THE NERVES AND MUSCLES OF MEDUSAE

V. DOUBLE INNERVATION IN SCYPHOZOA

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INTRODUCTION

The first paper of this series (Horridge, 1954*b*) was a study of the through-conducting, unpolarized net of relatively large nerve cells which propagates a single nerve impulse over the whole bell of *Aurellia* at each beat, and by virtue of its symmetrical properties and relatively rapid conduction rate is responsible for the mechanically desirable symmetry of the contractions. This is the net, common to Rhizostomeae and Semaeostomeae, which was originally described by Shäfer (1878) in *Aurellia*, and which has provided a model for much discussion of the physiology and the phylogeny of the elementary nervous system. However, this nerve net is only a specialized part of the peripheral nervous system. Its action as a conducting system is confined to the excitation of the main muscles of the bell and it may be considered as equivalent, on functional and physiological grounds, to the motor giant fibre systems found in some other invertebrate groups. This limitation of the function explains the relatively straightforward physiological properties. In fact, under normal conditions, this whole net acts on the muscle as a single motor nerve.

In the literature one can find descriptions of several different kinds of other excitation which cross the subumbrellar epithelium without giving rise to a contraction wave *en route*. These fall into four groups. First, historically (Romanes, 1877), is the 'excitational wave' of tentacle retraction which crosses the bell of *Aurellia* at half the velocity of the contraction wave and initiates a beat from the first tentaculocyst that it meets. This is a special case of the indirect acceleration of the rhythm by a mechanical stimulation at a distance from the ganglion. Secondly, Bozler (1926*b*) has described the response of the oral arms of *Pelagia* to stimulation at the margin of the bell and also the outward propagation of slow waves of contraction across the circular muscle with a velocity of about 1 cm./sec. In confirmation and extension of Fränkel's (1925) work on *Cotylorhiza*, Bozler has also described the compensatory movement of *Cotylorhiza* (1926*a*) and *Pelagia* (1926*b*) in which, after displacement, an asymmetrical component added to the beat serves to bring the animal back to an even keel. Considering these different examples of conduction, Bozler came to the conclusion that each reaction had its basis in a separate nerve net. Thirdly, Horstmann (1934*a*, *b*), besides briefly describing the compensatory movement of *Cyanea*, noticed that for a short time

after removal of a marginal ganglion of *Aurellia* the neighbouring part of the margin remained bent inwards during the pauses between contractions. Following Fränkel, he inferred a regulatory effect of the ganglia upon the 'tonus' of the surrounding circular muscle, and assumed that this was also the mechanism of the compensatory movement. Finally, Mayer (1906), observing a contraction wave continuously circulating round a ring cut from the bell of *Cassiopea xamachana*, found that the amplitude of the contractions increased for a short time after a general mechanical disturbance, though the frequency of the contractions remained the same. This observation escaped the attention that it deserved from later workers on medusae, who confined themselves to the influence of the frequency upon the amplitude of the contraction.

In the ephyra larva of *Aurellia* it has been found (Horridge, 1955*c*) that the extra-ganglionic nervous system is divided into two nerve nets which meet and interact at the marginal ganglia. The first, named the diffuse nerve net, spreads over both oral and aboral surfaces. It contains primary sense cells and motor fibres, and is responsible for the contraction of the radial muscle of a single arm, together with the co-ordinated movement of the mouth in the feeding response. It is reminiscent of the oral disk conducting system which controls the tentacles and the feeding reaction in anemones (Pantin, 1935). The diffuse nerve net, besides having a local sensory and motor function, also acts on the rhythm of the marginal ganglia and inhibits the beat during the feeding response. The other nerve net is the giant fibre system, corresponding histologically and functionally with Shäfer's net in the adult *Aurellia*, but the anatomical arrangement is in accordance with the larval division of the muscles into radial and circular blocks. This through-conducting net is superimposed histologically and functionally on the diffuse nerve net, and it brings with it the rapid co-ordination essential for the swimming movement. In the adult *Aurellia* the feeding response is lost, but the diffuse nerve net persists as the subumbrellar conducting system, which is presumed to be responsible for Romanes's 'excitational continuity', i.e. to conduct the tentacular waves and to act indirectly on the rhythm.

In *Cyanea* there are two nerve nets which are histologically similar to those of the ephyra of *Aurellia*. Here the feeding response of the larva has again disappeared, but the asymmetry of the compensatory movement makes it likely that double innervation has persisted into the adult in muscles sufficiently powerful to give a record.

For *Cassiopea* (Rhizostomeae), there is no histological evidence available, but in the related *Rhizostoma* (Bozler, 1927) there is a network of large bipolar cells corresponding to the giant fibre net of semaeostomes, and also a network of smaller multipolar cells that corresponds with the diffuse net. Mayer's observation, quoted above, requires some extension of the simple all-or-nothing action of the giant fibre net, and it will be shown that the relevant reactions of *Cassiopea* are closely similar to those of *Cyanea*. Also in *Nausithöe* (Coronatae) it is now found that the responses can only be explained in terms of two overlying nerve nets which interact at the marginal ganglia, and these two nets are similar to those

of the other orders. In these three orders, at least, there is emerging a common plan which includes double innervation of some muscles where an asymmetrical movement of the bell is mechanically necessary for feeding or for the compensatory movement.

MATERIAL AND METHODS

Large specimens of *Cyanea* were obtainable at Millport in July 1955. They were of the red variety and usually 20–30 cm. diameter. The specimens of *Cassiopea* were studied at Ghardaqa, Red Sea, in September 1955. The *Nausithöe* appeared at Naples in March 1952, and the results there obtained could not be interpreted until recently when they were found to be closely comparable with the results obtained using the ephyra of *Aurellia*.

The methods employed have been of the simplest kind, electrical stimulation by condenser discharges and recording by isotonic levers on a smoked drum. Fresh material was found to be essential.

