

THE RESPONSES OF *HETEROXENIA* (ALCYONARIA) TO STIMULATION AND TO SOME INORGANIC IONS

By G. A. HORRIDGE

Gatty Marine Laboratory, St Andrews

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The Red Sea Alcyonarian *Heteroxenia fuscescens* (Ehrb.) consists of a pinkish brown fleshy syndete up to 5 cm. in length, firmly attached to a stone or dead coral, from which grows a mass of autozooids with columns 2-4 cm. long and 2-3 mm. in diameter (Fig. 1). The first physiological observations on a xenid were made by Keller (1883) on a brown species from Suez that he considered to be *H. fuscescens*. He described the rhythmical movements in which the tentacles are clapped together (Fig. 2A). The zooids beat independently of one another, and the two halves of a disk, split longitudinally, continued to beat as before but independently. Leaning on the Hertwigs' (1879) statement that the nervous system of actinians is best developed in the disk, Keller came to the conclusion that there is here a diffusely spread rhythmical centre. He also pointed out the resemblance to the beat of medusae.

Krukenberg (1887) worked on colonies which he identified as *Xenia umbellata* at Massawa and *H. fuscescens* at Suez. From the results of operations on the disk he inferred that a rhythmical nerve centre was located in the peristomial region. He also studied the transmission of excitation. A weak mechanical stimulation of a tentacle produced a response of only one tentacle, which curled over the mouth. Following stronger stimulation the whole crown of tentacles temporarily folded together as in Fig. 2B, and neighbouring zooids behaved similarly. In the present paper this reaction is called a 'spasm'. Krukenberg inferred a 'ganglionic nerve net', best developed in the disk and tentacles and condemned Keller's conclusions (a) that the nervous centres of the polyps are entirely independent, and (b) that the rhythm is unaffected by external stimuli. He finally concludes that it seems unlikely that the rhythm corresponds to that of medusae.

A few other authors refer to the rhythmical beating of xenids; for example, Saville Kent (1893, p. 197) says 'all the eight tentacles move synchronously, opening out and contracting in a continuous measured rhythm', and Hiro (1937) gives the rhythm of *H. elizabethae* as 30/min. for large and 37/min. for small colonies. Hiro describes the spasm and agrees with Keller, *contra* Krukenberg, that other zooids are not affected when one is stimulated or even cut off. In his review of the Red Sea xenids, Gohar (1940) added some physiological observations. He found a temporary acceleration of the rhythm when small inert particles fell upon the tentacles and observed the folding of a single tentacle while the rest continued their rhythm. On the transmission between zooids he agrees with Krukenberg.

In the present paper the rhythmical beat and the co-ordinated spasm provide a means of studying the propagation of excitation. The intimate mechanisms of the beat and of the spasm are unknown, but it will be shown that the first has much in common with the rhythm of medusae, and that the second has an absolute refractory period of 0.08 sec.

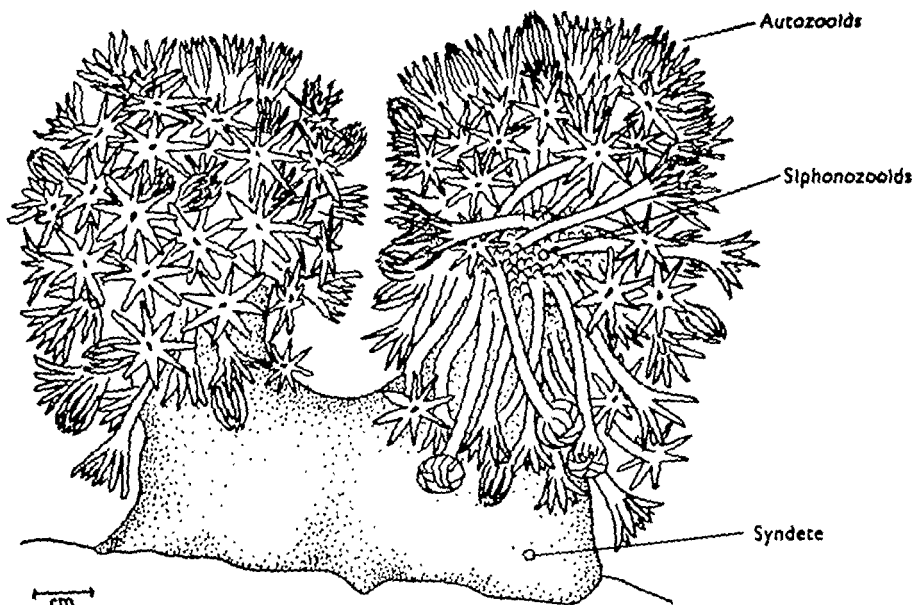


Fig. 1. A colony of *Heteroxenia fuscescens* (Ehrb.).

