49: (a) and (b): The action of picrotoxin at 1.10^{-3}M.

normal sea water, the excitatory effect would be removed and allow the depressant action to predominate. But if the effects observed can indeed be interpreted as a depressant effect, it is, at most, not at all striking.

Picrotixin: (Fig. 49 (a) and (b)).

Interest in this compound follows the discovery that it blocks peripheral inhibition in crayfish muscle (Van der Kloot, Robbins and Cook, 1958). In Planocera it is found to have an effect at a concentration of 1.10^{-3}M only, being ineffective at lower concentrations. At this concentration, there is a rise in both tone and activity rate. The increased tonus may be maintained, or it may wane after about one hour, but the increased rate of activity continues and may even persist for a considerable period after washing out.

Glutamic acid: (Fig. 50 (a) and (b)).

There is evidence that glutamic acid stimulates the centres of adrenaline production in vertebrates (Weil-Malherbe, 1950; Mann and Quastel, 1940), although the mechanism is not clearly understood. Further, L-glutamic acid has been found to excite crustacean muscle (Robbins, 1959).

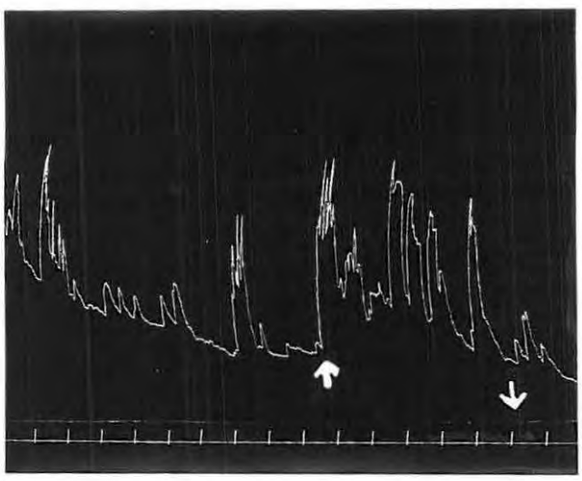
There is very little evidence for any excitatory effect of L-glutamic acid on this preparation. At a concentration of 1.10^{-3}M there was, in two of five tests, a temporary rise in tone, which was, however, not marked, and in one of these tests an indication of an increase in rate of activity, while below this concentration, this substance was ineffective.

Cadmium Chloride: (Fig. 51 (a) and (b)).

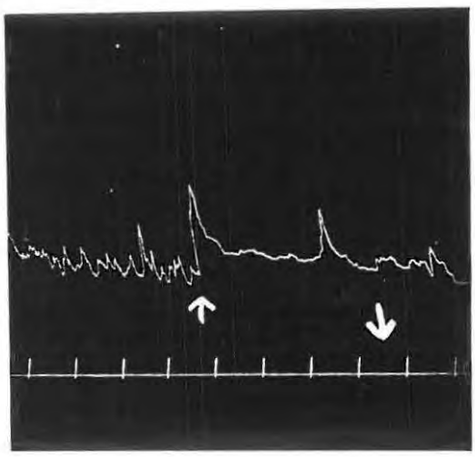
The results of experiments on Cucumaria sykion Lampert and on a sciatic-gastrocnemius preparation of Xenopus laevis Daudin have shown that cadmium ions can act as an effective myoneural block (Ewer, Pople and Ross, 1961). Similarly, they have demonstrated an inhibition of rhythmical activity and responses to mechanical and electrical stimulation by cadmium ions in Actinia equina L. and Calliactis parasitica.

A number of tests were made in which preparations of P. gilchristi were exposed to a solution of 1.10^{-3}M cadmium chloride for periods of 2 - 2½ hours. In only one of five tests was there any sign of abolition of activity (fig. 51 (b)), and in

Fig. 50.



(a)

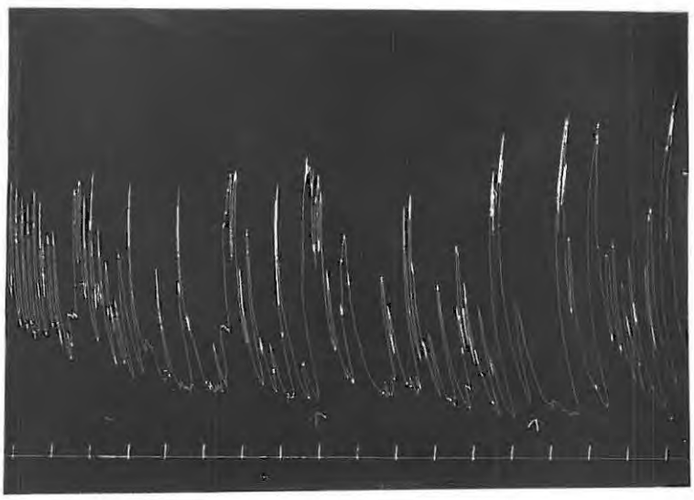


(b)

Fig. 50 (a) and (b): The action of l-glutamic acid at 1.10^{-3}M.

Figs. 51 and 52.

(a)



(b)

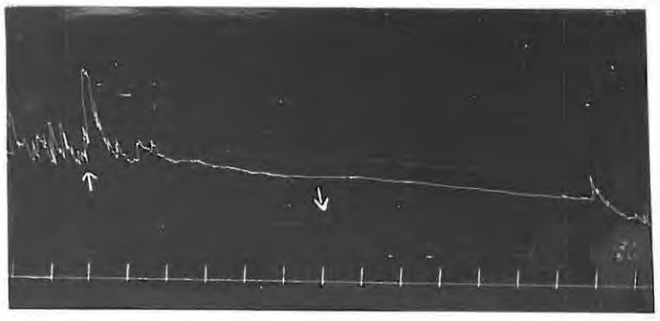


Fig. 51: (a) and (b): The action of cadmium chloride at 1.10^{-3}M. In (a) the second arrow indicates a second wash through with the same solution.

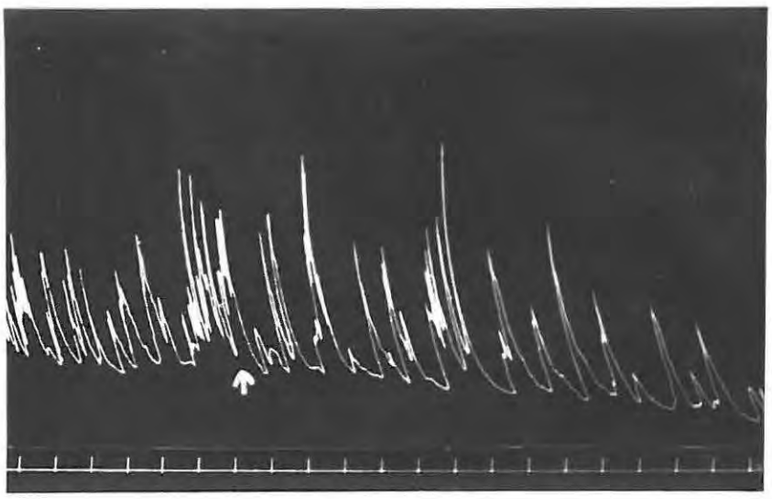


Fig. 52: The action of cysteine at 2.10^{-3}M.

this preparation all activity was abolished within 20 min., recovery, on return to normal sea water, was very slow as can be seen in the figure.

Some experiments were then done in which free, cerebrate animals were placed in a 1.10^{-3}M cadmium chloride solution. These animals at first showed enhanced activity in the form of continuous swimming or crawling, but within 15 - 20 min. co-ordination was abolished in four of six animals: these animals could no longer orientate themselves and remained in an inverted position from which they could not right themselves; swimming movements, too, became unco-ordinated; the remaining two animals remained normal for about 40 min; one of the above four animals showed complete cessation of activity after 20 min., but this animal did not recover when returned to normal sea water, even after a period of 36 hr. The remaining five animals were only partially narcotised after a period of two to three hr., performing occasional unco-ordinated movements and responding to mechanical stimuli.

These results would appear to indicate that cadmium chloride has a central rather than a peripheral blocking action in P.gilchristi, affecting co-ordination long before there is any clear sign of narcotization.

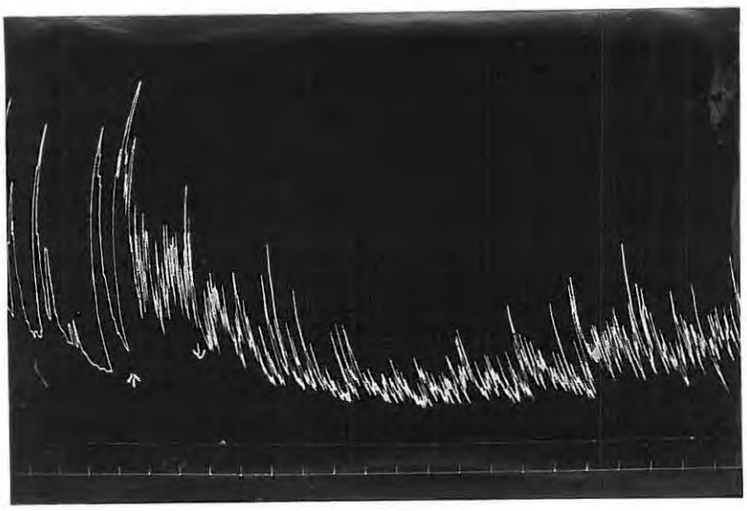
Cysteine: (Fig. 52).

A number of observations have suggested that the rhythmical activity displayed by hearts and the myo-nerual systems of some invertebrates may depend upon the presence in the tissues of substances containing sulphydral groupings. Thus the inhibition of the frog's heart by heavy metals such as cadmium or silver can be corrected by the addition of cysteine which also temporarily enhances the activity of the heart after such inhibition. (Del Castillo-Nicolau, Hufschmidt and Konecci, 1951). A similar effect has been observed in the sea anemones Actinia equina and Calliactis parasitica (Kostayantz and Smirnova,1955; Ewer, Pople and Ross, 1961); in both these anemones cysteine may also stimulate rhythmical activity in the absence of previous inhibition.

Treatment with cysteine alone had no effect on a preparation of P.gilchristi, even at a concentration as high as 1.10^{-2}M. In the above tests with cadmium on free animals, half the animals were placed in normal sea water, and half in a 1.10^{-3}M

Fig. 53.

(a)



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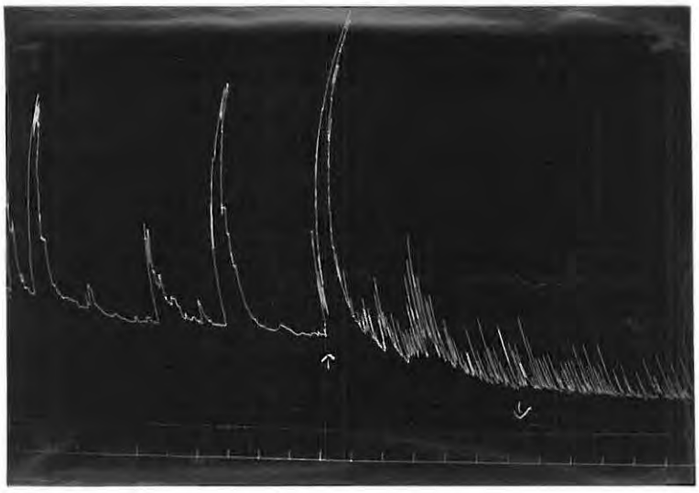

(c)

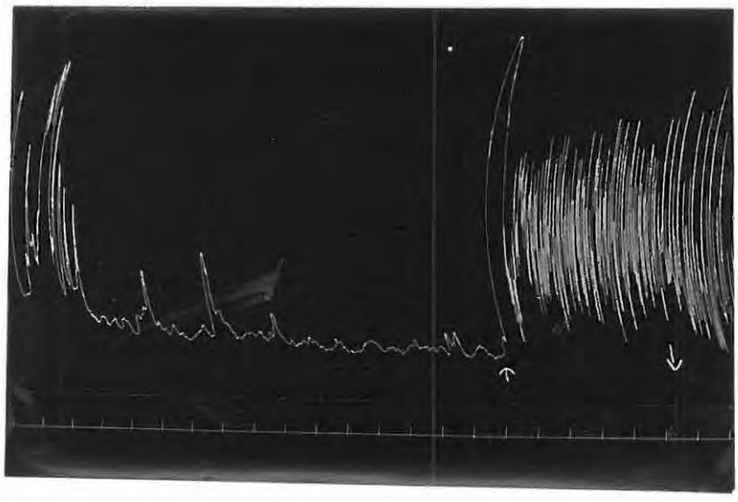

Fig. 53: The action of strychnine: (a) at 5.10^{-4}M
(b) 1.10^{-4}M; (c) 1.10^{-3}M.

solution of cysteine, after treatment with cadmium chloride. No consistent difference could be detected between the two sets of animals with respect to the time taken for co-ordination to be recovered.

A second compound containing a sulphydral group, namely glutathione, was also tested on the preparation and on free animals, but was without effect.

Strychnine: (Fig.53 (a) - (c)).

It has been shown that in vertebrates strychnine selectivelyblocks inhibitory synapses of the spinal chord. The rapidity and effectiveness of its action have led to the suggestion that it acts competitively with the inhibitory transmitter (Eccles, 1959).

The minimal effective concentration of strychnine on a preparation of Planocera is 1.10^{-5}M, and at this and all higher concentrations there is an increase in the rate of activity which becomes more marked the greater the concentration. The effect on tone is not the same at all concentrations. Thus at a concentration of 1.10^{-3}M there is a rise in tone which is maintained until the solution is washed out; at 5.10^{-4}M there is an initial rise in tone which is, however, not maintained, and falls off in about 20 min., while at a concentration of 1.10^{-4} or 1.10^{-5}M there is an almost immediate fall in tone, while the rate of activity continues at an increased level.

Fig. 54:

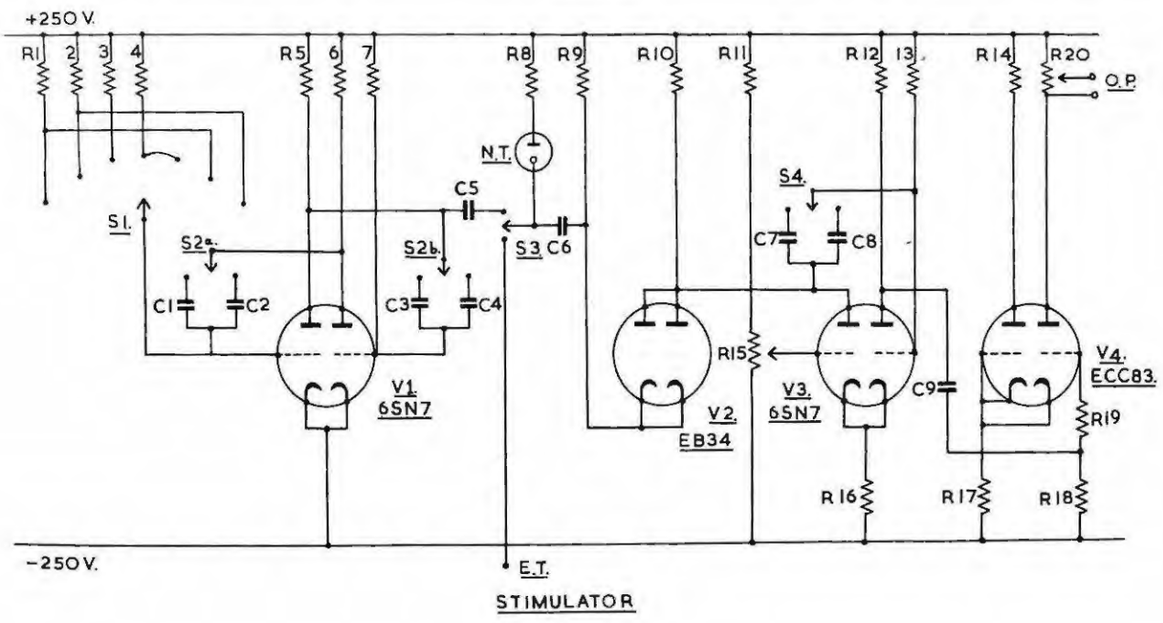

Fig. 54: Stimulator.
E.T. = external trigger; N. T. = neon tube;
O. P. = out-put;
Switches: S1, S2 a and b=frequency controls
S3 Internal-external trigger control;
S4 coarse duration control.
Resistors: R1=10 Meg; R2=4.3 Meg; R3=1.7 Meg; R4=20 Meg;
R5=47K; R6=47K; R7=20 Meg; R8=270K; R9=1 Meg;
R10=22K; R11=270K; R12=10 K; R13=1.4 Meg;
R14=14.7 K; R15=50 K; R16=10 K; R17=6.6K:
R19=220 K; R20=100 K;
Condensors: C1=2 f; C2=0.05 f; C3=0.05 f;
C4=0.001 f; C5=0.1 f; C6=100pf; C7=0.01 f;
C8=0.1 f; C9=1 f.