A. SEMAEOSTOMEAE. *CYANEA CAPILLATA* ESCHSCHOLTZ

The compensatory movement of Cyanea

The specimen of *Cyanea* is held immersed in a tank of sea water with the main axis horizontal, and maintained in this position by two small hooks through the exumbrellar part of the jelly. The support is arranged so that the bell can be rotated and any segment brought uppermost. In this position the spontaneous

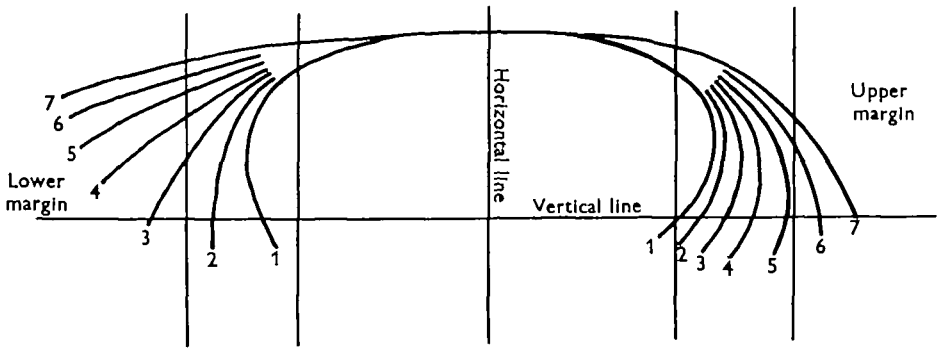

Fig. 1. Diagrammatic representation of successive stages, 1–7, in the relaxation of a bell of *Cyanea* which is mounted on its side. The uppermost margin, on the right, contracts to a greater extent, relaxes slower and less completely than the lower margin. The figure is turned on its side for convenience.

beats are consistently asymmetrical. At each beat the uppermost part of the margin contracts to a greater extent, remains in a contracted state for a longer time than the lower side, and also fails to relax as completely before the next beat. A representation of successive positions is shown (Fig. 1). The bell may be rotated about its axis so that any segment is brought uppermost, but this does not affect

the orientation of the asymmetry, which remains determined by its relation to the vertical. In freely swimming animals it is readily observed that the mechanical effect of this asymmetry is to bring a tilted animal back to an even keel, exumbrellar surface upwards.

After removal of seven of the eight tentaculocysts the asymmetrical character of the contractions persists as long as the remaining tentaculocyst is in the uppermost position, but now on rotating the bell the asymmetry is lost. Furthermore, the average rate of beating is greatest when the single tentaculocyst is in the uppermost position, and the spontaneous rhythm may stop altogether when the bell is rotated to bring the tentaculocyst to the lowest position. This agrees with another observation, that in tilted normal animals the contraction wave is often initiated at the uppermost marginal ganglion.

Confirming Fränkel (1925), these observations indicate that the local effect which forms the asymmetrical component is to be found at the uppermost margin in the compensatory movement. The mechanism that is important for the life of the whole animal is one for locally retarding the process of relaxation.

The slow waves

When a small specimen of *Cyanea* (5–10 cm. diameter) is spread out upside down in a dish of sea water it can be seen that relatively slow waves of contraction spread across all the muscles with intervals of ½–2 min. between successive waves. In their most characteristic form these waves begin at the inner edge of the circular muscle and spread outwards. Under a binocular microscope each wave can be seen as a regular sequence of contractions of the individual folds into which the muscle sheet is thrown. At any point the contraction lasts 2–4 sec. as in the normal contraction wave of the beat. Having traversed the circular muscle, the slow wave appears in the radial muscle, which means that excitation crosses the intervening muscle-free area. The radial muscle contracts largely as one unit with a relatively slow response that may last 15–120 sec. As recorded by writing levers this response of the radial muscle is of smaller amplitude than in the normal contraction wave.

In a small specimen these outwardly moving waves often succeed each other in a regular sequence, or, if the animal is quiescent, they may be provoked by a gentle movement or pinch of the oral filaments. At times spontaneous waves cross the bell from side to side, and in response to a small prod or pinch of a marginal lappet a slow wave may be initiated, first in the appropriate radial muscle, then followed by a centripetal wave across all or part of the circular muscle. Two waves travelling in opposite directions cancel out where they meet. Across the circular muscle fibres the velocity of the wave is 0·15–0·25 cm./sec., but along the line of the fibres it is about 2 cm./sec. However, here it must be borne in mind that the length of the pathway across the fibres is about ten times the apparent length on account of the deep folds of the muscle sheet. In the radial muscle a simple velocity could not be measured. Observed under a binocular

microscope the sustained, weak contraction of the whole muscle appears to be a combination of many irregular local contractions out of phase with one another.

In large specimens of *Cyanea* (up to 35 cm. diameter) the spontaneous slow waves are also found (Fig. 2). A contraction typical of the slow wave may follow a local mechanical stimulation, but the response is rarely propagated to the next muscle block. It is only in the radial muscle preparations, described below, that slow contractions regularly follow stimulation of a nearby muscle-free area.

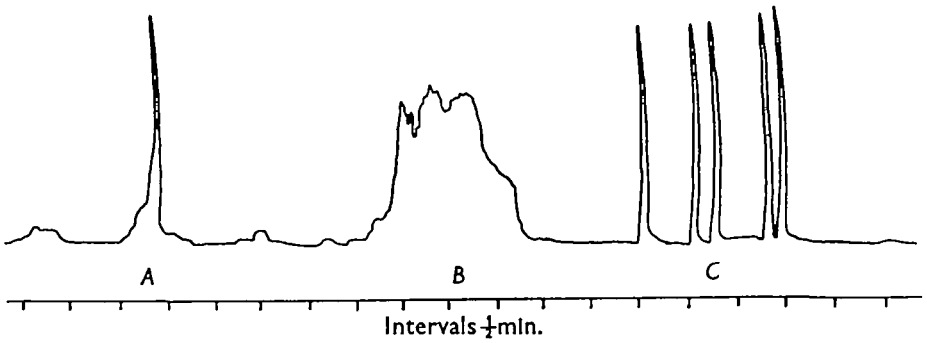

Fig. 2. Spontaneous responses of a radial muscle, showing the contrast between the record of a slow wave *B* and a series of normal beats *C*. At *A* is a small slow contraction terminated by a beat which it has initiated. Figs. 2 and 4–7 are traced from smoked drum records.