MATERIALS AND METHODS

Naturally growing colonies of *Heteroxenia fuscescens* were examined by diving at Ghardaqa, Red Sea, and specimens were removed with their substrate. They thrive in an outside tank but soon disintegrate if kept out of the light. Colonies may not be torn from their substrate, for any small damage to the syndete releases the internal water pressure and the circulation through the colony is interrupted. For the same reason experiments which require section through the colony are impracticable. Details of the care of xenids are given by Gohar (1940).

The arrangements for electrical stimulation and measurement of the refractory period were as figured by Pantin (1935), with a condenser shock as stimulus. With this arrangement the form of the stimulus depends on its intensity, but the simple circuit is a great advantage.

The zooids do not all beat at the same rate, and for any one of them the interval between beats is not quite constant. Accordingly, an arbitrary but convenient measurement was employed; the time for twenty (or for thirty) beats was taken for a number of zooids. This is given as an average for ten zooids with its standard deviation between zooids.

Solutions of potassium sulphate and of sodium, magnesium and calcium chlorides were prepared isotonic with the local sea water, taken as 120% normal sea water.

QUALITATIVE OBSERVATIONS

In the normal rhythm the eight tentacles are bent stiffly and symmetrically inwards by muscles on their upper sides. The contraction is a single co-ordinated twitch which brings the tentacles together (Fig. 2A). The relaxation suggests that they spring back under elastic forces. This continues day and night at 30-45/min. It is not co-ordinated between zooids and the continuous activity is a striking sight.

A different movement is the maintained spasm of one or more tentacles. Apparently the same muscles contract as in the beat but with a much greater intensity

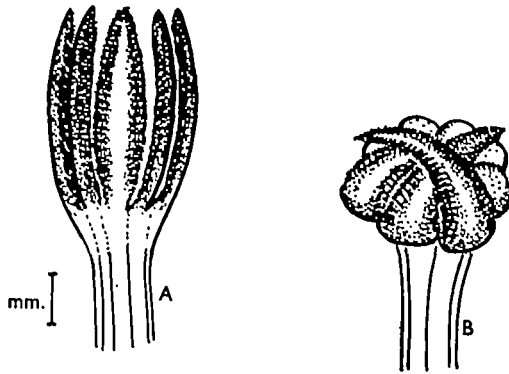


Fig. 2. A single autozooid; A, contracted in a beat; B, contracted in a spasm.

(Figs. 3 B, C, D). This can occur in response to a touch, but sometimes an occasional tentacle bent inwards spontaneously while the others beat unaffected. When lightly touched with a needle a tentacle will sometimes respond alone but usually the excitation spreads round the disk and the eight tentacles contract together (Fig. 2B). In most colonies the spasm affects all zooids when any part is stimulated strongly. The transmission across the syndete is not prevented by a cut which excludes transmission in the superficial layer and is therefore not ectodermal.

A difference in the anatomical arrangement of two conducting systems is shown as follows. An autozooid disk is split vertically and allowed to recover from the shock; each half now beats independently, as Keller found, but a pinch to one half produces a spasm of *both* halves. The responses were also examined for any interaction between the rhythm and the spasm. It sometimes happened spontaneously that two opposite tentacles would bend inwards by chance at the same time (Fig. 3C). They would remain bent for many beats, but the groups on either side would continue in perfect co-ordination. Further, six tentacles could respond independently to delicate touches while two were unaffected (Fig. 3D). These two continued in unison, though not necessarily regularly, until the others again joined their rhythm. These simple qualitative observations were repeated many times.

The rate of the rhythm is found to be slower in severed parts than in whole zooids. It is also more variable, both between parts and from time to time in single parts. These effects are more marked the smaller the severed piece (Fig. 4). This is interpreted by considering that the origin of the rhythm lies in a population of

units, each with its own rhythm and that the rate of the whole is the rate of the fastest. The simple picture is not entirely borne out by experiment, for, if a zooid disk is divided into eight segments, the minimum interval between beats is always greater than in the original zooid. The same result is usually found with medusae, and implies the existence of excitation other than that within the rhythmical system.