B. RESPONSES TO ELECTRICAL STIMULATION.

1. METHODS:

1.1) Apparatus:

(a) Stimulator:

The stimulator which is represented in fig. 54, was designed with the following objects in view:

(i) that the output should be in the form of negative-going square wave pulses, the amplitude of which would reach a maximum of 200 volts or more, controlable from zero to maximum;

(ii) that the pulse should be stable for repetition rates variable from 1 pulse in 10 sec. to 10 pulses per sec.;

(iii) that the three variables - amplitude, frequency of repetition rate and pulse duration - should be completely independent.

These were achieved in the following way.

The repetition rate is determined by a standard free-running multi-vibrator circuit, pulses from which are differentiated by means of the differentiating circuit C6 - R9. The repetition rate can be varied by adjustment of the time-constants of the grid circuits of the valve. Such adjustment is achieved by means of the switches S1, S2a and b, which enable appropriate combinations of resistance and capacity to be selected from the resistor and condenser banks R 1-4 and C 1-4 respectively. One of the resistors R 1-4 in conjunction with C1 and 2 determines the time-constant of the grid circuit of the left-hand section of the valve V1, while the resistor R7 combined with either C 3 or 4 determines the time-constant of the grid circuit of the other section.

The double diode, V2, both sections of which are connected in parallel for convenience, allows only negative going pulses from V1 to appear at the anode of the first section of V3. The latter is a 'one-shot' cathode-coupled multi-vibrator, which produces pulses whose duration may be altered by variation of the time-constant of the grid-circuit of the right-hand section of the valve and adjustment of the grid bias of the left-hand section. The switch S4 provides a coarse adjustment of the pulse

length, enabling the value of the time-constant of the grid-circuit of the right-hand section to be changed from R13 x C7 to R13 x C8 or vice versa, while fine adjustment of the pulse length is accomplished by varying the bias of the first grid by means of potentiometer R15.

The values of R 1-7, R9, R11, R12, R13, R15 and C 1-8 are the same as those used by Ead (1951), since these values give the desired repetition rates and pulse lengths.

To achieve the desired form of output it is necessary to follow the one-shot multi-vibrator with a suitable amplifying stage. This consists of an ECC83 valve (V4) whose cathodes are tied together and whose second grid is connected to the common cathode. With appropriate choice of resistors R14, R15 and R18 it is possible to arrange that the right-hand triode is normally biased beyond cut-off.At the same time the resistance R15 is sufficiently large to ensure that the anode potential of this section will drop by almost its full value if its grid comes up to zero potential. The positive square pulses from the appropriate section of the'one-shot' multi-vibrator are applied to the first grid of the amplifying stage via the resistor (R20) which serves to prevent the potential of this grid rising appreciably above the HT-negative potential. In the circuit used the load resistance (R15) of the amplifying stage consists of a 100K potentiometer, enabling negative going pulses of any desired amplitude from zero to almost the full value of the supply voltage to be obtained. The positive side of the HT is earthed to the chassis.

A small neon bulb is connected across R8 and C5 so that when pulses arrive from V1 they cause the voltage across the neon tube to exceed its striking voltage, thus giving a visual indication of each stimulus.

(b) Power Supply:

A regulated power supply of standard design was used (Terman, 1955).

(c) Calibration:

The amplitude and duration controls were calibrated and an exact measurement of the pulse repetition

rates at various settings of the switches S1 and S2 were made on a 545A Tektronix oscillograph.

(d) Electrodes:

Two simple platinum electrodes were used throughout the experiments.

(e) Recordings:

All recordings were made as in the previous experiments, using the same standard apparatus.

1.2) The Preparation.

The decerebrate animal was ligatured as described on p.10. Several attempts were made to stimulate a preparation when immersed in sea water, but this proved extremely difficult, for the following reasons:

(a) the buoyancy of the sea water allows the sides of that part of the animal which is between the ligatures to move quite considerably. In the absence of the electrodes this activity subsides within about 5 min., but if the electrodes are touching the body, the activity continues indefinitely so that the animal is constantly changing its position relative to the electrodes. These movements take the form of vigorous twistings and undulations of the sides of the body, the latter resembling the swimming movement. It is as if the animal were trying to push itself away from the electrodes.

Further, experiments using this procedure showed the threshold to be both very variable and very high (of the order of 100 volts), while the size of the response to consecutive stimuli of the same magnitude was extremely variable and unreliable.

(b) a better control of stimulation might be effected by inserting into the animal the exposed tip of a stimulating electrode, coated with a non-conducting substance. This procedure could, however, not be followed, because movement of the animal resulted either in its pulling away from the electrodes or moving in such a way that the tip went right through the animal and only the coated part was then in contact with the preparation.

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Doubtless the technique of stimulating the animal in sea water could be perfected, but for the present purposes the following method was found to be satisfactory. The preparation was set up in the dry, by fastening the foam plastic of the lower ligature on to a cork, while the upper ligature was suspended from the arm of a lever as before. A rather large counterweight had to be applied to take up the weight from the wet foam plastic of the upper ligature and also the slack of the animal in the absence of the support of sea water. The animal was kept moist with sea water which was allowed to drip slowly from a Mariott bottle. The two electrodes were arranged in one of two ways:

(i) they were placed one on each side of the animal between the ligatures;

(ii) the cathode was laid against the side of the animal between the ligatures while the anode wire was extended by a thread of wool soaked in sea water. This thread rested against the portion of the animal exposed above the upper ligature and could thus remain undisturbed by the movements of the preparation.

This method proved satisfactory and after an initial response to the tactile stimulation from the electrodes there was very little sign of irritation. Spontaneous contractions, similar to those described earlier (p. 13) persist in these preparations.

I.3) Experimental Approach:

It has already been seen (p. 8 , fig.10) that the nervous system of P.gilchristi is in the form of a network. The coelenterates also have a nerve net which has certain clearly defined functional characteristics; it is obviously of interest to determine whether that of P. gilchristi shows the same characteristics.

This investigation has thus been made with the following four main points in mind:

(1) In many anthozoa, both a "quick" and a "slow" response can be elicited: thus Ross (1957) has shown that in Calliactis parasitica both types of response may be elicited from the sphincter region of the anemone, while the mid-column shows only the slow response. The latency of the quick sphincter

response is of the order of 0.1 sec., while that of the slow response is usually about 30 sec., although it may be as long as 150 sec. It is characteristic of the quick response that it can normally only be elicited by two stimuli above a certain frequency, there being no response to the first stimulus even at an intensity of 10 times threshold; the slow response of the sphincter is characterised by the fact that it is elicited only by a relatively large number of shocks and at a low frequency of repetition rate (Ross, 1957 a).

(2) Pantin (1935 a) has demonstrated that above threshold the response of this anemone is entirely independent of the intensity of electrical stimulation.

(3) It is now widely recognised that the neuro-muscular system of sea anemones is very sensitive to the frequency with which successive shocks are applied. This was first shown by Pantin (1935 a) who demonstrated that the magnitude of the quick response of the sphincter depends on the time interval between successive shocks. Further, when a number of shocks are applied, the rate of contraction of the marginal sphincter of Calliactis parasitica increases with increasing frequency of stimulation. This is not due merely to mechnical summation of more frequent individual contractions, for the actual magnitude of each contraction is itself greater. The same type of effect may be demonstrated when the whole animal is stimulated, but here a further phenomenon can be observed, namely a greater spread of the response at higher frequencies of stimulation. These two phenomena Pantin has explained in terms of facilitation, recognising both neuro-neural and myo-neural facilitation as normal events.

(4) Pantin (1935 b) has demonstrated that, although the nerve net of the sea anemone Calliactis parasitica is differentiated into fast and slow conducting pathways, conduction may take place from any point of stimulation of the animal to any other part of the animal.

Thus the following four questions emerge:

(1) Does the response of P.gilchristi to electrical stimulation resemble either the quick or the slow response of anemones?

(2) Is the response dependant on the intensity of the stimulus?

(3) Is there any evidence for a facilitation phenomenon?

(4) Is the network a true nerve net allowing conduction to all parts of the animal?

Figs. 55 and 56.

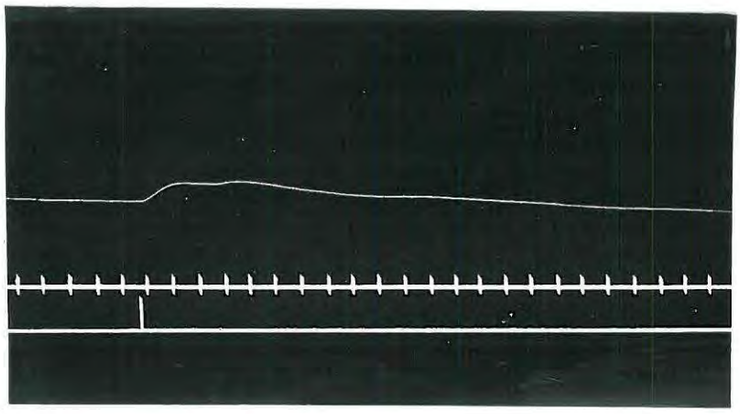

Fig. 55: Trace showing the development of a slow contraction after a response to electrical stimulation. Time trace, one sec.

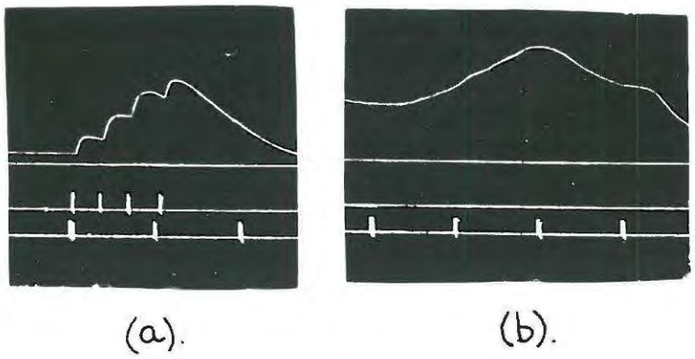

Fig. 56: Traces showing the difference between the rates of contraction during a response to electrical stimulation and during a spontaneous contraction.
(a) Responses to four electrical shocks;
(b) a spontaneous contraction recorded from the same animal. Time trace, 10 sec.

An attempt has been made to answer these questions and the results are presented below.

2. RESULTS:

2.1) The Nature of the Response:

In response to a single shock above the threshold of intensity and stimulus pulse length, a preparation of P.gilchristi gives a very rapid contraction;[1] the latency of this is of the order of 50 msec., that is, of the same order as the quick sphincter response of the anemones Calliactis and Metridium.

In a number of experiments it was observed that after the quick response to the stimulus, a slower contraction set in (fig. 55). Thus far it has not, however, been possible to separate this from the quick response, nor have conditions of stimulation been discovered which will elicit such a slow response alone. Stimuli consisting of a series of shocks at a low frequency and at an intensity level which was just below threshold for the quick response failed to elicit any contraction.

It is also of interest to note the difference between the rate of contraction during a spontaneous contraction and that during a quick response to electrical stimulation. The rate of contraction during spontaneous contraction and in response to a single shock at intensities below 60 volts were measured in traces from a number of different preparations. The rate of contraction in response to electrical stimulation varies with the intensity of the stimulus, but when records of the two types of contraction from the same animal are compared, it is invariably found that the rate of contraction of the quick response is from two to four times greater than the rate of contraction during a spontaneous contraction. At intensities above 100 volts this increased considerably, being as much as six to eight times greater than the rate of a spontaneous contraction. Thus fig. 56 shows the difference between a response to a stimulus and a spontaneous

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[1] Evidence will later be presented showing that this is effected by direct stimulation of the motor nerves.

Fig. 57.

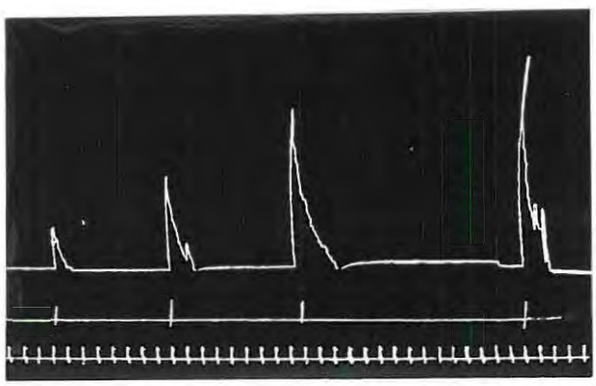


Fig. 57: The effect of increasing the intensity of the stimulus. The record shows four responses, each to a single shock, at intensities of 10, 60, 120, and 200 volts, reading from left to right. Pulse length constant at 60 msec. The upper signal trace shows the point of delivery of the stimulus; time trace below in [redacted] min.

The drum was stopped after relaxation from each contraction.

contraction in the same preparation; the two records were obtained at an interval of two and a half min. and measurement shows that the rate of contraction in response to an electrical shock is 4.02 times greater than that during the spontaneous contraction.

2.2) Effect of Intensity of the Stimulus:

Above threshold, a stimulus does not evoke an "all-or-nothing" response, and an increase in size of the response with increasing intensity can be readily demonstrated. Thus fig. 57 shows the effect of increasing the intensity from 10 - 200 volts, for a constant pulse length of 60 msec. As might be expected, increasing the pulse length for a constant value of intensity likewise gives an increasing magnitude of response.

The threshold thus depends on a sufficient magnitude of both intensity and pulse duration. Table VIII shows a number of combinations of duration and intensity which were found to be necessary to elicit the smallest observable response. These figures serve merely to illustrate the order of magnitude of the stimulus required to elicit a contraction sufficiently large to be recorded mechanically; very little attention has been paid to the determination of absolute thresholds for the following reasons:

(a) the rather large load against which the animal had to contract when set up as a preparation in the dry, renders it very likely that the magnitude of the stimulus required to elicit a recordable contraction is in fact well above the true threshold.

(b) threshold values were found to vary very considerably from preparation to preparation and from time to time in the same preparation; the latter may possibly be due to a variation in the state of excitation of the animal at different times.

2.3) The Effect of Frequency of Stimulation:

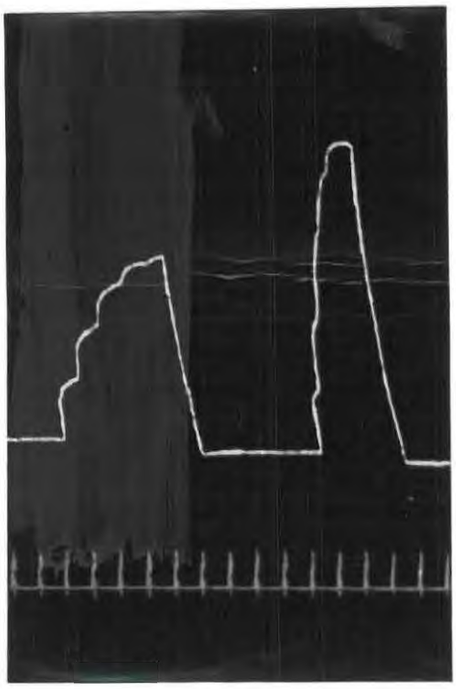
A number of experiments were done in which a series of either four or five shocks was applied, at frequencies ranging from one shock in five sec. to ten shocks per sec. In some of the records there seemed

TABLE VIII.