Action of the diffuse net on the marginal ganglia

The bell of a small specimen of *Cyanea* is pinned to wax on the bottom of a dish of sea water in such a way that the bell can beat freely, but with one of the tentaculocysts held firmly by pins through the jelly so that it can not tilt or vibrate. All the other tentaculocysts are removed and the preparation left for some hours to recover from the shock of the operation. It is consistently found that a gentle mechanical stimulation, such as rubbing the subumbrellar surface with a straw, is followed by the acceleration of the rhythm of the distant tentaculocyst. The excitation can spread across the whole bell. It is sometimes, but not necessarily, accompanied by a typical slow wave of contraction. A more severe stimulation, particularly within 3 cm. of the tentaculocyst is often followed by a long pause. Some preparations, especially those with the tentaculocyst upside down, show no acceleration but only the long pause. Comparable results, showing both acceleration and inhibition of the rhythm following distant stimulation, are found if the same experiment is repeated with *Aurellia*, but here there are no slow waves; instead, the spread of excitation in the diffuse nerve net is marked in some specimens by the contraction of the marginal tentacles. As a special case of the acceleration of the ganglion some preparations give, after a few seconds delay, a single beat in response to a single mechanical stimulation of the diffuse nerve net. For *Aurellia* this observation is described in detail by Romanes (1877); in *Cyanea* examples occur in the records shown.

The responses of the radial muscle

A pair of radial muscles, with a muscle-free area and tentaculocyst between them, is cut from the bell of a large specimen of *Cyanea*. Most of the jelly is carefully removed from the back of the muscles and the segment is pinned to wax under sea water as shown (Fig. 3). Two small hooks attached to writing levers are set in the jelly behind the remnants of circular muscle. The levers are loaded with

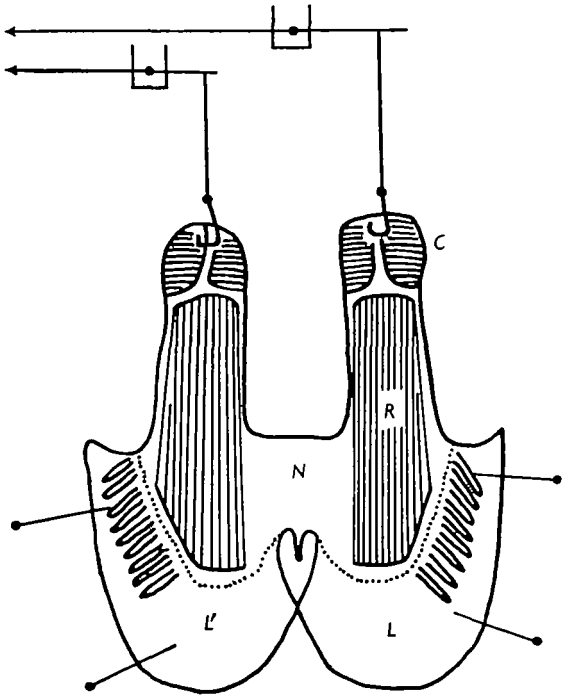

Fig. 3. The preparation of a pair of radial muscles of *Cyanea* with a tentaculocyst and muscle-free epithelium *N*. The dotted line shows the approximate lower limit of the giant fibre net. The italic capital letters indicate the alternative positions of the stimulating electrode; *N*, the giant fibre net; *L*, *L'*, the lappets; *C*, the circular muscle; *R*, the radial muscle.

a few grams to maintain a small tension in the muscles. To achieve satisfactory results it is necessary to use the lightest possible system with minimum friction on the drum. Stimulation throughout was with a fine silver wire, insulated to its tip, excited by a simple neon oscillator at 5 shocks/sec. The method of stimulating the lappet is important. The stimulator probe is moved gently along the lappet margin and inwards towards the limit of the radial muscle and is removed the moment any movement begins. Unless the contrary is stated, the muscle is not stimulated directly. The sensitivity of the lappet varies greatly from point to point, but an effort has been made to use minimal strength stimuli.

A single radial muscle gives a variety of responses, the characteristics of which

depend largely on the position of the stimulus. In some preparations, e.g. Fig. 4A, the height of the contraction is almost constant. In this record the first contraction at no. 1 is a spontaneous beat after a pause. It is followed at no. 2 by a localized contraction as a result of stimulation on the lappet, i.e. at *L* in Fig. 3. This is immediately followed at no. 3 by a 'spontaneous' beat which is initiated from the marginal ganglion as excitation in the diffuse nerve net arrives there from the lappet. The stimulus was then applied to the remnant of the circular muscle, i.e. at *C* in Fig. 3, and the response was normal. This trio is repeated at nos. 7–9; it