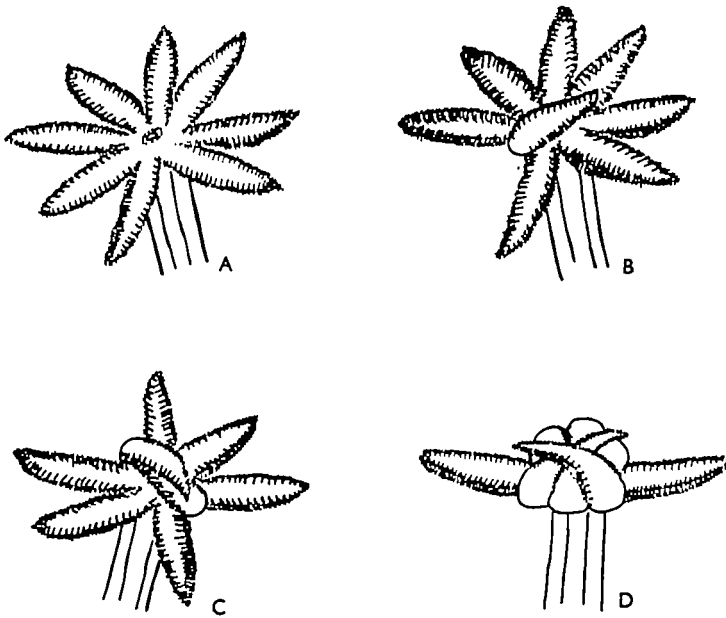


Fig. 3. A single autozooid; A, relaxed; B, C, D, in a partial spasm as described in the text.

ELECTRICAL STIMULATION

It proved impossible to introduce an extrasystole into the rhythm by a single shock because a spasm always intervened. The response of an isolated tentacle to a weak shock is a small jerk. An increase or a decrease of the rate of the rhythm may follow weak electrical stimulation. After a spasm the normal rhythm is resumed, but *Xenia macrospiculata* Gohar takes several beats to regain its original frequency after a spasm.

A single shock above threshold applied on the head of a zooid produces a spasm that normally lasts 6–9 sec. There is no retraction of the column. The response is independent of the strength of the stimulus except for a small stimulus to a tentacle, which may then contract alone; at high intensities spasms of 15–30 sec. were regularly found.

Two shocks at the same point on the disk at an interval of 0.1–2 sec. produce a similar spasm, but now the column of the zooid partially withdraws; neighbouring zooids are not usually affected. With three shocks, intervals 1 sec., several neighbouring polyps go into a spasm for 6–9 sec., and the stimulated one retracts its column. Four shocks, intervals 1 sec., produce a spasm of about thirty zooids over an area 3 × 3 cm. for 6–9 sec., and perhaps this time a few retract in the neighbourhood

characteristic of some alcyonarian colonies in contrast to madreporarian and zooanthid colonies. With stimuli at, say, 1/sec. there is at first only a local spread of excitation, and in some alcyonarians, *Lobophytum*, for example, 10–12 stimuli may be required before the wave of zooid retraction suddenly begins to spread over the whole colony. On account of their small size the colonies of xenids are not suitable to demonstrate the details of co-ordination over the colony, and all the zooids contract before the third or fourth shock.

In certain colonies with large zooids a single shock on the column wall of a zooid produced no visible result, but a second shock, interval 1 sec., initiated a spasm in this zooid alone. In other colonies, usually less developed and with smaller zooids, a single shock on the column produced a spasm in that one zooid and a second shock (interval 1 sec.) produced a spasm in neighbouring zooids. In another series of experiments a small colony was used which responded to the second of a pair of stimuli, interval 1 sec., on the column or syndete by a co-ordinated spasm of all zooids. The second stimulus was then applied at a point on the syndete as far as possible (i.e. 5 cm.) from the first. The second stimulus now produced no effect. However, when the second stimulus was applied 2 cm. away from the first a co-ordinated spasm followed. Such results varied greatly between colonies, but most experiments with one group of colonies showed that the second stimulus must be within 2 cm. of the first to produce a wave of spasm; but in some specimens two stimuli produced such a wave only if applied within a few millimetres, and again in many colonies two stimuli produced no result at all.

The strange result of Keller, who found no co-ordination of the spasm over the colony, could possibly be explained by the following observation. A colony from a shady place in 3 m. of water at low tide had very large zooids 6–8 cm. long with an 'etiolated' appearance. Strong shocks on the syndete at $\frac{1}{2}$ sec. intervals produced no spasm. Stimulated disks showed the spasm as usual, but no transmission to other zooids could be induced.