Approximate Threshold Values of Intensity and Pulse Length.

Intensity (in volts)	Pulse Length (in msec.)
3	70
5	60
10	40
15	20

Fig. 58.



A. B.

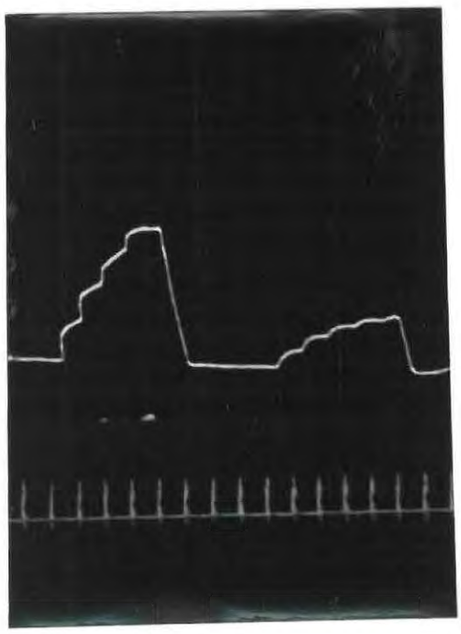
Fig. 58: The responses to two stimuli at the same intensity and pulse duration, but different frequencies.
A. is the response to 5 shocks at one per sec.
B. is the response to 5 shocks at two per sec.

to be a clear indication of a facilitation phenomenon: thus fig. 58 shows the effect of increasing the frequency from one shock per sec. to two shocks per sec. There is an obvious increase in the rate of contraction, for the overall contraction in response to the same number of shocks is greater at the higher frequency, and when such a trace is measured, it is found that the responses to either one or all of the second, third, fourth and fifth shocks are greater at the higher frequency.

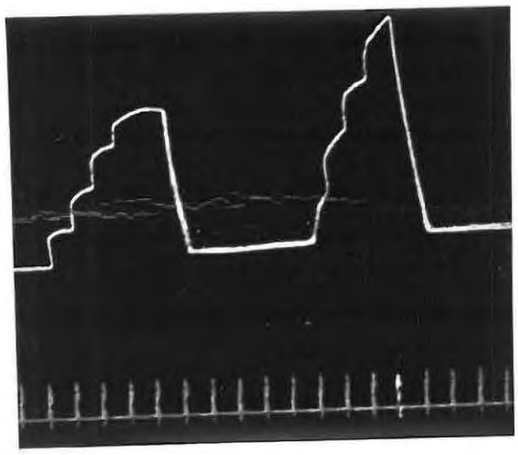
However, it is not possible to draw any conclusions from the few recordings which showed this phenomenon for, from each preparation which showed such an effect, records were also obtained in which successive tests at a constant frequency of stimulation displayed marked variation in the rate of contraction; thus figs. 59 (a) - (c) illustrate the effects of successive stimuli, all at a frequency of one shock per sec., while figs. 60 (a) and (b) show the effects of successive tests with shocks at a constant frequency of two per sec. Furthermore, in some cases, increasing the frequency of stimulation up to 10 shocks per sec. had no effect at all on the rate of contraction. The latter observation in particular would appear to rule out the likelihood of a facilitation phenomenon.

Hoyle (1960) has shown that a preparation of the parietobasilar muscles of the sea anemone Stomphia coccinea Müller will respond to a series of successive shocks in two different ways, either showing facilitation or not, and that this can be correlated with the size of the contraction in response to the first shock: if the first contraction is large, the second and subsequent contractions are all smaller than the first and no facilitation is apparent; on the other hand, if the first contraction is small, the response to the second shock is always much larger. It was considered possible that this might be the explanation of the variability of the response in P. gilchristi, but an analysis of the tests performed over a wide range of intensities, and with first responses of variable size, revealed no consistent relationship between the size of

Fig. 59.

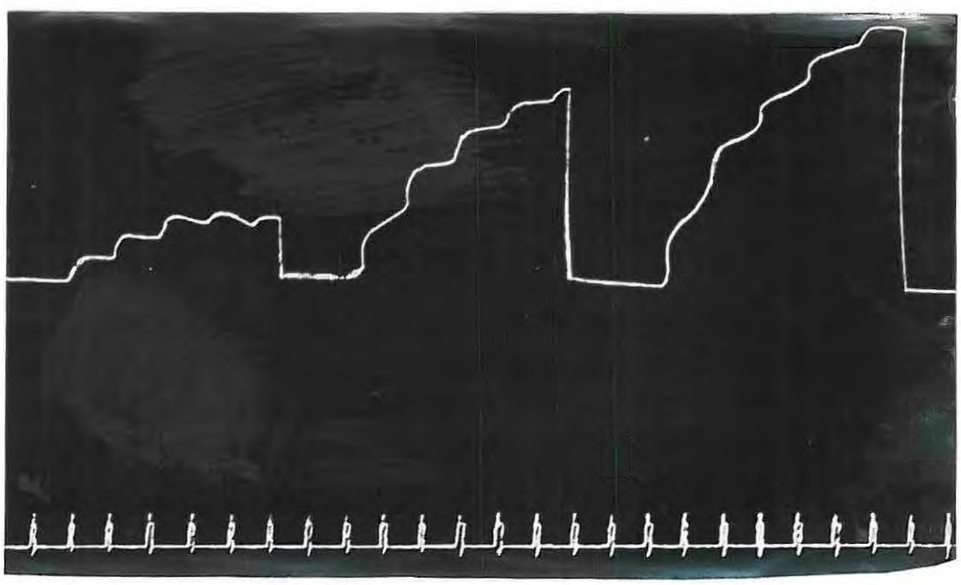


(a)



(b)

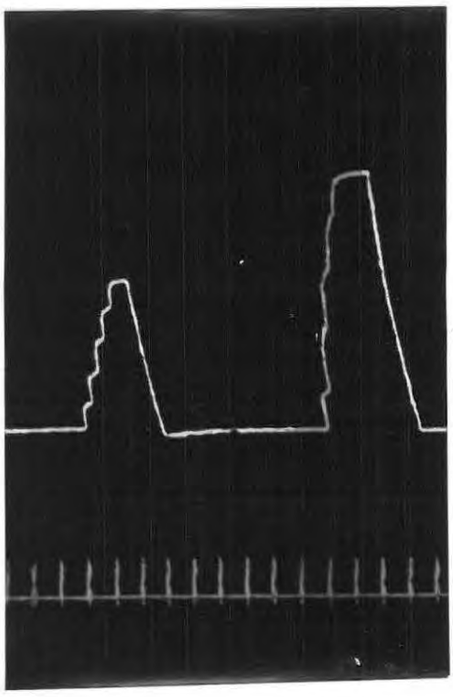
(c).



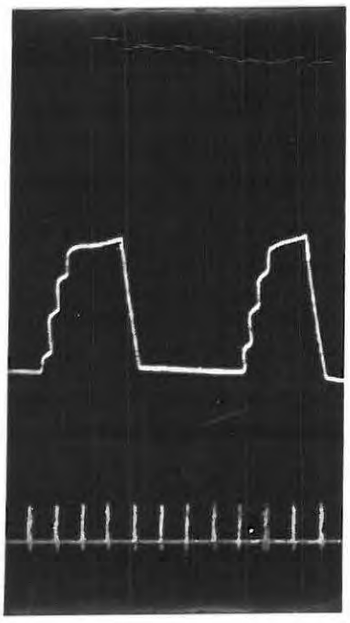
(i) (ii) (iii)

Fig. 59: (a), (b) and (c): Three records, all of responses to successive stimuli consisting of shocks of constant intensity and duration, and delivered at the same frequency of one shock per sec.

Fig.60.



(a)



(b)

Fig. 60: (a) and (b): Two records of responses to successive stimuli consisting of shocks of constant intensity and duration and delivered at the constant frequency of two per sec.

the first contraction and the rate of contraction.

It may be suggested, however, that both the variability in the magnitude of the response to successive stimuli at the same frequency and those records which appear to show facilitation may be due to differing states of excitation of the animal. Indeed a closer study of the records presented in fig. 59 adds weight to this interpretation. In all three of these traces it can be seen that in the larger response, a further contraction is being superimposed on the response to each shock; for example, in response (i) of fig.59 (c) the trace levels off after the response to each shock, while in responses (ii) and (iii) the trace continues to rise after the initial response. It is quite possible that the same is taking place in the larger responses to stimuli at a frequency of two shocks per sec., but that this is obscured because the records have been made at too slow a speed.

It seems possible that the superimposed contraction is a reflection of a greater state of excitation of the preparation, and a variation in the state of excitation of the animal may possibly be related to the time interval after the last spontaneous contraction. In further experiments it would be desirable to stimulate the animal at different times after spontaneous contractions, but thus far such experiments have not been done.

2. 4. The Nature of the Nerve Net:

(i) The Role of the Brain:

Before describing the experiments which were performed in an attempt to analyse the pathways of conduction in the nerve-net of *P.gilchristi* it is necessary first to present a brief account of the importance of the brain in this nervous system. This has been analysed by comparing the behaviour and responses of a decerebrate animal with those of the intact animal.

(a) Locomotion:

It has been seen that the same type of mechanical record of activity is obtained from a decerebrate as from a cerebrate animal (p.12). When a free, decerebrate animal is observed it is seen to move around partly by muscular movement, but also very largely by gliding with the cilia of the ventral body surface. When dropped into a large volume of water such an animal may, in falling to the bottom, show movements which are clearly components of the swimming movements of the intact animal. However, these movements are completely unco-ordinated and the regular passage of transverse swimming waves antero-posteriorly down the body never takes place. Likewise, a mechanical stimulus may cause the animal to perform movements which are clearly components of the ditaxic crawling of normal animals, but again this lacks co-ordination: there is no regular alternation between the extension of left and right sides of the anterior end of the animal, but a number of different portions of the anterior end may extend forwards or slightly side-ways in an irregular manner; the posterior end remains passive and is often held free from the substratum. Thus, although the components of both swimming and crawling movements are apparent, the complete locomotory patterns are never seen in decerebrate animals.

(b) Righting Reaction:

When a normal intact animal is placed in an inverted position, with the dorsal surface in contact with the substratum, it quickly rights itself by flexing the anterior end dorsally, gripping the substratum with the ventral surface of this part of the body, and pulling the rest of the body over into the normal position, with the ventral surface in contact with the substratum. This appears to be a thigmopausic response, for if an object is placed in contact with the ventral surface of an animal in the inverted position, it clings to this and makes no attempt to right itself. A decerebrate animal is able to right itself, but this

does not take place in the swift, co-ordinated manner described above. In an inverted position, when the ventral surface is not in contact with any other surface, a decerebrate animal seems to be generally excited and performs unco-ordinated writhing and twisting movements. These eventually result in some part of the ventral surface of the lateral margins of the body coming into contact with the substratum, and the rest of the body is slowly pulled over into the normal resting position. In the decerebrate animal there is thus no co-ordination of this movement and any part of the ventral surface which comes into contact with the substratum may initiate a slow and clumsy righting movement.

(c) Response to Mechanical Stimulation:

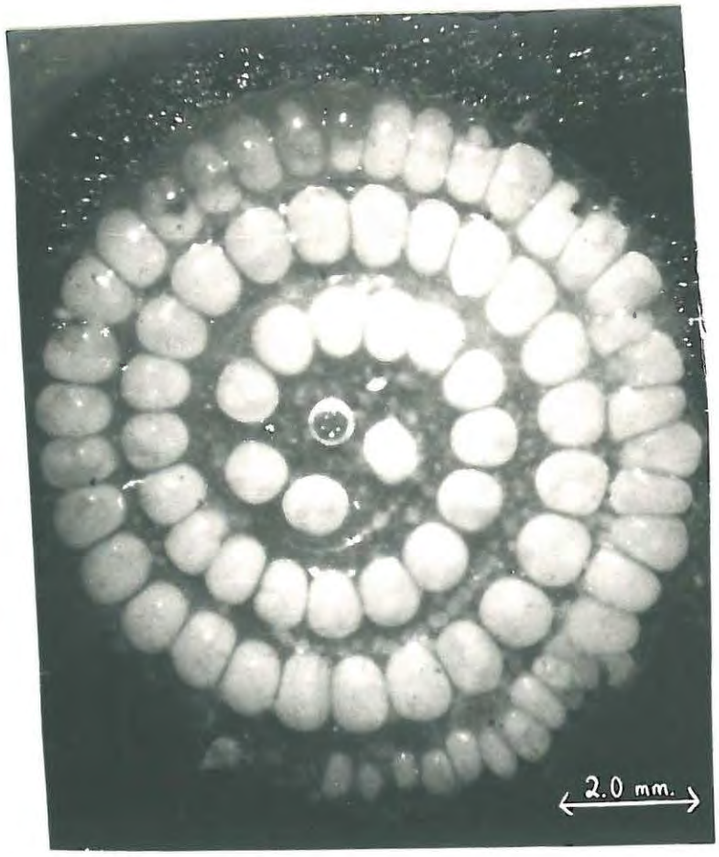
When a slight mechanical stimulus is applied to any part of the lateral edge of either a cerebrate or a decerebrate animal there is a local contraction, resulting in a retraction of this edge of the body. A stronger mechanical stimulus applied to a cerebrate animal elicits rapid ditaxic crawling of the animal away from the point of stimulation. In a decerebrate animal, strong or repeated mechanical stimulation may have one of two effects: either there is never any response other than a retraction of a localised edge of the body, as described above, or movement may be elicited. This, in some cases, took the form of movements of the anterior end of the animal which were clearly components of the normal crawling movement, but more usually such stimulation evoked unco-ordinated writhing movements of the lateral margins of the whole body. In the latter case it could be clearly seen that there was a gradual and rather slow spread of excitation, first down the side of the body stimulated, and then across to the other side. The factor which seems possibly to determine which of these two responses is elicited - either a localised contraction, or movement, is the state of excitation of the animal. The first response was invariably elicited from animals which were lying still on the bottom of the dish, with no sign of movement, and the second from animals which were showing signs of some spontaneous movement.

(d) Feeding:

To the chemical stimulus of food extract ejected from the tip of a pipette near to the animal, a cerebrate individual normally responds by an outward extension of the edge of the body in the direction of the stimulus, orientation of the body towards the source of stimulus, followed by a prolonged outburst of locomotion. If any part of the body comes into contact with a piece

Fig. 61.

(a)



(b)

Fig. 61: The eggs of P. gilchristi in a strand of transparent matrix. (a) Eggs laid in a coil by an animal with the brain left intact; (b) eggs laid in a disorganised strand by a decerebrate animal.

of food, the animal twists rapidly so that the anterior end is brought into contact with the food; the anterior edge of the body is then lifted and thrown over the food which is drawn rapidly under the body into position beneath the pharynx; the pharynx is then extruded and engulfs the food.

In a decerebrate animal, food extract elicits a lifting and extension of the edge of the body towards the source of the stimulus, but there is no orientation and locomotion does not follow. When a piece of food is brought into contact with the edge of any part of the body of the animal, the edge of the body in that particular region is lifted and the food very slowly drawn in under the animal by a curling movement of the margin. There is, however, no orientation of the anterior end towards the food, nor does the characteristic lively engulfing movement of the body edge occur; extrusion of the pharynx, however, follows as usual.

Thus, although a decerebrate animal will feed there appears to be no co-ordination between the response to chemical stimulation of food extract and locomotion, or between response to food itself and an orientation of the animal towards food.

A further observation which was made is as follows: when an intact animal has fed, there is no response either to chemical stimulation of food extract or to food itself for approximately 24 hrs; all components of the pattern of behaviour accompanying feeding are apparently inhibited. Thereafter there is a gradual return of components of the response over a few days, until, after defaecation, the complete feeding pattern may again be elicited. In a decerebrate animal, however, there is no such inhibition and the animal will respond to and accept food continuously, even if unable to take it into the pharynx.