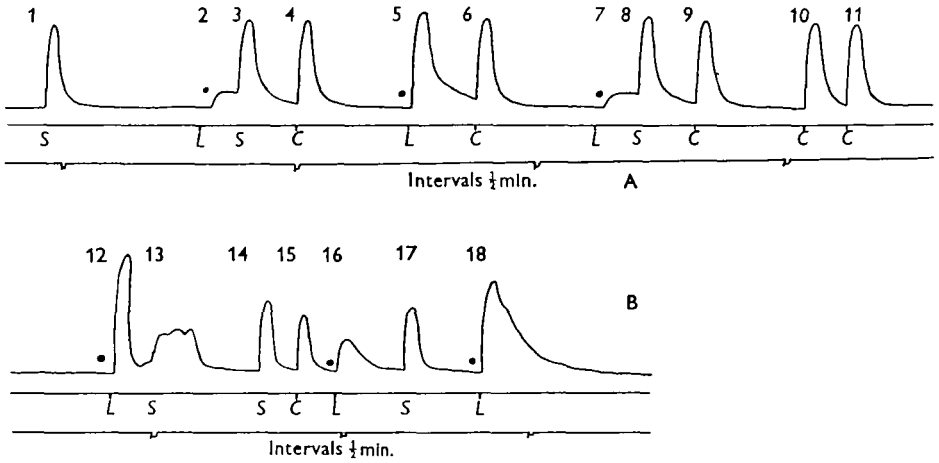

Fig. 4. Various responses of a single radial muscle as described in the text. The letters indicate the origin of the excitation: *S*, spontaneous beat; *L*, stimulus on lappet; *C*, stimulus on circular muscle.

was also attempted at nos. 5 and 6, and here stimulation at *L* produced a contraction wave directly, although the contraction no. 5 is drawn out by a slower relaxation. The shape of the contraction at no. 1, and especially no. 10, discount the possibility that the slower relaxation of no. 5 is explained by the lack of a previous contraction, i.e. to facilitation of relaxation.

A record showing a greater variety of forms of contraction is given in Fig. 4B. Here the first stimulus on the lappet is too close to the muscle and excites the giant fibre system directly at no. 12, but it also initiates a slow contraction at no. 13. Similar stimulation later produces at no. 18 a contraction where slow and fast waves are combined and at no. 16 a small slow wave is produced. The normal contraction waves at nos. 14, 15 and 17 again set a standard for comparison.

A difference in strength of the contraction waves is also noticeable in Fig. 4B. This effect is better shown in Fig. 5, from a different preparation. Here there is a large contraction with rapid relaxation at nos. 1 and 16–18, following stimulation of the giant fibres at *N* (Fig. 3), but smaller contractions follow stimulation of the circular muscle remnants at *C*. A slow contraction alone occurs at no. 15 and a lengthening of the quick contraction at nos. 10 and 12, possibly also at nos. 2 and 6.

An essential difference between the fast contraction and its slow component lies in the contrast between the through-conducting, all-or-nothing character of the contraction wave and the local, more variable character of the other. This is shown in Fig. 6 by a record of two neighbouring radial muscles *T* and *B*. Except for the symmetrical contraction, no. 4, which follows stimulation of the giant

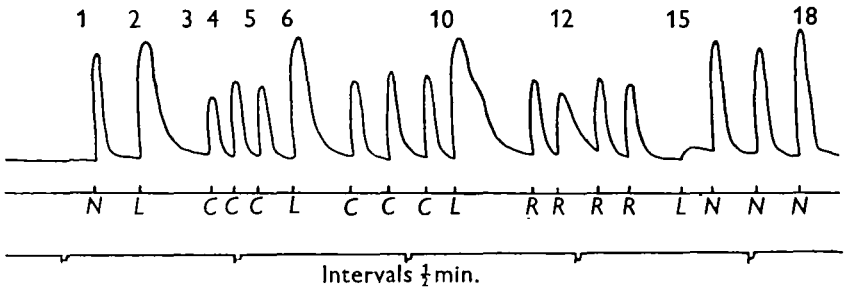

Fig. 5. The influence of the position of the stimulus on the height of the contraction. The letters indicate the point of stimulation: *N*, on the giant fibre net; *L*, on lappet; *C*, on circular muscle; *R*, on radial muscle.

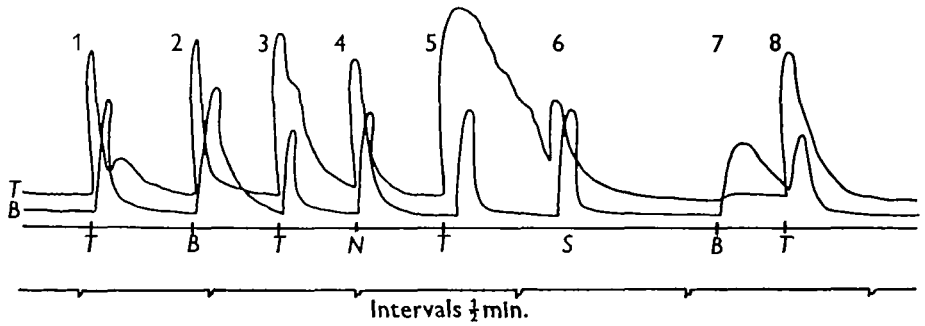

Fig. 6. Simultaneous records from a pair of radial muscles showing that the delayed relaxation is varied and local. The letter *T* indicates when the stimulus was applied to the lappet corresponding to the top trace, and similarly *B* for the bottom trace. The symmetrical contraction at *N* followed stimulation on the nerve net and *S* was a spontaneous beat.

fibre net at *N* (Fig. 3), the stimuli are applied at the points *L* or *L'* (Fig. 3). It is important to point out that in Fig. 6 the stimulus was never directly applied to the muscle. In the record a local effect is superimposed on the contraction wave and this effect here takes the form of an extension of the relaxation process. The muscle *T* shows the effect better than *B*, especially at no. 5, but the muscle *B* delays its relaxation at no. 2 and shows only a slow contraction at no. 7.

Other asymmetrical movements of Cyanea

In Fig. 7 is a recording from radial muscles on opposite sides of the bell of a large *Cyanea*. The upper trace intermittently shows abnormally great relaxations, but no importance can be attached to the contrast with the relative stability of the

lower trace, for this may be due to the recording system. However, where changes of the contraction height or of the base-line occur in the lower trace they appear to be independent of the changes in the upper trace. This can be interpreted in terms of the varied responses of the radial muscles already described, if we allow a little spontaneous or extraneous excitation in the diffuse nerve net.