A refractory period of the 'colony' (as distinct from the 'disk') conducting system was measured in some colonies, which responded by a spasm of at least a few zooids to the *second* of a pair of shocks applied on the syndete. The apparatus was as figured in Pantin (1935), with a rotary arm and mercury cups to provide the variable interval. No result followed a single stimulus. For several colonies, with stimulus strength at least four times the threshold, there was no response to the second of the pair of stimuli at an interval less than 0.08 sec., but with intervals greater than 0.1 sec. a spasm of some zooids always followed.

EFFECTS OF IONS

Potassium

The effect of excess potassium ions on *Heteroxenia* is twofold. First, there is a slowing of the rhythm, which stops after 50 min. immersion in sea water containing 5% of isotonic K_2SO_4 . Secondly, the responses of the tentacles, the sensitivity of the colony surface to touch and the co-ordination of the spasm over the colony progressively

disappear. The final result is a *relaxed*, paralysed colony, effectively narcotized, and turgid as a result of the hyperactivity of the cilia. As an example, ten counts of one colony initially gave an average of twenty beats in 29.7 ± 0.9 sec. S.D. Isotonic potassium sulphate was gradually added up to 5% of the total volume. For 20 min. there was little effect, but after 30 min. many zooids took 40 sec. for twenty beats and after 40 min. the few that remained active took more than 60 sec. for twenty beats. Some examples showed a slight initial acceleration. If the potassium sulphate is quickly added the chemical stimulation produces temporary spasms.

In the relaxed and insensitive condition produced by excess potassium ions neither the longitudinal muscles nor the tentacles will respond to electrical stimuli at 1/sec. The circular muscle of the column, however, will still contract very slowly. This produces an interesting result, for now the sphincter formed by a contracted band of circular muscle isolates hydraulically the distal part of the zooid, which shrivels, while the basal part inflates to a sphere. During recovery in normal sea water the shrivelled polyp head recovers its rhythm before the rest of the colony because it is isolated from the reservoir of potassium ions in the fleshy syndete. A series of stimuli now applied to the syndete fails to produce a spasm in this polyp, though the polyp itself responds normally. This shows that excitation which normally co-ordinates the spasm of the colony is also stopped by the excess potassium ions. During recovery the local response of tentacles and the spasm appear before the onset of the rhythm.

Sodium

An excess of sodium ions in the medium first accelerates the rhythm, then, in strong enough solutions, slows it. In one experiment with a slowly beating colony the initial count was ten beats in 22.2 ± 2.4 sec. S.D. Isotonic sodium chloride was added to make 14% of the total volume. After 2 min. the average for ten counts in the next 5 min. was ten beats in 18 ± 1.34 sec. S.D., and after 25 min. the average for ten counts was ten beats in 26.7 ± 3.0 sec. S.D. In all observations there is first an acceleration but the interval between beats is never less than 1.1 sec. Then, as the sodium concentration in the tissue rises, the zooids become more liable to respond to the slightest touch by a long-sustained spasm, and with mixtures containing more than 10% sodium chloride permanent spasms prevail. This contrasts with the relaxation produced by potassium ions and suggests that sodium ions are not directly slowing the rhythm. No effect on the co-ordination of the spasm over the whole colony was found.

Magnesium

Excess magnesium ions anaesthetize *Heteroxenia*. Isotonic $MgCl_2$ solution is slowly added to make 25% of the total volume. After 2 min. the decline of the frequency and amplitude of the rhythm is noticeable. At the same time the co-ordination of the rhythm between tentacles is progressively lost. Simultaneous beats of the tentacles become less frequent, and give way to contractions of greater amplitude and duration more characteristic of the spasm. Soon only temporary spasms of individual arms are seen. These spontaneous movements finally stop after 2 hr.

Meanwhile the co-ordination of the spasm between zooids is lost after a gradual decline, as tested at intervals by electrical stimulation on a part of the syndete not immersed in the solution. After 4 hr. the insensibility is complete, and when the head of an autozooid is cut off neither the head nor the stem contract. During recovery from magnesium the irregular movements of tentacles reappear first. These are replaced by an abnormally slow co-ordinated rhythm. The unco-ordinated movements do not merge into the rhythm, but for a time they are distinct as in normal life when a zooid is touched or shaken.