(e) Egg-laying:

A normal, intact animal lays its eggs in batches of up to 100, embedded in a strand of fairly firm, transparent matrix which is attached to the substratum. The whole is arranged in a neat coil, as is shown in fig. 61 (a). A decerebrate animal will also lay eggs, but they are attached to the substratum in a long disorganised strand, as shown in fig. 61 (b). Here, as in the feeding response, some inhibition seems to be abolished with removal of the brain, for a sexually mature animal with well-developed gonads will often lay eggs within as short a time as 15 min. after removal of the brain. Decerebrate animals have never been seen to copulate.

Fig. 62.

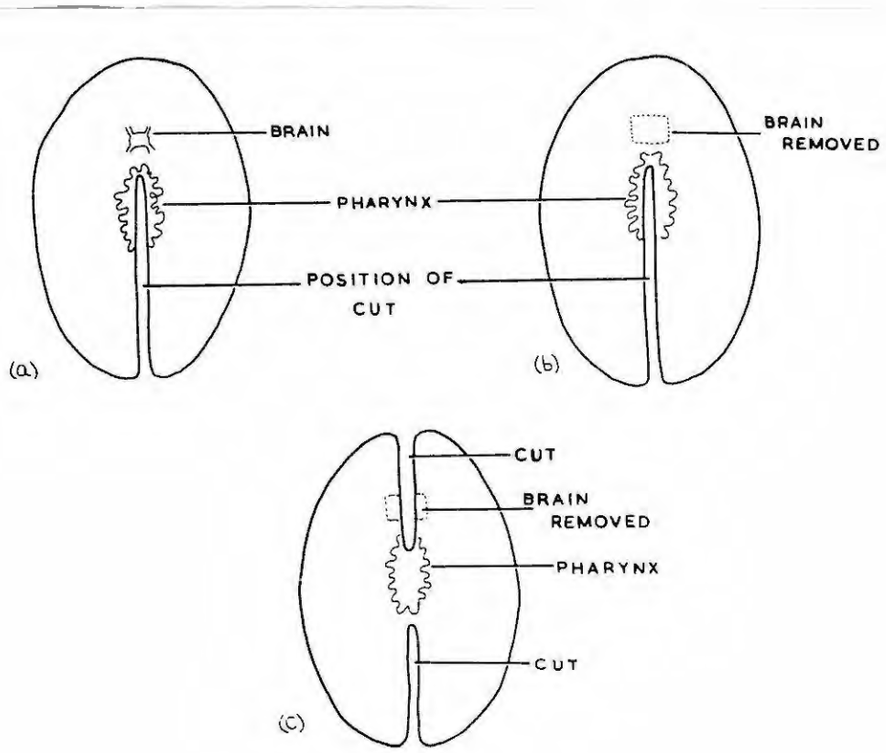


Fig. 62. Diagrams showing the positions of cuts in hemisected animals.

Thus, while most of the normal activities are shown in decerebrate animals, they are only in the form of incomplete patterns of behaviour; the decerebrate animals lack the co-ordination of such activities exhibited in the intact animal. The role of the brain may thus be said to be primarily that of a co-ordinatory centre, though co-ordination in the rest of the nerve network is sufficient to allow the basic components of normal activity to be displayed.

(ii) The Nerve Network:

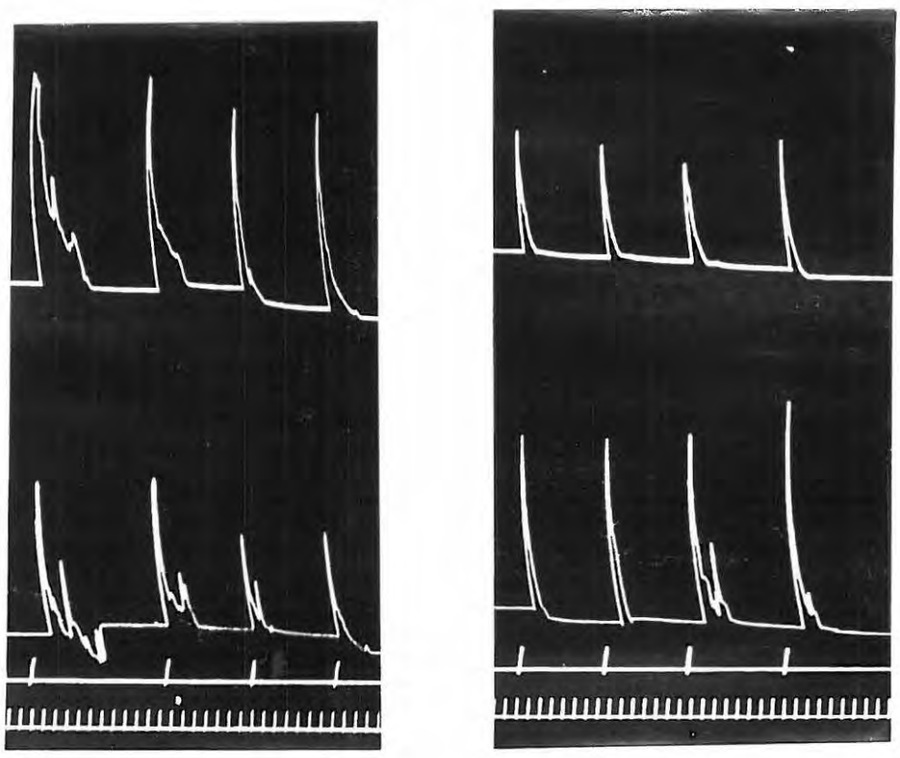
This conclusion leads directly to problems of the mechanisms of peripheral co-ordination as opposed to co-ordination through the brain, and the first question which can be approached is that of the functional plan of the nerve network. Can it be regarded as a true nerve net, comparable with that of the coelenterates, and carrying motor information to all parts of the animal?

This question has been investigated in the following way: an animal was partially hemisected as in fig. 62 (a), so that the brain was left intact; the animal was ligatured anterior to the brain and fastened down; each posterior half of the animal was then suspended from a separate lever. The levers were equally counterweighted, gave the same magnification of the response and were so arranged as to write on a smoked drum, the one directly below the other.

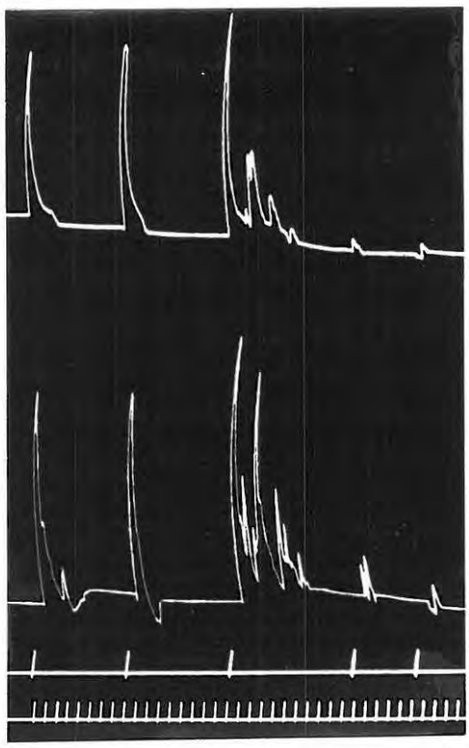
When both electrodes are placed on one half of such a preparation, both halves respond immediately and with equal, or almost equal magnitude to a singgl shock above threshold. This is illustrated in figs. 63 (a) - (c). When a slow kymograph speed is used, there is no detectable difference in the latency of the response of the two sides. In some cases the responses of the two sides were equal in magnitude, while in others there was a small difference figs. 63 (a) - (c). It is possible that the larger response frequently, but not invariably, observed from the side on which the electrodes were placed could be due to direct stimulation of the muscles on this side, but the difference in magnitude might be due to some decremental effect in the brain.

If the same procedure is followed, but in addition to partial hemisection, the brain is removed (fig. 62 (b)), there is no response from the side that is not directly stimulated. This is true, even for 10 successive shocks at a frequency of two per sec., up to a stimulus intensity of approximately 120 volts and 60 msec. duration. If, however, the intensity is raised to 150 volts or

Fig. 63.



(a) (b)



(c)

Fig 63: The responses of both sides of a partially hemisected, decerebrate animal to electrical stimulation of one side. The electrodes were on the side recording on the upper trace in (a) and (c) and on the side recording on the lower trace in (b). All stimuli consist of single shocks, with intensity varied as follows: (a) the first two shocks at 50v. and 50 msec; last two at 30v. and 50 msec; (b) all stimuli at 50v. and 50 msec; (c) the first three stimuli at 50v. and 50 msec; the last two at 5v. and 60 msec. For description of signal traces see legent to fig. 57.

Fig. 64.

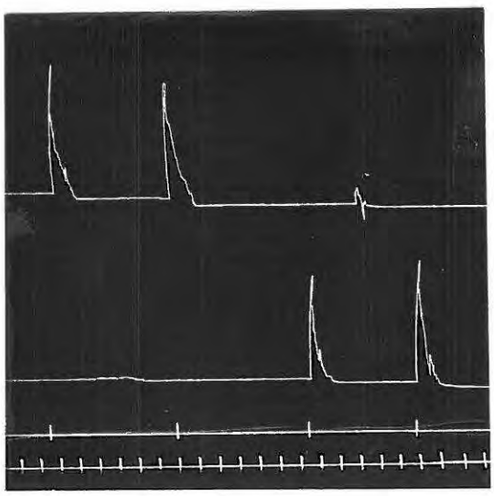


Fig.64: The responses of both sides of a decerebrate partially hemisected animal to electrical stimulation of one half of the animal. The half recording on the upper trace was directly stimulated by the first two stimuli; the half recording on the lower trace was directly stimulated by the second two stimuli. All stimuli at 50v. and 50 msec. For description of signal traces see legend to fig. [redacted]. 57.

greater, or if a series of shocks of 120 volts at a frequency of 10 per sec. is applied for 5 sec., a small contraction may be elicited from the other side. This is, however, an artifact, for it was found that if the electrodes were placed on the wet foam plastic at the base of the animal, so that they were not in contact with the animal at any point, and a stimulus of either of the above two magnitudes applied, both sides of the animal contracted. If, however, a series of shocks at an intensity of 120 volts and a frequency of two per sec., or 80 volts and a frequency of 10 per sec. be applied there is no response from either side no matter how long the stimulation is continued. To avoid this artifact, in all subsequent tests the stimulus intensity was kept below 100 volts. In no case could a response be elicited from the unstimulated side, even for a series of shocks applied at a frequency of 10 per sec. for 5 sec; this result was obtained on whichever side the electrodes were placed and irrespective of the position of the electrodes on that side (fig. 64).

These results clearly indicate that excitation does not pass from one side of the animal to the other via the nerve net, though it may be relayed through the brain. There remains however, the possibility that the nerve net had been damaged by the removal of the brain. This criticism is met by an experiment in which the brain was removed and the animal partially hemisected from the anterior towards the posterior end, so that the nerve net in the hind end of the animal remained intact. Again no response could be elicited from the side that was not directly stimulated. Finally, the possibility that the ligature damaged the intact end is excluded both by the fact that conduction occurred in the cerebrate preparation, that is, through the ligatured end, and by an experiment in which the animal was cut at the anterior and posterior ends, as in fig.62 (c), leaving a strip of tissue in the region of the pharynx intact; again no response could be elicited from the side not directly stimulated.

A final experiment was performed in which nerves VI and VII were cut through close to the brain, while the brain was left intact. In such a preparation there was no conduction from one side of the animal to the other, irrespective of which side of the animal was stimulated. This implies that nerves VI and VII are the motor nerves from the brain for the quick response of the longitudinal muscle and that motor information does not pass through the general network to the longitudinal muscles.

It is thus clear from these experiments that the nerve network of P.gilchristi is not a uniform network, allowing of conduction to any part of the animal, but that the essential link from one side of the animal to the other consists of a sensory input into and a motor output from the brain. However, it may be objected that the quick response of P.gilchristi does not involve any normal neuro-muscular mechanism of the animal, and that the above results are thus not a true reflexion of the functional characteristics of the nerve net in normal activity. To meet this objection, a number of observations were made on the effects on normal locomotion of cutting the large ventral nerve trunks which issue from the brain. These can be briefly reported as follows.

When nerves VI and VII (see fig. 9) are cut on one side of the animal, quite close to the brain, no swimming waves are elicited in the posterior half of that side of the animal, while the waves remain normal in the region anterior to the cut and also in the other side of the animal. These trunks are thus clearly responsible for the passage of the impulses from the brain to the posterior two thirds of the animal in normal swimming. It is also clear from this experiment that the motor impulses for the swimming movement cannot be carried from nerves I-V via the peripheral network to the posterior end of the animal. Likewise, cutting of nerves I-V leads to abolition of the swimming waves in the anterior region of the body on the side on which the nerve trunks had been cut, while swimming remains normal in the posterior end of the animal. It is thus clear that the motor impulses for co-ordinated swimming movements are carried to the different parts of the musculature along definite tracts, and that these impulses cannot be relayed from one of the anterior trunks via the peripheral network, to the posterior end, or vice versa. Similar effects of cutting the major nerve trunks have been described by Moore (1933) on Planocera reticulata and by Olmsted (1922) on Leptoplana saxicola. Thus the results from cutting the major nerve trunks confirms the picture presented by stimulation of hemisected animals.

The above results lead directly to the question of the relationship of the network system to the conduction of sensory information to the brain: that is, whether the sensory input can pass through the network and thus indirectly into the larger nerve trunks leading to the brain.

Fig. 65.

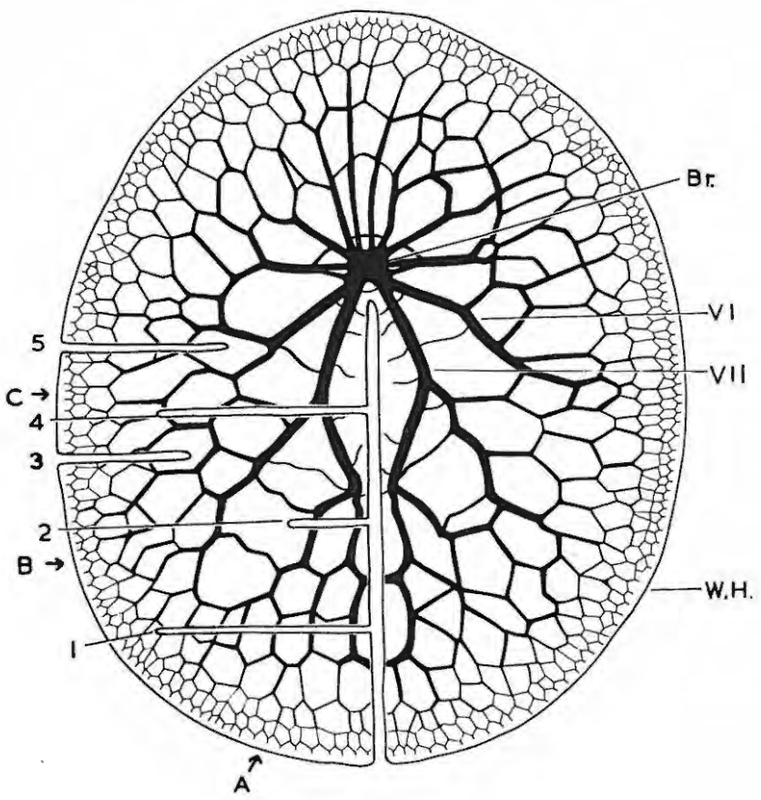


Fig. 65: Diagram showing the positions of sections through the nerve tracts of the ventral network in a hemisected animal.
A, B, etc. indicate successive positions of the electrodes; 1,2, etc. indicate successive cuts through the animal. Br. = the brain; VI and VII = main ventral nerve trunks.
W.H. witness half of the animal.