There is, however, an entirely artificial type of asymmetrical contraction of the bell which may be produced by electrical stimulation at certain frequencies. The refractory period of the radial muscle of *Cyanea*, measured by Bullock's (1943) method is 1·1–1·3 sec. at 16° C. If the interval between electrical stimuli is

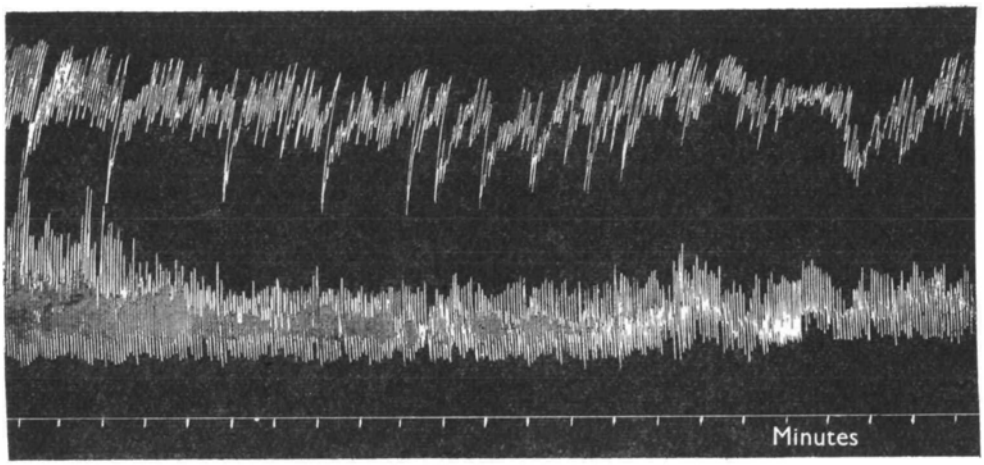

Fig. 7. Contractions of a pair of opposite radial muscles during a long series of spontaneous beats of an isolated segment of *Cyanea*. The difference in character of the traces may be partly due to the recording system, but this does not hide the disparity between the variations of the two sides. Read from left to right with contractions upwards.

adjusted to coincide with the refractory period of the muscle, or a simple fraction of it, asymmetrical contractions are readily recorded (Fig. 8). Presumably this happens because the muscles in various parts of the bell do not have identical refractory periods. This kind of experiment may be refined so that alternate large and small contractions are produced, as in parts of Fig. 8. The interpretation (Bethe, 1937) that such results disprove the all-or-nothing character of the nerve impulses is not valid. They are not concerned with the nerve impulse, but with the strength of the contraction. The alternative suggestion that the muscle consists of a population of units with a distribution of refractory periods gives a more satisfactory explanation.

The actions of the two nerve nets on the muscle appear to be independent, even when simultaneous. By comparison of muscles on opposite sides of the bell when first one muscle then the other is given a series of electrical stimuli, I have tried to find in *Cyanea* a local effect of the diffuse net on the neuromuscular facilitation of the giant fibre net. However, these experiments have always led to

a negative result as far as facilitation is concerned, though the preparations show the other effects described. This is in contrast to the situation to be described in *Cassiopea*.

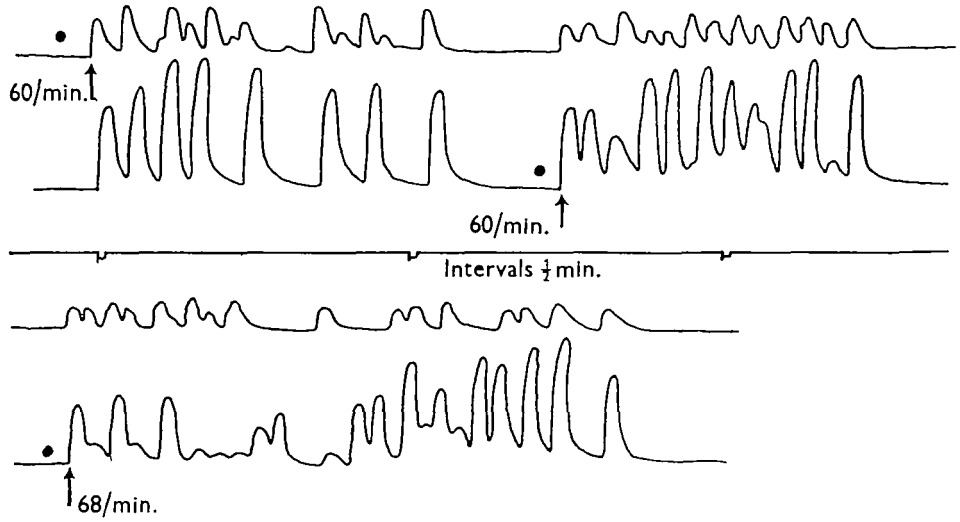

Fig. 8. Simultaneous records of a pair of neighbouring radial muscles of *Cyanea* when stimulated at the given frequencies, which are arranged to clash with the average refractory period of the muscle fibres. This is quite an artificial state of affairs. Stimulation is applied to first one muscle then the other, as indicated by the black spot.

B. RHIZOSTOMEAE. *CASSIOPEA ANDROMEDA* ESCHSCHOLTZ

The compensatory movement of Cassiopea

The specimen of *Cassiopea* is held on its side by hooks passing through the jelly of the exumbrellar surface. Under these conditions the asymmetry of the beat closely resembles that of *Cyanea* when similarly treated, although the arrangement of the effective muscles is quite different. The wave at each beat originates at the uppermost margin, which after each contraction remains more bent inwards than the lower margin and consequently has a smaller amplitude. However, after about half an hour in this position the pulsations and their origin often become quite symmetrical. When left still longer some specimens even reverse the initial asymmetry of the compensatory movement, which now tends to turn the animals upside down. These observations agree with the behaviour of freely swimming individuals, which turn to swim upwards when first disturbed, but which later turn upside down and settle on the bottom.