Calcium

The effect of excess calcium ions is primarily to slow the rhythm. No initial acceleration is found. In one particular colony counts of the rhythm gave initially twenty beats in 37.3 ± 2.3 sec. s.d. Addition of isotonic calcium chloride to make 1.2% and later 2.4% of the total volume was immediately followed by a slowing to twenty beats in 50 and 65 sec. respectively, from which there was some recovery after 15 min. in the mixture. On now increasing to 4.8% a spasm was produced in most autozooids, though occasionally there was some rhythm with long intervals of 5–10 sec. between beats. After 15 min. in the 4.8% solution there was some relaxation of the spasms and the zooids lay open with only occasional movements of single tentacles. The response of tentacles and zooids to touch and the co-ordination of the spasm over the colony remain normal long after the spontaneous rhythm has disappeared.

DISCUSSION

(a) *The co-ordination of the colony*

In some colonies a single shock produces no response, but a second at the same point within 3 sec. is followed by a wave of spasm. When the interval between the shocks is gradually reduced to less than 0.1 sec. the response suddenly disappears. With shocks well above threshold the minimum interval is of constant duration. It seems that we have here the absolute refractory period of the conducting system. In some coelenterates, of which *Calliactis* is the earliest example (Pantin, 1935), it is possible, by application of the second shock at a point distant from the first, to show that the whole pathway becomes refractory. This experiment is not successful with *Heteroxenia*, but at least the responding units include a link which works with an all-or-nothing action and the first excitation is a single discrete nerve impulse near its point of initiation. It is a suggestion that the excitation following the second stimulus is similar.

The durations of the spasms of the individual zooids are remarkably uniform over the colony and they recover their rhythm simultaneously. The excitation from the second shock has exactly the same effect as a single shock on the disk. It may be that a standard spasm is the only possible response, or this resemblance may imply that the conducting system brings a standard 'signal'. But observation soon eliminates a restriction of the response, for partial spasms and extra long spasms occur

frequently. A single shock to a disk always produces a symmetrical spasm whose duration is approximately constant for a range of intensities; but at high intensities or with several shocks, much longer spasms follow. All this suggests that the signal which reaches all the disks is a single impulse which in these colonies is initiated by the second shock on the syndete and is not a burst of impulses followed by a sensory accommodation. Variations in the spasm responses will then depend on local conditions and long spasms follow a sequence of impulses.

An observation on *Anthelia glauca* Savigny is relevant here. This is a xenid with no rhythm and the tentacles stand stiffly round the disk. A beautiful response is seen when condenser shocks at 30/min. stimulate the syndete. At each shock after the first the tentacles of all the forty or so zooids in the colony jerk simultaneously towards the mouth in a series of discrete contractions. The jerks correspond with the shocks and stop when they are discontinued.

(b) *The origin of the rhythm*

There is very little information about the conducting system which co-ordinates the beat. Artificial stimulation is here disappointing, for it produces only a spasm, never an extrasystole. The rhythm is reminiscent of the beat of medusae and the spasm is similar to the maintained contractions of the radial muscle of the ephyra larva of *Aurellia* (Horrige, 1955, Fig. 1) and of many Hydromedusae. Two lines of evidence suggest that the rhythm is similar. First, severed tentacles and parts of the disk show the rhythm, though slower than normal as though the part with

Table 1. *A summary of the reversible effects of ions on Heteroxenia, Cassiopea and Rhizostoma*

	<i>Heteroxenia fuscescens</i>	<i>Cassiopea andromeda</i>	<i>C. xamachana</i> from Mayer <i>Rhizostoma</i> from Bethe
Excess K ⁺ ions	Initial temporary spasms (indirect effect). Progressively slows then stops relaxed. Finally prevents transmission over colony	Initial acceleration (indirect effect). Progressively slows then stops. Finally prevents transmission in both nerve nets	Slows then paralyses. Oral arms paralysed (Mayer)
Excess Na ⁺ ions	Temporary spasms (indirect chemical stimulation). Accelerates the rhythm in strong solution. 10% produces paralysis in spasms	Temporary acceleration (indirect). Accelerates the rhythm	Weakly accelerates, then paralyses
Excess Ca ⁺⁺ ions	Initial temporary spasms (indirect chemical stimulation). Progressively slows then stops the rhythm, followed by relaxed paralysis	Initial temporary acceleration (indirect). Progressively slows then stops	Momentarily accelerates then slows and then stops
Excess Mg ⁺⁺ ions	Progressively slows then stops	Progressively slows then stops	Progressively slows then stops

(Paralysis ≡ not excitable and no transmission)

the fastest natural frequency drives the rest. The frequency of parts of various sizes support this (Fig. 4).