This question was answered by a series of experiments as follows: An animal was partially hemisected as before, and the brain left intact; one side of the animal, the witness half, was attached to a lever and the other side laid flat on a strip of wet foam plastic. The electrodes were placed at the end of this side of the animal, furthest from the brain, stimulation giving rise to a response from the witness half at low voltages and for a single shock. A series of cuts was then made, one at a time, on the side that was directly stimulated, and the responses of the witness half to this electrical stimulation noted. The positions of the cuts were later examined under a binocular microscope and plotted as in fig. 65, the successive positions of the electrodes being indicated by the letters A, B, etc., and the numbers indicating the successive cuts.

When the electrodes are in position A and cut 1 is made, there is no response from the witness half, even for a series of shocks at a frequency of 10 per sec. and at an intensity of 90 volts. If the electrodes are then moved to position B a contraction can again be elicited from the witness half.

When cut 2 is made, there is no effect on conduction to the other side, nor does cut 3 have any effect. When cut 4 is made, the response is again abolished. If the electrodes are moved to position C the response of the witness half returns, and cut 5 has no effect on this. It is clear from these experiments that cutting one of the major nerve trunks, as in cut 2, is without effect, and further, that cutting the network as in 3 and 5 also has no effect, but that sensory conduction to the brain from any area is abolished if both those major trunks which run directly to the brain are cut.

Thus all these experiments serve to show that there is neither a sensory or a motor system in the form of a functional nerve net. However, it will be remembered that it was earlier pointed out that mechanical stimulation at one point on the body of a decerebrate animal may activate all parts of the musculature. The latter observation indicates that there is indeed a functional nerve net. Clearly the two sets of observations conflict - the one indicating that there is no functional nerve net, the other that there is a functional nerve net. This apparent contradiction will be discussed presently.

One final point may be noted. It is clearly of some interest to be able to determine whether the response to electrical stimulation is due to direct stimulation of the muscles, or to stimulation of the nerve fibres. The experiments on hemisected animals clearly show that direct stimulation of sensory nerves to the brain is involved, and the response in the side not directly stimulated is a result of excitation conveyed by motor nerves from the brain. The possibility that some direct stimulation of the muscles takes place on the side directly stimulated cannot be excluded, and it has already been noted that the response on this side may be larger than that from the side not directly stimulated. However, the very precise correlations between thresholds and latencies of the responses from the two sides indicates that even in the side directly stimulated, the response is mainly due to direct stimulation of motor nerves. That the latter is a result of stimulation of motor nerves on that side of the animal and not to sensory nerves which then relay from the brain is shown by the fact that decerebrate animals respond in the same way and on the same time-scale, to direct electrical stimulation. This can also be extrapolated to preparations which consist of a whole animal.

TABLE IX (a).

Effect on Rate

Preparation	Potassium		Sodium		Calcium	
	High	Low	High	Low	High	Low
I. Myogenic Hearts						
Anomia	...	...	...	...	...	...
Aplysia	...	...	0	...	−	...
Helix	...	...	...	...	−	0
Octopus	...	...	...	...	...	+
Ostrea	+	+	...	...	−	+
Oyster	...	...	+	...	...	...
Pecten	...	...	...	...	−	+
Venus	irreg.	0,+	...	...	−	+
Frog	−, then +	−	+	−	−	+ or − in Ca^{++}-free
Raja	...	...	...	...	...	...
II. Neurogenic Hearts						
Cambarus	−	+	+	−	...	...
CrayfisH	...	...	...	...	−	+
Homarus	irreg.	...	...	−	...	...
Limulus	+	+	+	−	−	+
Lobster	...	...	...	...	−	+
Maia	...	...	...	...	...	...
Palinurus	...	...	...	...	...	...

Table IX: + indicates an increase in tone or activity rate;
− indicates a decrease in tone or activity rate;
... indicates no information available;
irreg. = activity irregular;
diast. = diastolic arrest;
syst. = systolic arrest.

TABLE IX (b).

Effect on Tone.

Magnesium		Potassium		Sodium		Calcium		Magnesium	
High	Low	High	Low	High	Low	High	Low	High	Low
...	+	...	...	...	...	...	...	...	..
...	...	+	...	+	...	...	+	...	+
...	...	+	...	...	...	−	+	...	..
...	...	...	...	...	...	...	...	...	..
...	...	+	+	...	...	+	...	...	+
...	...	...	...	+	...	...	...	...	..
−	0	...	...	...	...	+	+	...	+
...	...	−	...	...	...	...	...	...	..
...	...	0,−	...	+	+	+	−	...	..
−	...	...	...	...	...	...	...	+	..
...	...	...	+	...	...	...	...	...	..
...	...	...	...	...	...	+	...	...	..
...	...	+	...	...	...	...	...	...	..
...	...	...	...	...	...	...	...	...	..
−	...	...	...	...	...	+	+	−	..
...	...	...	...	...	...	...	...	...	..
+	...	−	...	...	...	...	...	−	..

TABLE IX (c).

Effect on Heart Arrest

Potassium		Sodium		Calcium		Magnesium	
High	Low	High	Low	High	Low	High	Low
syst.	diast.	...	...	...	diast.	diast.	syst.
syst. x7 diast.	...	...	...	diast.	syst.	diast.	syst.
syst.	diast.	...	...	diast.	syst.	...	...
...	...	...	...	diast	syst.	...	...
syst.	syst.	...	...	diast.	diast.	diast.	syst.
...	...	syst.	...	...	...	...	...
syst.	...	syst.	...	diast./ semi-syst.	diast.	diast.	diast.
syst.	...	...	...	diast.	semi-syst.	diast.	syst.
diast.	syst.	syst.	syst.	syst.	diast.	...	...
...	...	...	...	...	...	...	...
...	...	syst.	diast.	...	...	...	...
...	...	...	...	diast.	syst.	...	...
semi-syst.	diast.	syst.	...	...	...	...	...
syst.	diast.	...	...	diast.	syst.	diast.	...
...	...	...	...	diast.	...	diast.	...
...	...	...	...	...	...	...	...
diast.	...	...	...	diast.	syst.	diast.	...

4. DISCUSSION.

1. The Effects of Ions and Drugs:

It has been the main intent, throughout the course of this investigation, to provide some data on the responses of P.gilchristi, which could then be compared with the results of similar investigations on other invertebrate preparations. We are now in a position to review and analyse the available information.

There is an extensive literature describing experiments involving ion treatments on invertebrate muscular tissues, this commonly consists of accounts on the effects of one or a few ions only on some particular preparation. The greater number of these investigations have been made on hearts: the results of such experiments on molluscan hearts have been reviewed by Krijgsman and Divaris (1955), those on the hearts of tunicates by Krijgsman (1956), while Prosser (1950) has summarised the data from experiments on the hearts of a large number of invertebrates.

Although the spontaneous activity displayed by a preparation of P.gilchristi is often very irregular, nevertheless in some preparations the rhythmicity is striking and may continue for fairly long periods in a regular rhythm of contraction and relaxation. It is of interest, in considering any type of preparation which exhibits spontaneous activity, to compare the characteristic reactions of such a preparation with those of both myogenic and neurogenic hearts, in the hope that such an analysis might indicate whether the spontaneous activity being studied is itself myogenic or neurogenic. The effects of ion treatments clearly present a possible basis for such an analysis and comparison.

For this purpose, it is necessary first to examine and compare the effects of ion treatments on myo- and neurogenic hearts. To facilitate such a comparison, the data from the sources referred to above have been reassembled and grouped according to whether the heart beat is myogenic or neurogenic in origin. These data are presented in tables IX (a) - (c).

It can be seen that the available information is scanty and incomplete, but as far as it allows any conclusions to be drawn, it is clear that the overall picture presented is one of

TABLE X

A comparison of the effects of ions on invertebrate preparations.

(a) Effects on Tone

Preparation	Potassium		Sodium		Calcium		Magnesium	
	High	Low	High	Low	High	Low	High	Low
P. GILCHRISTI	+	0	+	-	+	-	-	+
ANEMONES	+	0 or +	+	0 or -	+	-	-	+
MYOGENIC HEARTS	+(or-)	+ ...	+	...	+(or-)	+	-	+
NEUROGENIC HEARTS	+(or-)	+ ...	...	...	+	+ ...	-	+
ARENICOLA EXTROVERT	...	...	...	...	...	...	-	...
CUCUMARIA CLOACA	+	slight +	...	...	...	...	...	...
EARTHWORM CROP/GIZZARD	+, then -	...	...	...	-	...	...	...

(b) Effects on tone and heart arrest

Preparation	Potassium		Sodium		Calcium		Magnesium	
	High	Low	High	Low	High	Low	High	Low
P. GILCHRISTI	+	0	+	-	+	-	-	+
ANEMONES	+	0 or +	+	0 or -	+	-	-	+
MYOGENIC HEARTS	syst. (diast.)	syst. or diast.	syst.	syst.	diast.	syst. or diast.	diast.	syst.
NEUROGENIC HEARTS	syst. or diast.	diast.	syst.	diast.	diast.	syst.	diast.	sy
ARENICOLA EXTROVERT	...	...	...	...	...	...	-	+
CUCUMARIA CLOACA	+	slight +	...	...	...	...	...	...
EARTHWORM CROP/GIZZARD	+, then -	...	...	...	-	...	...	...

(c) Effects on rate

Preparation	Potassium		Sodium		Calcium		Magnesium	
	High	Low	High	Low	High	Low	High	Low
P. GILCHRISTI	+	+, then 0	+	- or 0	+	-	-	+
ANEMONES	+	-	+	- or +	+	-	-	+
MYOGENIC HEARTS	+ ...	+(or-)	0 or + ...	- ...	-	+	-	+
NEUROGENIC HEARTS	- or + ...	+	+	-	-	+	-	...
ARENICOLA EXTROVERT	...	...	...	...	...	...	-	+
CUCUMARIA CLOACA	+	0	...	...	...	...	...	...
EARTHWORM CROP/GIZZARD	...	...	...	...	...	...	...	...

similarity between the effects of ions on the two types of heart: there is no consistent difference, for any of the ion treatments, between myo- and neuro-genic hearts. Thus, in fact, an analysis of such experiments does not allow one to draw a closer parallel between some particular rhythmically active preparation and one type of heart rather than the other. Nevertheless, a more general comparison can be made, as will be seen presently.

A few other invertebrate muscle preparations have been studies in the same way: the work of Ross (1960 a and a) on midcolumn and sphincter preparations of the sea anemones Calliactis and Metridium has already been mentioned: Wells (1942) investigated the effects of potassium ions on the cloaca of Cucumaria elongata; Wells and Ledingham (1940) the effects of magnesium ions on the extrovert of Arenicola marina and Ambache et al (1945) the effects of both potassium and magnesium ions on a preparation of the crop and gizzard of the earthworms Lumbricus terrestris and Allolobophora longa. These data,[1] together with the overall picture of the effects of ion treatments on the two groups of hearts are presented with the results of similar experiments on P.gilchristi in tables X (a) - (c).

It can be seen in the tables IX (a) - (c) that the data on the type of heart arrest induced by an ion treatment are somewhat more complete than those relating to tone and rate; it will also be noticed that the type of heart arrest does not always correspond with the effect on tone: thus, for example, the effect of high calcium ion concentration on the hearts of the crayfish and lobster is to increase tone and yet results in diastolic arrest. In the absence of the original sources of information, the actual effects are not clear, but it seems possible that such apparent contradictions may be the result of an initial effect of increasing tone, which is followed by relaxation and diastolic arrest, or vice versa. In other words, the type of arrest of the heart may be a more accurate reflexion of the effect on tone than some of the results presented in table IX (b). For this reason tables X (a) and (b) have been compiled separately, in an attempt to facilitate comparison and

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(1) The effect of ions upon the responses of the sphincter muscles of anemones to electrical stimulation are not considered here, as we are concerned only with effects on spontaneous activity.

to bring out any possible points that might otherwise be overlooked.

An examination of these tables reveals a number of significant points. The effects of K^+, Na^+ and Mg^{++} show a general consistency throughout. This is particularly so in the case of magnesium, which seems to have a general narcotising effect, and whose absence induces hyperactivity and a positive inotropic response. In spite of a number of variations, the excitatory action of potassium appears to be very widespread. In fact Wells (1942) on the basis of his specific experiments on the action of potassium on the crops of Aplysia and Helix, the hearts of Aplysia, Helix, Maia and Homarus, has suggested that all types of rhythmically active muscle are essentially alike in their responses to potassium. The few exceptions recorded in the tables presented here rule out the validity of too wide a generalization, but such a statement appears valid as a first approximation.

In the case of sodium there is again a certain amount of variability in the response to an excess or defect of the ion, but the overall picture presented by the tables is that high Na^+ concentrations are excitatory, while low concentrations or Na^+ deficit may have a depressant action.

The most interesting point which emerges from this survey is the consistent difference between the effects of excess and defect of Ca^{++} on both myo- and neuro-genic hearts and the earthworm preparations on the one hand, and on preparations of both the sea anemones and Planocera on the other. This, as well as the general correspondence between the effects of all the ion treatments on these two preparations, warrants a more detailed examination of the responses of the anemones and further comparison with those of P.gilchristi.

The responses of P.gilchristi and of mid-column preparations of Calliactis and Metridium to single and multiple ion changes are summarised in tables XI (a) and (b). Representation of the effects on the rate of activity in these tables has presented some difficulty, for it has become clear that Planocera shows far more marked increases in the rate of activity than do the anemone preparations; thus Ross records increases in rate of the order of 25%, 50% and 75% of the original rate as '+', '++' and '+++', while it will be remembered that increases of the order of from two to eight or more times the normal activity rate

TABLE XIII.

A Comparison of the effects of drugs on P. gilchristi with those on column preparations of the anemones Calliactis and Metridium.

Drugs.		Effects of Treatments: On Tone		On Activity.	
		Anemones	Polyclad	Anemones	Polyclad.
Acetylcholine	1.10^{-4}	0	0	0	0
Acetylcholine (Eserinised)	1.10^{-4}	+	0	0	0
	6.10^{-4}	+	0	+	0
Eserine	1.10^{-4}	0	0	0	0
	1.10^{-3}	+	0/++++	0	+++ then —/Ab.
D-tubocurarine	1.10^{-4}	0	0	0	0
Adrenaline Cl	1.10^{-7}	0	0	0	0
	1.10^{-6}	+	0	+	0
	5.10^{-6}	...	+	...	+
	1.10^{-5}	++	++	+	++
	1.10^{-4}	+++	+++	+	+++
Nor-adrenaline	1.10^{-5}	0	0	0	0
	1.10^{-4}	+	+++	0	++
Tyramine	1.10^{-5}	0	0	0	0
	1.10^{-4}	+	0/+	0	0
	$2.5.10^{-4}$	+	...	0	...
	1.10^{-3}	...	+	...	0
3-Hydroxytyramine	1.10^{-4}	0	0	0	0
	5.10^{-4}	...	+	...	0
	1.10^{-3}	...	+	...	0
Yohimbine	1.10^{-4}	0	0	0	0
Tryptamine	1.10^{-5}	0	0	0	0
	5.10^{-5}	+	0	0	0
	1.10^{-4}	+++	0	+	0
	1.10^{-3}	...	+++	...	++++
5-Hydroxytryptamine	1.10^{-5}	0	+	0	0
	1.10^{-3}	0	++	0	0
L S D - 25	1.10^{-5}	0	+	0	0
	1.10^{-4}	0	++	0	0/+
Stryohnine	1.10^{-4}	+	- -	-	+++
Gamma-amino-butyric acid	1.10-4	+	0/-	0	0/-

are recorded from P.gilchristi. Nevertheless, the most important points to be noted are the relative effects of one ion or drug to another in each type of preparation, and for this purpose it is obviously more profitable to use a grading which will reveal the difference in effects of different solutions on each preparation, than to attempt to reduce the results from both preparations to the same scale of representation, and thereby obscure many significant points. Where the effects on tone are concerned, Ross has used the same scale of grading as has been used for Planocera (p. 20), with the exception that while Ross records all effects over a 75% increase as '+++', in this investigation effects which show an increase of over 100% are recorded as '++++'; for the reasons expressed above, this additional representation will be retained for the results on P.gilchristi recorded in tables XI (a) and (b).