The slow wave of Cassiopea

A single electrical stimulus applied anywhere on the subumbrellar sheet of muscle fibres produces a normal contraction wave, but within the narrow clear band round the margin, outside the area of the circular muscle, the effect of a

stimulus is quite different. The neighbouring lappets, up to a distance of 10 cm., are drawn together by a local contraction of the circular muscle. With successive stimuli at intervals of 10 sec. this excitation spreads farther and in one case, for example, reached all round the bell at the third stimulus. The velocity is about 8 cm./sec. at 25° C. compared with 40–50 cm./sec. for the contraction wave. Under a binocular microscope the movement appears to be a contraction of the same fibres that contract at each beat, and subjectively it appears to occupy at any one place the same contraction time as the faster wave, but the slow wave only affects the circular muscle. If stimulation is continuous, the slow wave becomes a maintained contraction, but in *Cassiopea* this applies also to the contraction wave, and in each case the contraction of any small group of fibres comes in jerks, though the units are out of phase. A cut inwards from the margin through only the circular muscle has no effect on either of the two waves, and the cut is continued inwards without effect until only a narrow bridge is left in the radial muscle sheet.

Simultaneous slow and fast contraction in Cassiopea

A continuously circulating wave of contraction is maintained indefinitely once it is initiated in a large ring cut from the bell. The frequency of the contractions depends only on the velocity of the wave and the length of the closed circuit. The amplitude of the contractions soon settles down to a constant value as measured by the movement of an isotonic lever. Mayer (1906) has already found that when such a preparation is taken out of the water and thrown back, the amplitude but not the frequency increases. This experiment may be refined by use of electrical stimuli, but the result is effectively the same (Fig. 9). However, it can now be seen that besides the contraction wave circulating with a velocity of 40 cm./sec., the additional effect is an elevation of the line of peaks and usually also of the base-line. The second effect is propagated with a velocity of 8–10 cm./sec. and it lasts for more than one cycle of the contraction wave. These facts by themselves require for their explanation at least two independent pathways for the operation of any possible mechanism. In addition, the stimuli are equally effective even if applied outside the area where an electrical stimulus produces a contraction wave.

The question of what is happening at the point where the two nerve nets converge on the muscle fibres can hardly be touched for lack of data. The strength of a contraction depends on the activity of the diffuse net and on the interval since the previous contraction, but at present it is impossible to separate the effects of these two factors.

The value of the circulating ring preparation lies in the fact that here a contraction wave is available without any accompanying excitation in the diffuse nerve net. Normally an electrical stimulus produces an effect in both systems, but in the ring the effect is maintained in the through-conducting system, while in the diffuse nerve net it dies out. This shows an important difference between the two nerve nets, and makes possible the demonstration of the interaction of the two waves as they affect one group of muscle fibres.

C. ORDER CORONATAE. *NAUSITHÖE PUNCTATA* KÖLLIKER

The behaviour of the adult *Nausithöe* is closely similar to that of the ephyra larva of *Aurellia*. They have a similar habitat in the surface water with continual active swimming and a macrophagous feeding method. Both have two distinct systems of co-ordination over the subumbrellar surface, and it is sufficient to set out briefly the reactions of *Nausithöe*.

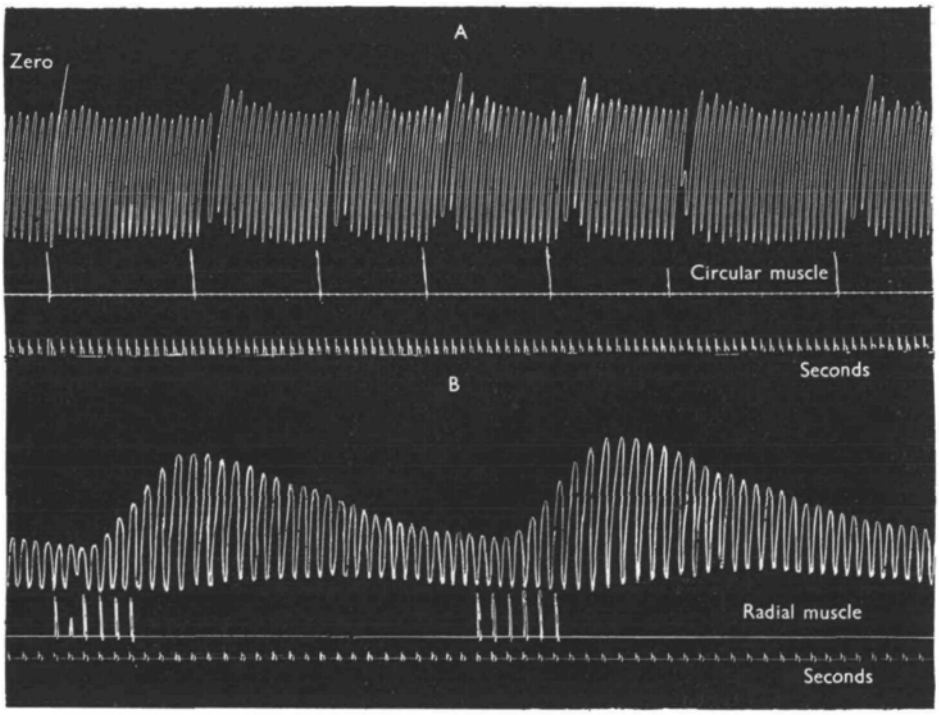

Fig. 9. *Cassiopea*. The responses of a ring which carries a continuously circulating contraction wave. A, top; single electrical stimuli applied 18 cm. from the recording point produce both a momentary dislocation of the circulating wave and also a shortening of the muscle, at both contraction and relaxation, for several cycles. B, five electrical stimuli at 1 sec. intervals produce a long-lasting effect on the height of the contraction, but here do not disturb the circulation of the wave. Both the radial and the circular muscle give the above reactions.