Secondly, *Heteroxenia* and the jellyfish *Cassiopea* (Rhizostomeae) react in a very similar way to excesses of potassium, sodium, calcium and magnesium ions if the primary effect on the rhythm alone is considered. But in *Cassiopea*, as in adult semaeostome medusae, excitation in the diffuse net accelerates the rhythm, whereas in *Heteroxenia* a spasm follows. Because of this difference the indirect effects on the rhythm contrast strongly. The results of earlier work (Mayer, 1906; Bethe, 1908) and my observations on *Heteroxenia* and on the Red Sea species *Cassiopea andromeda* are compared in Table 1.

The effect of an excess of the physiologically common cations on the rhythm of medusae, discovered by Loeb (1899), has been studied in detail by Mayer (1906, 1910) on *Cassiopea xamachana*, and Bethe (1908) on *Rhizostoma pulmo*. Mayer's theory of the origin of the rhythm has held the field for fifty years, but he argued that the rhythm arises from excess sodium in the ganglion because an access of sodium accelerates. Clearly this does not follow. The failure of excess external sodium to influence other nerve preparations leads to the conclusion that in coelenterates it acts by diluting the other ions.

The effects of ions are better known in medusae than in *Heteroxenia*, but the situation is confused and it is difficult to reach any conclusion. The results with some ions in excess of their normal concentrations can be interpreted in terms of the effects of these ions on the membrane potentials of other excitable tissue. Excess external potassium ions depolarize all excitable membranes known. For a rhythmical system this could increase the rate, or retard the repolarization of the membrane and decrease the rate. On various heart preparations excess potassium usually accelerates; but it usually slows down coelenterate rhythm. Excess calcium ions slow the rhythm of Purkinje fibres (Weidmann, 1955) by a rise of the threshold without change of resting potential, so that more depolarization is required to initiate an impulse. Excess calcium slows coelenterate rhythms and also a variety of heart preparations (data on hearts from Prosser, 1950, Table 69). According to Weidmann the effect of magnesium is similar to that of calcium. Excess sodium appears to have only small effects. In conclusion, the effects of ions on coelenterate rhythms suggest a polarized membrane mechanism, and in *Heteroxenia*, it is impossible to attribute the rhythm either to nerve or to muscle.

(c) *The two conducting systems of the disk*

The effects of ions on *Heteroxenia* suggest that the rhythm originates in a system with a polarized membrane as in nerve or muscle fibres. Two observations suggest the existence of separate systems of conduction for the beat and the spasm in the disk in addition to the contractile elements. First, in the beat the part with the fastest rhythm drives the whole. Therefore the excitation originates in the through-conducting pathway which conducts it. Secondly, there is the observation (Fig. 3D), that two opposite tentacles can show a co-ordinated rhythm while the intervening tentacles are in a spasm. The difficulty is to explain how the momentary

excitation of the beat can cross the regions of the disk where there is the excitation of the spasm. The inadequacy of a single conducting system in the disk rests on this observation, together with the generalization that in either a nerve net or a conducting membrane a long-lasting excitation travels further than a momentary one of the same kind. Also the spasm can be confined to one tentacle and also can be co-ordinated over the colony by a nerve impulse. The muscle fibres involved appear to be identical in both the local, maintained contraction of the spasm of a single tentacle, and the co-ordinated momentary contraction of the beat. It requires two lots of one-way transmission from two conducting systems to one set of muscle fibres (or alternatively to two sets of intermingled muscle fibres) to explain the observation that these two kinds of excitation remain distinct. The ephyra larva of *Aurellia* gives exactly comparable data, but the two conducting systems can be histologically demonstrated as two nerve nets (Horridge, 1955). In *Heteroxenia* two conducting systems are only inferred; the refractory period of part of one has been measured, the other reacts to common anions as a typical membrane conductor.

SUMMARY

1. In *Heteroxenia* there are two distinct kinds of response; one is the unco-ordinated rhythm of the autozooids; the other is the maintained spasm which is co-ordinated over the colony.
2. The absolute refractory period of the conducting system which co-ordinates the spasm of the colony is found to be 0.08 sec.
3. The actions of excesses of potassium, sodium, calcium and magnesium ions suggest that the rhythm resembles that of Scyphozoa.
4. The organization and overlap of the two conducting systems in the autozooid disk recall the scyphozoan pattern of two nerve nets. However, in *Heteroxenia* there is little evidence of interaction between the two systems.

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