It can be seen that there is a close parallel between the relative effects of different ions. Thus, for example, while both calcium and potassium ions have more marked effects in excess on the P.gilchristi preparation, on both preparations calcium ions are less excitatory than potassium ions. But the following points of difference between the reactions of the two preparations may be noted.

(i) As has already been pointed out, most of the treatments have a more marked effect on the polyclad preparation than on the anemone preparations; this is true not only of excesses of calcium and potassium ions, but also of an excess of magnesium ions. However, the effect of Ca^{++}-lack appears to be more marked on the anemone preparations, apparently causing complete abolition of activity.

(ii) Ross (1960 a) reports that the effect of excess magnesium ions cannot be offset by simultaneously increasing the concentration of either the calcium or potassium ions. However, the figures tabulated by Ross, which are also presented in table XI (b), show only the effects of raising the potassium ion concentration to four times the normal, with a magnesium ion concentration of twice the normal, while it has been shown that in P.gilchristi an even higher concentration - six times the normal - is required to balance the action of magnesium excess really effectively. It is uncertain whether higher concentrations of potassium ions were tested with such an excess of magnesium ions. The effect reported for the anemones is, however, of further interest in that

it emphasises the importance of magnesium ions in maintaining the normal activity of the animal, an emphasis which has already been observed in the results from tests on P.gilchristi.

(iii) As recorded in table X I (b), the responses of the anemones and Planocera to pure NaCl appear to differ. However, although activity is abolished in the anemones after 15 - 30 min. in pure NaCl, the tone is maintained at a high level. Further, while preparations of P.gilchristi usually displayed prolonged and marked hyperactivity as well as a rise in tone, there was a single preparation in which activity was abolished after about 20 min., although tone was maintained at the new level. The effects of pure NaCl are, however, difficult to interpret and hence to compare in the two preparations. It has been shown that in P.gilchristi the effect of pure NaCl is more likely to represent an effect of magnesium ion lack rather than any marked excitatory action of the sodium or chloride ions; such a possibility does not appear to have been investigated in the anemones, but mention may be made of the effect of replacing the NaCl of artificial sea water with some substitute substance. When replaced with sucrose, the effect on the anemone mid-column preparations is identical with that on Planocera, but when, with an anemone preparation, it is replaced by choline chloride, the depressant action is entirely absent, and there are even signs of hyperactivity, which are not due to the choline chloride itself. Thus in the anemones it must be concluded that the depressant action is due to lack of chloride ions rather than to a deficit of sodium ions, and the same may also be true for Planocera, on which the test with choline chloride could not be performed. Thus the precise action, if any, of sodium itself remains obscure. Further, since the cations magnesium, calcium and potassium have been shown to be of considerable importance in maintaining the conditions for normal functioning of the animal, it is quite possible that the effects witnessed in a solution of pure NaCl are a result, at least in part, of generally unphysiological conditions in such a solution. It seems unlikely that the difference in the responses recorded should be regarded as an important difference between the two types of preparation under consideration.

All in all, the differences which have been pointed out appear to be ones of degree only, and as far as one could legitimately expect identical responses to be shown by two preparations, not only of animals from two different phyla, but also

differing in that one consists of a preparation of the whole animal, and the other of a ring of the body wall of the animal, the parallels between the responses of the polyclad and the anemones Metridium and Calliactis would seem to be not without significance. But before leaving this comparison of ion effects, it is necessary to discuss another set of such experiments, those of Horridge (1956) on the alcyonarian Heteroxenia fuscescens (Ehrb.).

Firstly, with respect to the effects of potassium, Horridge finds that the overall effect of a potassium ion concentration of 5.02 times the normal is to relax and paralyse the colony, and that initial excitation usually only results from rapid addition of the excess potassium to water surrounding the colony. These observations are interesting in view of the action of excess potassium on sphincter preparations of Calliactis and Metridium, where excess potassium causes an early enhancement of the quick sphincter contraction in response to electrical stimuli, but long exposure to excess potassium ions induces a general depression and abolishes all response to stimuli. This suggests that the rhythmical beating shown by the zooids is mediated by spontaneous activity within the "fast conduction system" and is therefore not comparable with the slower spontaneous activity of the mid-column of Metridium and Calliactis. This is partly reflected in the fact that the beating occurs roughly every two sec., compared with intervals of several minutes which separate major contractions both in mid-column preparations of Metridium and Calliactis and in the present preparation of Planocera.

The effect of Na^{++} at 1.2 times the normal concentration is first to accelerate the rhythm and then to slow it down, but the latter effect seems to be due to the production of permanent spasms over the colony, and not to a direct depressant action. Thus again the response of Heteroxenia to an excess of Na^{+} appears to be comparable to that of sphincter preparations of Calliactis and Metridium to a solution of NaCl only, although it also resembles the response of the P.gilchristi preparations to pure NaCl.

Magnesium at 2.72 times normal has essentially the same effect on the coral as on the anemone and polyclad preparations.

The effect of excess Ca^{++} is difficult to interpret. Horridge reports a slowing of the beat of the zooids, but since the sphincter preparations of Metridium and Calliactis are not spontaneously active, no effective comparison can be made in this respect. Horridge further reports that excess Ca^{++} produces spasms of the zooids: the exact nature of these events, apart from the greater amplitude of contraction involved, is not clearly described, but it is perhaps of significance that Ca^{++} in excess greatly enhances the quick response of the anemone preparations. It may perhaps be added that while in his Table I, Horridge speaks of excess Ca^{++} causing "relaxed paralysis", it is clear from the text that the responses of the colony to mechanical stimulation are not prevented: the "paralysis" is limited only to the rhythmical beating.

In discussing his results, Horridge points out that he has obtained the same responses on the scyphozoan medusa Cassiopea andromeda and then shows his results on this medusa and on Heteroxenia to be identical with the results of Mayer (1906) on Cassiopea xamachana. However, the particular results from Mayer's experiments which have been selected and tabulated by Horridge as the effects of excesses of K^+, Ca^{++}, Na^+ and Mg^{++} are actually the effects observed by Mayer when medusae were placed in solutions of pure K_2SO_4, $CaCl_2$, NaCl or $NaCO_3$ and $MgSO_4$ or $MgCl_2$. When the rest of Mayer's results are examined, a rather different picture emerges. The greater number of his experiments were done by placing medusae in artificial sea waters lacking one or more ionic components: in other words, the effects recorded are those of ion deficit and not excess. A survey of these results reveals the following points:

(i) In all these artificial sea water solutions the normal pulsations eventually cease, irrespective of the initial response;

(ii) K^+-lack causes more rapid cessation of pulsation, but the beat can be restored by adding the required amount of a K^+ salt.

(iii) Ca^{++}-lack causes a very markedly rapid cessation of pulsation, and pulsations are restored with almost equal rapidity when the Ca^{++} is restored in the correct proportion.

(iv) Mg^{++}-lack causes hyperactivity, which is supressed on restoring the Mg^{++}.

These results seem to indicate very clearly that this medusa responds in the same way to the same ions as do the anemone mid-column preparations. Further, the effects of lack of more than one ion in a single test present the same general picture as for the P.gilchristi and anemone preparations, and this is particularly so for medusae which have been deprived of their marginal sense organs.

The fact that in each case, even where the effect is one of initial excitation, the normal pulsations cease after a variable period of time, as they do when the animal is put in pure isotonic solutions of potassium, calcium or magnesium salts, seems to point to the possibility that prolonged exposure to unbalanced ion solutions produces general physiological disturbances of the medusae, which obscure the action of the ions on the myoneural system. This is likely to be most marked where single ion solutions are used. It thus seems more reasonable to base any comparisons on the results of Mayer's tests involving ion lack, and in these to accept only the initial effect of each treatment as significant; when this is done, no valid reason can be seen for supposing that C. xamachana behaves in a way that is very different from the anemones in its responses to ion treatments. Furthermore, if the suggestion that only the initial responses of jellyfish be taken as significant, Horridge's observations on the behaviour of C. andromeda also broadly agree with the behaviour of anemone mid-column preparations, both K^{+} and Ca^{++} ions being excitatory and Mg^{++} depressive.

In sum there are striking resemblances between the effects of ions upon the preparation being studied and upon preparations of coelenterates where slow rhythmical activity is displayed. The responses in both cases share particularly an excitatory action of Ca^{++}, an effect not reported elsewhere among invertebrates.

The parallel between the reactions of anemones and Planocera preparations is not confined to the action of ions, but is further emphasised by the action of drug treatments.

Before such a comparison can be made it is necessary to discuss certain aspects of the effects of drug treatments on P.gilchristi in more detail. One of the most striking features of these results is the very high minimal effective concentrations of nearly all the substances tested, concentrations which could, in fact, be considered as unphysiological. In other words,

the criticism may be raised that some of the effects which have been witnessed are not the result of the action of the drugs on nerve, muscles or the synaptic junctions themselves, but instead, the result of unspecific sensory stimulation of the animal. This is a question which is difficult to resolve directly. Ross (1960) has suggested and used an experimental technique which, he states, provides information on the site of action of both ion and drug treatments. This involves using a ring preparation of the mid-column of an anemone, but with a strip of the column attached, then exposing the strip alone to certain solutions and observing the presence or absence of a response from the ring. In no case did he find that a response could be elicited from the ring, when only the strip and not the ring was immersed in the test solution. From this, Ross concludes that the effects arise in the muscle itself or in that part of the nerve net in immediate contact with the muscle. This technique does not, however, eliminate the possibility of direct chemical stimulation of sensory structures, causing localised responses which are not conveyed to the column ring from the strip, nor the possibility that some of the treatments are simply the result of the summation of such localised responses. For this reason, no attempt was made to experiment with P.gilchristi in this way, and it is difficult to see how this criticism can be resolved.

On the other hand, the high concentrations required may be a reflexion of the relative impermeability of a preparation consisting of the whole animal: most other preparations which have been subjected to such tests consist either of hearts or isolated smooth muscle preparations from vertebrates and invertebrates, which are likely to offer less resistance to penetration. One observation which tells against this possibility is that preparations that were exposed for fairly long periods to concentrations just below the observed minimal effective concentration did not show any signs of a delayed response. This problem, however, requires further investigation in the form either of even more lengthy exposures to the drug, or making a small lateral incision in the preparation to facilitate penetration of the drug.

In the absence of further investigation of this point it seemed that the overall picture of drug action on the preparation might be clarified by a table in which effective substances are grouped according to their minimal effective

TABLE XII.

Minimal Effective Concentrations of Drugs active on a preparation of P.gilchristi.

Concentrations.					
5.10^{-6}M	1.10^{-5}M	5.10^{-5}M	1.10^{-4}M	5.10^{-4}M	1.10^{-3}M
Adrenaline					
	5-H T				
	L S D - 25				
	Strychnine				
		Nor-adrenaline			
			G A B A		
				Tryptamine	
				3-Hydroxy-tyramine	
				Eserine	
				Glutamic acid	
					Tyramine
					Picrotoxi
					Yohimbine
					Cadmium Chloride

concentrations. But as can be seen by comparing table XII with tables IV - VII, the picture presented is a complex one, for the order of magnitude of the minimal effective concentration of a drug does not always correspond with the relative order or magnitude of its effect. Thus LSD-25 and 5-HT have lower minimal effective concentrations than nor-adrenaline and tryptamine, but also a weaker action on the preparation. Clearly such a table does not allow a simple division into substances which are likely to be having a "true" effect and those which may be having an effect by virtue of sensory stimulation. There is one other means by which it is considered that the action of a drug may be estimated, namely the time required after washing out before the preparation returns to normal activity. Clearly this cannot be taken as the only basis for the validity of an effect, for there will be some substances which will wash out more readily than others, but where the effect is questionable, this may be of some assistance. To facilitate the analysis, those substances in which the effect continues for longer than 15 min. after washing out have been indicated by a broken line, while those in which the effect waned after a short period (15 to 30 min.) before washing out have been indicated by a solid line in table XII.

A consideration of this table together with tables IV-VII (pp.34a, 37a, 40a, and 44a) which show the magnitude of the effects does, however, serve to bring out the relative importance of some substances rather than others.

In the first place, adrenaline stands out as being not only one of the few substances with a really marked effect, but also one which acts at a concentration far lower than any other substance. Among the sympathomimetic amines, nor-adrenaline falls in very close behind adrenaline, while the other sympathomimetic substances are of relatively far less significance in producing direct effects.

Among the indolalkylamines, tryptamine stands out as having by far the most marked effect: in fact, its action on activity rate is greater than that of adrenaline. However, this effect only occurs at a concentration of 1.10^{-3}M, the drug having only a small effect on tone at 5.10^{-4}M. It seems unlikely, however, that this substance is acting only by virtue of superficial sensory stimulation, for two reasons: first, this whole group of substances shows signs of being effective, and although

TABLE XI.

(a) A Comparison of the Effects of Single Ion Treatments on P. gilchristi with anemone and mid-column preparations.

		Effects of Treatments.			
		On Tonus		On Activity Rate.	
		Anemone	P.gilchristi	Anemone	P.gilchristi.
K^+	x 2	+	+/++	0	0/+
	x 4	++	+++	0	+++
	x 8	+++	++++	+	....(1)
Ca^{++}	x 2	0	0	0	0
	x 4	0	0/++	0	0/++
	x 8	+	+++	+	+++
Mg^{++}	x 2	0	- - -	- -	- -/Ab.
	x 4	-	- - - -	Ab	Ab.
K^+-free		0	+/+++ then 0/-	Ab	0/+++., then 0/-
Ca^{++}-free		-	- - -	Ab	- - - -
Mg^{++}-free		+++	+++	+++	++
Na^+ -free (replaced by sucrose)		-	- - -	-	- -

(b) A Comparison of the Effects of Multiple Ion Treatments on P.gilchristi with anemone and mid-column preparations.

		Effects of Treatments.			
		On Tonus		On Activity Rate.	
		Anemone	P.gilchristi.	Anemone	P.gilchristi.
K^+	x 4	. .		.	. .
Ca^{++}	x 4	+++	++++	+	+++
K^+ Mg^{++}	x 4 x 2	0	++, then -	- -	0/+, then -
Ca^{++}-free Mg^{++}-free		+++	+/+++	++/+++	0/++
NaCl only		+	++++	Ab	++++

not dramatic, the effect of 5-HT occurs at a relatively low concentrations; secondly, although the effect on tone is rapidly abolished after washing out, the effect of tryptamine on activity rate continues for long periods. Although 5-HT and LSD-25 act at much lower concentrations than tryptamine, their effect is never so dramatic. It is thus rather difficult to decide on the relative importance of these three drugs to one another - that is, whether tryptamine is to be regarded as relatively more important because of its very dramatic effect at high concentrations, or LSD-25 and 5-HT because of their action at far lower concentrations. However, the significance of this whole group of substances is quite clear from the fact that all three have dictinct effects on the Planocera preparation.

The only other two substances which have a marked effect are strychnine and picrotoxin. The latter only acts at a concentration of 1.10^{-3}M, its effect on tone often wanes after about 20 min., but the effect on rate may continue for some time after washing out. Strychnine is effective at a relatively low concentration.

The effects of drugs on preparations of P.gilchristi and of the mid-column of Calliactis and Metridium are summarised and compared in table XIII, the same schemes of representation of the effects being used here as were used in table XI (see p. 67). As will be seen, the parallel between the two preparations with respect to their responses to various drug treatments is even more striking than is the case with ions.

The first most striking resemblance to be noted is that in the anemones, as in P.gilchristi, the responses to adrenaline and tryptamine stand out in strong contrast to the rather weak responses to other effective substances. A response is elicited at a somewhat lower concentration in the anemones in the case of each of these drugs, but the response to 1.10^{-4}M adrenaline seems to be as closely similar in magnitude as it is possible to judge a quantitative effect accurately in experiments such as these, while in the case of tryptamine, the response to a 1.10^{-3}M solution in P.gilchristi resembles the response of the anemones to a 1.10^{-4}M solution. The resemblance is most striking with respect to the action of the drugs on the tone of the preparations, but in P.gilchristi the effect on the rate of activity is not only more marked in the case of these two drugs, but a glance at the table will show that in general the activity of the Planocera

preparation is affected more than that of the anemone preparations.