(1) The beat of the bell. It can be shown, by making incisions in different directions, that the nerve net responsible for the spread of the contraction wave is diffusely spread over much of the subumbrellar surface, as in other Scyphozoa. Artificial stimulation with a single shock elicits a single contraction of both the circular and radial muscles and it is likely, by comparison with other forms studied, that there is a network of through-conducting motor nerves which are responsible only for the symmetrical beat, and that one nerve impulse through the net is sufficient.

(2) The feeding responses may be summarized as follows:

A. A swing inwards towards the mouth of one or more of the relatively large marginal tentacles. These usually act independently of one another.

B. An inward bend of one or more of the marginal lappets by a maintained local contraction of their radial muscle.

C. A symmetrical retraction of the four lobes round the mouth, opening it wide by a maintained contraction of the whole sheet of radial muscle which is round the mouth.

The different responses above sometimes occur separately in response to food or mechanical stimulation, but normally they are co-ordinated together in such a way that captured food is passed to the mouth from the tentacle or lappet that receives it. Strong or continual stimulation of the subumbrellar surface is followed by an exaggerated feeding response that affects the whole animal, which promptly folds into a spasm lasting for several seconds.

(3) The rate of the rhythmical discharge of the marginal ganglia is slowed by a touch to the subumbrellar surface, and there is a temporary complete inhibition while the feeding reaction is completed.

Apart from differences in the details of the feeding responses the pattern is exactly the same as in the ephyra larva of *Aurellia*, and there is no doubt that two independent nerve nets are present, corresponding to those of semaeostomes. The principal point at present is the simultaneous existence of two types of contraction of the radial muscle of the lappets which, as in the ephyra, may be local and maintained or symmetrical and temporary. This can only be explained in terms of double innervation or by two types of muscle fibre and the latter is unlikely.

THE DIFFUSE NERVE NET

The term 'diffuse nerve net' is used here to cover all the peripheral nervous system except the through-conducting giant fibre net of the contraction wave. At present it appears to be one interconnected system, but this may later prove to be an oversimplification. In its histological appearance in *Cyanea* and *Nausithōe* it is similar to the diffuse net described in the ephyra larva of *Aurellia* (Horridge, 1955) and the two are closely comparable both physiologically and functionally.

The fibres of the diffuse nerve net of *Cyanea* are very thin with many bends, and often appear continuous from cell to cell. This should not be taken as evidence of continuity because the giant fibres, which are known to have contiguity synapses, appear as a continuous net when over-stained with methylene blue. The cell bodies, 6–10μ long, are smaller and rounder than those of the giant fibre net and are frequently multipolar. They branch and interconnect to form a fine network that ramifies everywhere among the epithelial cells. From the margin longer fibres run radially towards the ganglion and towards the radial muscle. The diffuse net also connects with the typical bipolar primary sense cells, each of which has a process that extends into the surface of the epithelium. These sensory cells are particularly abundant round the margin of the lappet, and

towards the marginal ganglion they merge into the columnar epithelium of ectodermal sensory cells on and round the tentaculocyst. In the marginal lappet, where the diffuse net is most easily stained, the giant fibres are absent both histologically and physiologically, and stimulation here produces a beat only indirectly or a slow wave in the nearby muscle. It is now realized that large fibres on the marginal lappet in an earlier diagram of *Cyanea* (Horridge, 1954*a*, fig. 2, p. 88) are part of the diffuse nerve net. The outer limit of the giant fibre net, as now determined by stimulation, is shown in Fig. 3 with a dotted line. The stimulation technique in the present experiments was intended to excite first the diffuse net and then follow with a contraction wave as the electrode moved into the area of the giant fibre net. The nature of the excitation in the diffuse net is unknown.

The histological appearance of the diffuse net of *Cassiopea* is not known, but physiologically it is very similar to that of *Cyanea*. It extends over the whole subumbrellar surface, including the oral arms. If the mass of the oral arms is removed and placed in sea water, aboral side upwards, it relaxes after an hour or so. A single electrical stimulus at any point initiates a wave of retraction of all the arms, which shorten by 2–3 cm. in 7–9 cm., and there is a slow bending of the stimulated arm and its branches.* In the quiescent animal this wave of excitation in the diffuse net initiates a series of beats from the marginal ganglia, but conversely it may slow the rhythm of a beating animal although the amplitude is at the same time increased.

On the other hand, the diffuse net of *Nausithöe* resembles that of the ephyra of *Aurellia* histologically, physiologically and in its anatomical plan. First there are primary bipolar sense cells, abundant in the tentacles and lappets of the margin. Connected to these is a fine net of bipolar and multipolar cells which is regionally differentiated. Round the mouth the fibres run principally in a circular direction over the inner sheet of radial muscle, but towards the marginal lappets long fibres run radially, which is in agreement with the co-ordination of one lappet and the whole mouth during the feeding response.

The network of multipolar cells and thin fibres of the diffuse net, stains with methylene blue in all Scyphozoa so studied, more easily in small specimens. The nerve cells in the tentacles and frills of the mouth are histologically similar, and are physiologically part of this net. When in company with the giant fibre net in the epithelium, it looks very similar to the net of multipolar cells coloured blue among the giant fibres in Bozler's (1927) figures. My own brief experience in staining *Rhizostoma* with methylene blue has convinced me that the diffuse net of semaeostomes is also present in *Rhizostoma* and is the net of multipolar cells that Bozler figures. In fact the diffuse net seems to be, histologically, physiologically and functionally, the generalized combined sensory-and-motor ectodermal net described in most groups of Coelenterates. As for the giant fibre system, Bozler (1927) divides his bipolar cells into three size groups, but I find no justification for separating these physiologically and consider them as all part of one and the same net.