As can be seen from the table, the parallel is not limited to the actions of adrenaline and tryptamine. In neither preparation is there any evidence for a general cholinergic mechanism of synaptic transmission. The effect of eserine on P.gilchristi has already been discussed and if this is indeed a sensory stimulation effect, the same may be true of the anemones, and the slight and rather insignificant effect of eserinised acetylcholine may be explained in the same way, as a sensory excitation due to the eserine.

Among the other sympathomimetic amines tested on both anemone and polyclad preparations, nor-adrenaline, which has an effect on P.gilchristi, has no significant effect on the mid-column preparations of the anemones; however, it does give some enhancement of the slow contraction in response to electrical stimulation in sphincter preparations of both Calliactis and Metridium, and in the Metridium sphincter it gives rise to a slight increase in tone of the preparation. Tyramine has a weak effect on both anemone and polyclad preparations, but has also been shown to have a marked enhancing effect on the quick sphincter response. It would be interesting to know the effect of this drug on the response of P.gilchristi to electrical stimulation, but although this experiment has been attempted, satisfactory observations have not yet been obtained.

Among the indolalkylamines, 5-hydroxytryptamine has no direct effects on the anemone preparations, while it does have an effect, although not very marked, on P.gilchristi; however, it enhances the responses of the column and both the quick and slow responses of the sphincter of Calliactis. Again it will be necessary, in further experiments, to give completion to this comparison by testing the effect of 5-HT on the responses of P.gilchristi to electrical stimulation. In its response to LSD-25, P.gilchristi differs from the anemone preparations.

The anemone and polyclad preparations are both further characterised by the fact that substances which have depressant or inhibitory actions on other preparations have either no effect at all, or at best, only a weak and ill-defined depressant action: such is the case of BOL and GABA on P.gilchristi preparations. In fact, no drug has been found which effectively inhibits either of the two types of preparation.

The sphincter preparations of Calliactis and Metridium show far fewer direct responses to the drugs which could be compared with those of P.gilchristi. The most outstanding effects are those of adrenaline, tyramine and tryptamine on the responses to stimulation. In the Calliactis sphincter, adrenaline causes great enhancement of the slow sphincter response, but this effect appears to be absent in the Metridium sphincter, where it causes direct effects by way of increased tone and some direct contractions. Tyramine has no direct effects, but enhances the quick response of the sphincter of both anemones. Tryptamine elicits some direct contractions and causes a rise in tone in Metridium. It enhances both the quick and slow responses of Calliactis sphincter, but only the quick response in Metridium sphincter. As has already been said, since experiments on the effects of drugs on responses to electrical stimulation in P.gilchristi have not been undertaken, these results cannot be compared, but they serve further to emphasise the importance of the above substances in the myo-neural system of anemones.

A further substance which has been tested on both anemone and polyclad preparations is strychnine. The effects of this drug are interesting in view of the finding of Moore (1918) that the normal reaction of the triclad Bdelloura sp. to a mechanical stimulation, namely, a contraction giving rise to a shortening of the body, is reversed in animals placed in a strychnine solution: that is, a mechanical stimulus now gives rise to an inhibition of the longitudinal muscle fibres and contraction of the circulars. An attempt was made to repeat these observations on P.gilchristi, but without success: in each case, mechanical stimulation of an intact animal in strychnine resulted in normal locomotion, while with a decerebrate animal, the stimulus invariably resulted in a localised contraction of the transverse muscles. However, the fact that in P.gilchristi there is a fall in tone, while in the anemone there is a rise in tone at comparable concentrations of strychnine is interesting because in the anemone it is the action of the circular muscles which is being recorded, while in P.gilchristi the action of the longitudinal muscles is being recorded. If we follow Moore's interpretation, at the same time taking into account the fact that in vertebrates strychnine is thought to act competitively with the inhibitory transmitter, the effects on P.gilchristi and the anemones would appear to be a result of the abolition of some inhibition of the

circular muscles in each case. It is doubtful, however, whether these observations are of great significance, for the same sort of effect has been witnessed in earthworms (Moore, 1918).

In sum, there is a marked similarity between the responses of P.gilchristi and the anemone preparations studied by Ross to adrenaline, tryptamine and tyramine. On the basis of these observations the very tentative suggestion may be made that we are dealing in anemones with two transmitting systems: this might be carried further to the suggestion that the one is mediated by a catechol amine, the other by an indolalkylamine. It seems reasonable to attempt to link each transmitting system with a particular type of response - either the quick or the slow response. But if we attempt to do this on the supposition that one transmitting system is mediated by a catechol amine, the other by an indolalkylamine, certain difficulties arise. First, in the sphincter we find that an indolalkylamine - tryptamine - affects both the quick and the slow response. However, since the effect on the quick response is greater than that on the slow, this may be explained as follows: while it is possible that, when drugs are added, enhanced tone follows stimulation of the specific type of myo-neural junction, it is also possible that a general excitatory condition might stimulate both types of junction. However, when it is also remembered that tyramine, a catechol amine, also effects the quick and not the slow response, the picture becomes very confused, for if we return to the hypothesis that there are two transmitting systems, each mediated by a specific type of substance, and associated with a particular type of response, we have here a quick response which is mediated by both types of transmitting system. Such a possibility cannot, of course, be excluded but it seems unnecessarily complicated. Further, the slow response of the sphincter is affected by another catechol amine - adrenaline, so that while the quick response is affected by an indolalkylamine, both the quick and the slow are affected by catechol amines. A suggestion made by Ross (1960 b) is helpful in this connection. He points out that in anemones the action of excess K^+ is very closely similar to that of tryptamine, and that since excess K^+ usually has depolarising effects on excitable tissues, the effects of this ion and of tryptamine may be due to an unspecific depolarisation of the muscle membrane. This would explain the effects of tryptamine on both types of response, as well as on the mid-column, and might also explain the very high concentration required for the

drug

to have effect on a preparation of P.gilchristi.

If this explanation of the action of tryptamine is accepted as being the most likely, the alternative suggestion can be made, that, while we are possibly dealing with two transmitting systems, each may be mediated by a different type of catechol amine. In the sphincter of Calliactis the slow response is enhanced by adrenaline, and the quick response by tyramine. A difficulty seems to arise, however, when it is remembered that in the mid-column, with its seemingly single type of response, both tyramine and adrenaline affect the slow spontaneous activity, although the effect of the former is far less significant in magnitude than the latter. However, Ewer (1960) has shown that the response of the mid-column to electrical stimulation is double in nature, with two responses of different latency being recognisable. One, of a very brief latency, is poorly developed in Calliactis, but in Bunodosoma (Ewer, unpublished observations) it is the dominant response of the mid-column. Thus it may be possible to interpret the slight effect of tyramine on the mid-column preparations of Calliactis and Metridium as an action on this very poorly developed quick response, the lack of any marked effect of the drug being directly correlated with the lack of development of the quick contraction mechanism of the mid-column.

Such an hypothesis must inevitably be regarded as very tentative and its extension to P.gilchristi lacks proof. It is, however, of considerable interest to recall the distinction between the slow periodic spontaneous contractions and the quick response to electrical stimulation (p.53), suggesting that here too we are dealing with two systems, and a closer study of the effects of these drugs upon the quick response to electrical stimulation is clearly very desirable. Finally, it must be noted that although 5-HT and LSD-25 may be acting in the manner suggested above for tryptamine, the possibility that some indolkylamine-like substance is acting as a transmitter substance in these animals cannot be finally excluded. In fact there is little to suggest at present whether, if there are two transmitting systems in this animal, they are mediated the one by an indolalkylamine and the other by a catechol amine, or each by a different catechol amine.

Indeed, it is not possible to extrapolate these results on drug treatments to any clear conclusions about the actual transmitter substance or substances in these animals. In the first place, the precise action of the effective drugs, whether on muscle, nerve, or the synaptic junctions, remains obscure. Further, although

Both anemone and polyclad preparations are sensitive to adrenaline, neither of the preparations appear to be affected by substances which normally antagonise the action of adrenaline: it thus cannot be concluded that adrenaline is acting in a manner functionally similar to its action on sympathethc effectors; neither can it be assumed that adrenaline itself is a transmitter substance in these animals. Nevertheless, the fact that a number of sympathomimetic substances have effects on these animals is undoubtedly significant. In this connection, Östlund's (1954) discovery that an unidentified catechol amine can be extracted from the tissues of Metridium is of considerable interest.

In the case of indolalkylamines too, although these substances, notably tryptamine, do have an effect, substances which are said to antagonise their action in other muscle preparations are without any definite effect on these two preparations. The suggestion of Welsh (1957) that 5-HT is a neuro-humour in a number of invertebrates cannot be extended to either the anemone or the polyclad preparations which have been considered here. In this connection it is interesting to note that while rather large amounts of 5-HT can be extracted from Calliactis and Metridium, it has recently been shown (Welsh, 1960) that the distribution of 5-HT in Metridium can be correlated very clearly with the distribution of the nematocysts, suggesting that any 5-HT extracted from these animals is likely to be associated with the nematocysts and not with any neural transmitter action.

Finally, it may be possible to relate the distinctive action of calcium ions on P. gilchristi and the anemones with the nature of the transmitter substance.

A number of different roles have been suggested or demonstrated for calcium ions. Thus there is some evidence that it has an unspecific action on the permeability of cell membranes to water and ions (Heilbrunn, 1952; Robertson, 1941). Further, Hodgkin (1958) has suggested that in the resting muscle cell the carrier substance is blocked by calcium ions, while Bailey (1942) has shown that calcium is associated with the contractile process in muscle and activates myosin ATP-ase. But there is also some evidence that calcium ions are assocoated in some way with release of the transmitter substance.

Harvey and McIntosch (1940) have shown that Ca^{++} are essential for the release of acetylcholine in vertebrate skeletal

muscle. In view of this, and also the very considerable evidence for cholinergic transmission of inhibition on the molluscan heart (Krijgsman and Divaris, 1955), it would appear that the depressant action of calcium ions on the molluscan heart may be due to enhanced liberation of acetylcholine as a result of the ion, and hence inhibition. The picture is, however, not simple, for while Ca^{++} are also depressant on the few arthropod hearts for which we have any information, it is also clear that acetylcholine is not an inhibitory transmitter substance in these hearts and in fact has an excitatory action (Krijgsman, 1952). The evidence includes work on the hearts of Palinurus and Limulus both of which have been shown to be depressed by calcium ions (Table IX). Further, the experiments of Ambache et al (1945) on the crop and gizzard of the earthworm have shown that while acetylcholine has a marked excitatory effect, calcium ions have an inhibitory effect: they associate the latter effect with a reduction in release of acetylcholine.

Thus, although it is not possible to make any generalisations concerning the action of calcium, there is evidence that it may be associated in some way with the release of the transmitter substance, and in this connection it may not be without significance that not only do the anemone and Planocera preparations differ from all others in their response to calcium ions, but at the same time show so marked a parallel in their responses to the same drugs.

It may be noted too that Ross (1960 b) has made the interesting suggestion that the action of excess calcium is very closely similar to that of tyramine, while the tryptamine response is almost identical with that of excess potassium. In P.gilchristi however, while the responses to potassium and tryptamine are closely similar, both substances having a marked effect on both tone and activity, the analogy between the effects of calcium ions and tyramine is not so clear: it is true that both have rather weak effects and that calcium only affects the rate of activity in a rather high concentration, but tyramine appears to be without effect on the rate of activity at all. Until we have some information on the effects of excess calcium on the responses of P.gilchristi, this analogy does not lead anywhere.

2. Responses to Electrical Stimulation:

It is now necessary to analyse and compare the physiological characteristics of the nervous systems, as revealed by experiments using electrical stimulation, of the anemone and polyclad preparations. But before commencing such a discussion, it is desirable first to examine the functional relations of the nervous system of P.gilchristi itself in more detail. We may well start with the most obvious difference between the nervous systems of coelenterates and platyhelminths, namely the possession by the latter of a more condensed central nervous system in the form of a brain from which a number of large nerve trunks arise.

Observations on the role of the brain and main nerve trunks of P.gilchristi in the normal activities of the animal have already been reported, and it is of some interest to compare these with the results of similar investigations on a number of other polyclads and triclads.

Olmsted (1922) found that in Planocera californica, Phylloplana littoricola and Leptoplana saxicola the role of the brain is that of a co-ordinatory centre for the normal locomotory activities of the animal, while the same has been shown by von Levetzov (1936) on a number of polyclad worms, including Thysanozoon brocchi, Stylochus neapolitanus and Stylochus pilidium. On the other hand, Moore (1923, 1933) has suggested that the brain inYungia and in Planocera reticulata is not necessary for co-ordination of locomotory movements, but serves mainly as an amplifier of sensory impulses. He reports that if decerebrate animals are stimulated by placing them in a solution of phenol in sea water in the proportion 1:40,000, or in dilute solutions of strychnine or nicotine, co-ordinated locomotion is elicited. This he interprets as indicating that the nerve network in these polyclads contains the necessary neural apparatus for co-ordinated action, and that the only effect of absence of the brain is that the general state of excitation in the reticulation of nerves is so low that spontaneous co-ordinated locomotion cannot take place. These experiments were repeated on P.gilchristi, and while dilute solutions of phenol or strychnine stimulated the animals into movements which were clearly components of normal swimming and crawling, these were definitely not co-ordinated. Von Levetzov (1936) too, reports that co-ordinated locomotion could not be obtained by artificial stimulation in the absence of the brain. It is very

difficult to determine what Moore regards as 'co-ordinated' locomotion, but it is doubtful whether any great weight can be placed on his conclusions, for later in the same paper (Moore, 1933), he states that in a solution of Phenol there is a return of spontaneous movement which "closely resembles the normal".

Von Levetzov (1936) reports that decerebrate animals can right themselves when placed in an inverted position, but that, as in P.gilchristi, this movement lacks the co-ordination seen in intact animals. He also reports that when a decerebrate animal is creeping slowly and meets a mechanical obstruction, there is no attempt to change direction, so that the normal co-ordination between this stimulus and the turning reflex is absent in decerebrate animals. This absence of co-ordination between stimuli and muscular action is also shown by the observations of Bardeen (1901) on the triclad Planaria maculata. In this animal co-ordination between the chemical stimulation of food and muscular action is entirely abolished after decerebration. Thus it is clear that the brain of both polyclads and triclads can be regarded as a co-ordinatory centre.

The further suggestion has been made that the "central nervous system" of Planocera is comprised, not only of the brain, but also of the major nerve trunks, VI and VII, of the ventral network. Experiments with electrical stimulation of hemisected animals give no indication that there is a functional nerve net in Planocera. On the other hand, it has also been shown that, in the absence of the brain there are some sensory-motor links, suggesting that there is a functional nerve net. This apparent contradiction may be resolved by supposing that sensory-motor co-ordination in the absence of the brain occurs by way of the two main ventral nerve trunks and not through a nerve net. This hypothesis would appear to receive some support from the observation of Bardeen (1901) that in the triclad Planaria maculata a decerebrate animal may show spontaneous movement and react to light, but that these reactions are only shown if at least part of one of the major nerve trunks (corresponding to nerves VII of P.gilchristi) is present. However, this idea is ruled out as far as P.gilchristi is concerned, for if a small piece of the lateral margin is removed from the rest of the body, it will right itself if placed in an inverted position and will also respond to mechanical stimulation, thus showing that there are, in

the peripheral network, sensory-motor links, independant of any part of the major ventral nerve trunks. Further it will be remembered that in a decerebrate animal the response to a strong mechanical stimulus can be seen to spread around the body, so that all parts of the lateral margins perform movements which are components of the normal swimming. It is clear from these observations that there is, in P.gilchristi, a functional nerve net.

A problem is thus presented by the apparent contradiction between the above conclusion, and that which must be drawn both from the experiments on hemisected animals, and from observations on the effects on swimming of cutting one or more of the nerve trunks as they issue from the brain. Let us consider first the nature of the quick response to electrical stimulation. It has been suggested that the parapharyngeal muscle sheets are primarily responsible for the quick protective curling up of the animal when disturbed; it is clearly probable that they are also the chief effectors of the quick response to electrical stimulation. Further, it has been shown that the nerves responsible for this response run through trunks VI and VII. It seems likely, therefore, that the same myo-neural apparatus is concerned in both the protective reaction and the quick response to electrical stimulation. Such a quick protective reaction of the animal clearly requires a rather high degree of specialisation in the neuromuscular system, with motor impulses which pass rapidly along definite tracts. Likewise the swimming behaviour of normal animals involves quick contractions, a rapid co-ordination between excitatory and inhibitory impulses and further obviously requires that the motor impulses responsible for such co-ordination should pass along definite tracts to different parts of the musculature.

It is thus clear that the observations which have led to the suggestion that there is no functional nerve net in P.gilchristi have been concentrated on two specialised quick reactions. It is clear that the passage of impulses releasing a quick contraction, either in response to electrical stimulation, or during swimming, does not take place through the nerve net, but it is equally clear that a functional nerve net is involved in the response of a decerebrate animal to mechanical stimulation and in the reactions of a narrow strip of the lateral margins of the animal. There are clearly two levels of co-ordination in the nervous system of P.gilchristi, the one mediating very quick

and specialised behaviour patterns, the other on a slower time-scale. It is likely that further observations will reveal that electrical stimulation of one side of a decerebrate and hemisected animal will evoke a contraction from the other side, but with considerably longer latency than the quick response, for it seems probable that concentration on observation of the presence or absence of quick responses has led to neglect and hence lack of observation of slow responses from this preparation.

This analysis has served to emphasise the distinction in P.gilchristi between certain specialised neural pathways and the general nerve net. This contrasts rather markedly with the observations of Pantin (1935 b) on the sea anemone Calliactis parasitica. Pantin demonstrated the existence of"through- conducting"pathways which allow of rapid conduction from any point on the column to the oral sphincter, and which are concerned with the quick protective closing reaction of the polyp. Experiments in which the pedal disc, together with the base of the column were separated from the body except for a small connection, showed that although a greater number of stimuli was usually required, a conducting pathway could be established between the end of the tail and the through-conducting system. In further experiments a vertical strip of the column was cut so as to include the underlying mesenteries. Pantin points out that if, now, the underlying mesenteries are cut through, through-conduction to the sphincter is not established very readily. However, an examination of his results reveals that some conduction is very clearly established, whereas in P.gilchristi cutting of the main nerve trunks completely abolished conduction of the quick response between the two sides of the animal. The greater degree of differentiation in the nervous system of P.gilchristi is clear.

In this light we may now re-examine the differences which have emerged in this investigation between the characteristic responses of P.gilchristi and the anemones to electrical stimulation.

In the first place it has been seen that the quick response of P.gilchristi has a latency of the same order of magnitude as that of the quick sphincter response of the anemones Calliactis and Metridium. In addition there is no evidence for the facilitation phenomenon, so characteristic of the anemones,

in P.gilchristi. But it follows from the above analysis and interpretation of the functional plan of the nervous system of P.gilchristi that the functional characteristics of the nerve net of the animal are being obscured by the responses of the specialised quick conduction pathways, which give rise only to a quick response and show no facilitation. Thus these characteristics cannot be compared with those of the nerve net of the anemones. Clearly this indicates the necessity for a preparation in which the major longitudinal nerve trunks VII as well as the brain have been removed, for in such a preparation we might expect the characteristics of the nerve net itself to be revealed.

The intensity sensitive nature of the preparation used here may also be a characteristic of the quick conducting pathways alone. In this respect it is important to note that a number of anemone preparations have been shown to be sensitive to the intensity of the stimulus, so that the 'all or nothing' rule cannot be regarded as a general characteristic of the Anthozoa. An intensity sensitive mechanism has been demonstrated in a preparation of the parieto-basilar muscles of the sea anemone Stomphia coccinea (Hoyle, 1960) and also in a preparation of the circular muscles of the sub-sphincter of Bunodosoma capensis (Ewer, unpublished observations).

In sum, it may be said that the present investigation has served only to reveal the characteristics of the quick-conducting pathways in this polyclad, and that the characteristics of the nerve net itself remain obscure. It is now necessary to relate these results to present views on the phylogenetic relationship between the Anthozoa and the Turbellaria. These will now be briefly discussed.

3. Phylogenetic Relations:

In recent years the question of the origin of the coelenterates has been the subject of some speculative discussion. While Hydra and the Hydroidea and also the actinula larva of the Trachylinae have, at different times, been suggested as being the nearest representative of the coelenterate ancestor, Hadzi (1944, 1953) has suggested that the Anthozoa are to be regarded as the most primitive group within the phylum. Further, that a number of their features, and especially their

trace of bilateral symmetry, indicate that this group should be regarded essentially as degenerate Turbellaria.

Pantin (1960), while accepting that the Anthozoa are probably the most primitive class of coelenterates, rejects Hadzi's argument that the bilateral organisation of the Anthozoa reflects a turbellarian ancestry, and points out that this can more reasonably be regarded as a reflexion of the mode of functioning of the gastral cavity as a hydrostatic skeleton in these animals. He further emphasises the differences in structural and functional organisation between the Anthozoa and the Turbellaria. Perhaps the strongest point he makes is that, while some musculo-epithelial cells may have been identified in the most primitive Turbellaria - the Acoela, the characteristic arrangement of mesenchymal muscles in the Turbellaria contrasts markedly with the strikingly rigid diploblasticity of the Coelenterata: here, double muscle layers are always formed either by folding of the epithelial layers, as in the anthozoan mesenteries, or by doubling of the dermal layer, as in some medusae (Krasinka, 1914).

Pantin also stresses the very great difference between the functional organisation of the muscle systems in the two groups. Here, however, a strict comparison is clearly not reasonable. The development of the use of the gastral cavity as a hydrostatic skeleton will obviously lead to specialization which will not be comparable with the platyhelminth system in which the parenchyma alone is the skeleton against which the muscles act.

A comparison of the anatomy of the nervous systems of the two groups reveals yet another rather marked difference in organisation. While the turbellarian system characteristically consists of a three dimensional reticulation, made up of bundles of nerve fibres lying internal to the mesenchymal muscles, that of the coelenterates is always in the form of a two dimensional nerve net, running within the epithelium. The difference, in anatomical terms, between the nervous systems of the higher Turbellaria and the Anthozoa is clear, but it must also be remembered that in the most primitive Acoela the nervous system consists of a layer in the base of the epidermis (Hyman, 1951). Nevertheless, as Pantin emphasises, even in the most primitive Acoela there is a simple brain in the form of an aggregation of nerves near the anterior end; this is a structure never found in the Anthozoa.

It is, however, likely that a comparison of the functional properties of the nervous systems will be more revealing than one limited to the anatomical arrangement, although, as in the case of the muscular system, we may expect to find specific adaptations to the two different modes of life of these two groups of animals. The most obvious is the possession of a brain by the Turbellaria. It has been shown that the polyclad brain may be regarded essentially as a locomotor co-ordinatory centre. In other words, it is concerned with activities which are not displayed by the anemones, and thus, in functional terms, one would not expect such a centre to be present in the Anthozoa.

Two further differences between these two nervous systems which have emerged in this investigation may likewise be correlated with the mode of life of the animals concerned. It has been seen that in P.gilchristi a single shock will elicit a response, whereas in the anemones, the quick response is characteristically only elicited by two shocks. This may be regarded as an adaptation to sedentary life, for it is clearly a disadvantage for a sedentary animal to respond by movement to every slight mechanical stimulus. The imposition of this 'binary law' may clearly be regarded as a mechanism whereby some differentiation between responses to sensory input is achieved. This interpretation is strengthened by the fact that the same binary system is found in sea squirts (Hoyle, 1952); clearly it is an adaptation which has arisen more than once in sedentary animals.

The facilitation phenomenon may be similarly interpreted, for it is possible to regard it not as a mechanism for increasing the effectiveness of a given stimulus, but rather as a device for preventing rapid and even conduction over the whole animal. Pantin (1952) has pointed out that the phenomenon of facilitation allows a considerable variety of response, since different parts of the nerve net show different degrees of facilitation. It has, however, still to be seen whether the nerve net of the Turbellaria shows facilitation or not.

Thus far this discussion has followed a rather negative trend. It is all very well to show that the functional differences which have been observed between the nervous systems of the Anthozoa and the Turbellaria may be regarded as adaptations to specific modes of life, and hence to conclude that the differences have no bearing on the question of a phylogenetic relationship. But this does not bring us much closer to any more positive argument.

In one respect the organisation of the nervous systems in these two groups shows a similar basic plan. It is clear that in both the Anthozoa and the Turbellaria there is a nerve net, which allows conduction to take place to all parts of the animal; as well, there are, in each case, specialized quick-conducting pathways. It has already been pointed out (p.85) that while there is some indication of separation between the nerve net and the quick-conducting pathways in the anemones, this is far more complete in Planocera. Indeed, this is what one would expect in an animal in which there are rapid and rather complex locomotory patterns. It is of some interest in connection with this point to note the observations reported by von Levetzov (1936) on the polyclad Thysanozoon brochi. In this case, when the posterior nerves are separated from the region which they normally innervate directly, swimming waves appear in the latter region after a few passages of the wave down the rest of the body. In other words, it seems that in this animal impulses for swimming waves can pass through the periphery, although it takes longer for this to be established than by direct passage through the usual pathways. Thus, in Thysanozoon at least, it would appear that the degree of separation between the nerve net and the more specialised pathways is not as complete as in others, notably P.gilchristi.

The present investigation does not allow the comparison between anthozoan and turbellarian nervous systems to be taken any further. The quick responses are specialisations which cannot reasonably be compared. In the anemones, the quick response is a protective reaction, mechanical stimulation resulting in withdrawal of the whole animal. In P.gilchristi the quick protective response of curling up ventrally is only seen when the animal is dislodged from the substratum; mechanical stimulation of the whole animal results either in a retraction of the edge of the body in the region of the point of stimulation, or in locomotion. Moreover, the quick-conduction pathways of Planocera are very clearly associated with the complex locomotory patterns, of which there is nothing comparable in the Anthozoa.

It is more reasonable to turn to a comparison of the functional properties of the nerve nets of the two groups, but here, as has been seen, we have no information on the characteristics of the turbellarian nerve net. The only comparative data

available are the pharmacological results, which possibly represent effects on the nervous system as a whole, and which have revealed striking parallels. However, we must be wary of jumping to conclusions on the basis of pharmacological results alone, for history has revealed the dangers that lie therein. One classical example that may be cited is that of Gaskell's (1914) hypothesis that the annelids are related to the vertebrates, which was based on the evidence for cholinergic and adrenergic mechanisms of transmission in the annelids. The unreliability of pharmacological results as a basis for phylogenetic hypotheses is further emphasised by the fact that the protochordates, commonly held to be ancestral to the vertebrates, show no evidence of any cholinergic mechanism of transmission (Bacq, 1939).

Thus in the case of the Anthozoa and the Turbellaria we must await a closer study. What would be of very great significance would be the discovery that the same catechol amine could be extracted from and used as a stimulant or inhibitor on both the Anthozoa and the Turbellaria. Until such positive evidence is obtained, and until a study is made of the properties of the nerve net of the Turbellaria, it is not possible to draw any radical conclusions.

5. SUMMARY.

1. The need for a study of a platyhelminth myo-neural system, particularly to enable a comparison with the anthozoan system to be made, is pointed out.
2. A preparation consisting of a whole animal is used and the general anatomy of the muscular and nervous systems of the animal is presented to facilitate an understanding of the preparation.
3. The spontaneous activity and general characteristics shown by the preparation under 'normal' conditions are described. A basis for assessment of the experiments which follow is detailed.
4. The effects of certain ionic solutions and drugs on the normal activity of the preparation are presented and later discussed. It is clear that these results are closely similar to the effects of the same treatments on the anemones in particular.
5. A stimulator for the controlled electrical stimulation of the preparation and the nature of the preparation used in these experiments are described.
6. An investigation into the responses of the preparation to electrical stimulation is made with the specific aim of comparing these characters with those of the anemones.
7. The preparation is found to respond above threshold intensity to a single shock with a response of short latency, comparable with the quick sphincter response of the anemones. The magnitude of this response increases with increasing intensity of the stimulus.
8. No evidence was found for a frequency sensitive system in this preparation.
9. The nervous system is found to show considerable differentiation between quick-conducting pathways and the nerve net itself.
10. The results on electrical stimulation are discussed and it is concluded that the characteristics of the nerve net itself are not being revealed in this preparation. A possibly more useful preparation from this point of view is suggested.

11. Anthozoan-Turbellarian relationships are discussed and it is shown that the conclusions which can be drawn from the results of this investigation are extremely limited. The necessity for a study of the characters of the Turbellarian nerve net is emphasized.

A P P E N D I X I.

The drugs used in the experiments recorded in section 3A (4) are given here in the form in which they were used, together with the name of the manufacturer.

Acetylcholine chloride (B.D.H.)
Adrenaline hydrogen tartrate (B.D.H.)
Bromo-lysergic acid 148 (Sandoz)
l-Cysteine hydrochloride (B.D.H.)
Ergotamine tartrate (Sandoz)
Eserine (B.D.H.)
Gamma-aminobutyric acid
l-Glutamic acid (B.D.H.)
Glutathione (B.D.H.)
Harmine hydrochloride (Light)
5-Hydroxytryptamine creatinine sulphate (Light)
3-Hydroxytyramine hydrochloride (Light)
Lysergic acid diethylamide - 25 (Sandoz)
dl-Nor-adrenaline (Light)
Picrotoxin (B.D.H.)
Strychnine hydrochloride (B.D.H.)
Tryptamine hydrochloride (B.D.H.)
Tyramine hydrochloride (B.D.H.)
Yohimbine hydrochloride (Light)

Fig. 66.

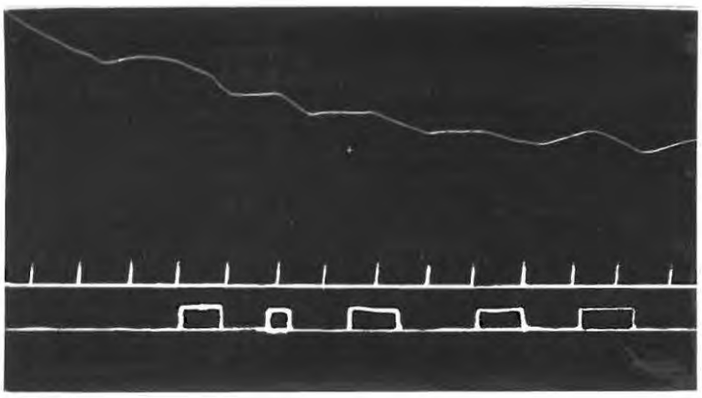

Fig 66: Trace showing inhibition in response to electrical stimulation. Upper signal trace a time trace in min. Lower signal trace indicates the duration of stimulation. All stimuli of 20 volts, applied at a frequency of one per sec.

A P P E N D I X II.

There is one further observation which was made and which it is felt should be reported. It was found that in one preparation, electrical stimulation by way of electrodes laid up against the sides of the animal in the usual way, elicited not an excitation, but an inhibition. This preparation could be made to relax when in a state of steady tone, or during a spontaneous contraction (fig. 66). The exact position of the electrodes with respect to the major nerve trunks could not be ascertained, but since the anode was lying close to the pharynx and the cathode directly opposite to this on the dorsal side of the animal, it is possible that the nerve trunks VII were being directly stimulated. The stimuli used were in no way different in either intensity, pulse length, or frequency from those which elicited excitation.

Since this result could not be repeated, it has not been reported together with the results on electrical stimulation, but it is felt that it is necessary to put it on record. It has been seen that a preparation of P.gilchristi will perform fairly regular rhythmical contractions, and the above result may not be without significance in view of the current interpretation of rhythmical activity in sea anemones (Ewer, 1960) and an echinoderm preparation (Pople and Ewer, 1958) as a result of a balance between excitatory and inhibitory states in the preparation.

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