* This observation reduces the value of the arm length as a specific character.

Absence of double innervation in Aurellia aurita

In *Aurellia* the conspicuous action of the diffuse nerve net in the feeding behaviour of the ephyra is lost at metamorphosis, and the evidence for any form of double innervation in the adult is very slender. There is Horstmann's (1934) observation that removal of a marginal ganglion is temporarily followed by a locally increased curvature of the bell, and his 'tonus' can be replaced by an explanation in terms of the two nerve nets, i.e. that local excitation of the diffuse net gives a local maintained contraction; but this is not supported by experimental evidence, and an explanation in terms of stress relief following the osmotic disturbances of the operation is equally tenable. The experiments that demonstrate double innervation in other species give negative results with *Aurellia*. No asymmetrical component has been found in the contraction of tilted animals and no slow waves across the bell have been described. In his study of facilitation in medusae, Bullock (1943) analysed the relation between the amplitude of contractions and the intervening intervals and he found the same relation satisfactory for both spontaneous and electrically initiated movements. However, by comparison with *Cyanea* and *Cassiopea* it is clear that this result applies only to those species where double innervation has a negligible effect, and of this *Aurellia* is the only known example.

CONCLUSIONS

The all-or-nothing character of the impulse at each beat is compatible with the consistent asymmetry of the compensatory movement if there is a separate local effect superimposed on the total effect of the giant fibre system. The local effect is the slow contraction in the muscle initiated (and perhaps there propagated) by the diffuse nerve net. The two responses may occur separately or simultaneously in one muscle. When they occur together the local effect in *Cyanea* takes the form of a lengthening of the relaxations; in *Cassiopea* the amplitude is increased.

Having before us the mechanism of the two nerve nets there still remains a problem to explain the compensatory movement when the animal is tilted. The asymmetrical component is consistently localized during a long succession of beats, but the diffuse net may in *Cyanea*, and usually in *Cassiopea*, conduct excitation across the whole bell. What is required is a mechanism which limits the area in which the double innervation is effective. This is possible if two impulses are initiated together from the upper ganglion and spread out, one in each independent net. However, because of the disparity in the two velocities it will only be in an area round the ganglion of origin that the excitation in the diffuse net will find muscle fibres that are not refractory. This suggestion has not been tested by experiment, but it brings together all known observations.

On a histological level there is no evidence of two types of muscle fibre, and on a large scale the muscles appear to be homogeneous and capable of both responses in every part. The only conclusion is that a double innervation is present. The innervating nerves here have the form of nets, which can be histologically

distinguished and separately stimulated. However, there is no histological picture of the nerve endings in the muscle. There is also the possibility, which has not been eliminated, that over short distances the propagation of the slow waves is from muscle fibre to muscle fibre, although the contraction is initiated by the diffuse net. It is therefore impossible to discuss in detail the innervation of the muscle fibres by both nerve nets.

Looking more broadly at the known examples of double innervation in Coelenterates, it is seen that they correspond with the functional requirement that a muscle must give a symmetrical and also a local response. It is an essential part of the organization of the ephyra larva that the feeding response is a maintained contraction of a single arm, but the swimming movement momentarily involves all arms. This is reflected in the co-ordination by two nerve nets. In the adult *Cyanea* and *Cassiopea* the double innervation persists and co-ordinates the compensatory movement. In *Nausithöe* the functional requirement and the corresponding pattern of the nervous system are closely similar to those of the *Aurellia* ephyra. However, in the adult *Aurellia* the diffuse net no longer acts on the muscle but only on the tentacles and the marginal ganglia. The existence of two separate but overlying conducting systems is established in the Hydromedusae (Horridge, 1955*a*, *b*), where, as in the ephyra, there is one system which co-ordinates the asymmetrical feeding responses and one which co-ordinates the beat. But in Hydromedusae the corresponding functional division into radial and circular muscles is an arrangement which does not require the double innervation of one group of muscles. By contrast with medusae of all kinds the co-ordination of most polyps is effected by a single net which is regionally differentiated. There is no indication of overlying independent systems or double innervation in most anemones. A description of two exceptions, the rhythmical Xeniidae and the swimming Boloceroididae, is in preparation.

The point of interaction of the two nerve nets in medusae is of especial interest. In semaeostomes the rhythmical centres of the beat are within the giant fibre system (Pantin & Vianna Dias, 1952), and presumably all other medusae have this pattern. These rhythmical nerve cells are strongly influenced by excitation in the diffuse nerve net, but the impulse at each beat is not propagated in the reverse direction, into the diffuse net. This is evidence that the diffuse net acts at polarized junctions where it accelerates—or, with too much stimulation, inhibits—the rhythmical nervous centres. The situation is exactly paralleled in the Coronatae and Hydromedusae, where in all studied forms except the Geryonidae, the principal effect is an inhibition of the rhythm when the radial system is active. The existence of two overlying conducting systems and a polarized interaction between them seems to be functionally necessary for the simultaneous but independent co-ordination of two activities, and is an essential concomitant of the bell-shaped form of a freely swimming medusa.

SUMMARY

1. The compensatory movements of *Cyanea* (Semaeostomeae) and *Cassiopea* (Rhizostomeae) are described. The asymmetry of these responses is not compatible with the properties of the through-conducting giant fibre net which propagates the contraction wave.

2. Stimulation experiments with *Cyanea* show that a second nerve net, called the diffuse net, acts locally on the muscles of the bell and delays the process of relaxation.

3. In *Cassiopea*, the responses of a ring of tissue containing a continuously circulating contraction wave are also evidence of a double innervation.

4. The responses of *Nausithöe* (Coronatae) are described and are interpreted in a similar manner.

5. In all three orders the two nerve nets are in places superimposed but are independent except where they meet at the marginal ganglia. Here the diffuse net acts irreversibly on the rhythmical units, which are known to be within the giant fibre net.

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