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STUDIES ON HEXACTINELLID SPONGES. II. EXCITABILITY, CONDUCTION AND COORDINATION OF RESPONSES IN *RHABDOCALYPTUS DAWSONI* (LAMBE, 1873)

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CONTENTS

Rhabdocalyptus can arrest its feeding current. The response is initiated by mechanical or electrical stimulation, and is coordinated through the sponge by a conduction system, having a precise excitability threshold and conducting on an all-or-none basis. All parts are excitable and conduct. Individuals in colonial assemblages are coordinated. Spontaneous as well as evoked arrests are observed. There is evidence of scattered pacemaker sites.

Conduction is diffuse and unpolarized, and occurs with a velocity of 0.26 ± 0.07 cm s^{-1} at 11 °C. The conduction system is probably the trabecular syncytium. Isolated dermal membrane ('pure' trabecular tissue, without flagella or contractile elements) conducts. Mechanical and chemical signalling mechanisms are discussed. It is concluded that they cannot account for the phenomena observed, but that conduction must involve electrical impulses.

The effectors responsible for current arrests are almost certainly the flagella of the flagellated chambers. It is assumed that they stop beating on receiving an arrest signal through the conduction pathway. The waveforms of arrests recorded with a thermistor flowmeter are best interpreted in terms of sudden, all-or-none cessation of pumping, with slow, gradual recovery of full pumping power. The flagella probably beat feebly at first on becoming active again following an arrest. The effector response shows a refractory period of 30 s. Responses occur with short latency. Delays are attributable to conduction time. The system is fatigueable.

Numerous parallels exist with the behaviour of the stigmatal cilia in the ascidian branchial sac, both in the characteristics of the effector response and in the mechanism of coordination.

1. General introduction

Because of their inaccessibility, hexactinellids have not until quite recently been studied alive. The few comments to be found in the literature on the subject of their feeding and general physiology are therefore inferential, and are based upon structural comparison of hexactinellids with other sponges whose physiology is known. As an example, we may mention Bidder's (1923) suggestion that hexactinellids are incapable of creating a directional water current in through the walls and out through the osculum like other sponges. Instead, he envisaged the sponge as a passive filtering device placed across the path of slowly moving deep ocean currents, with the water flowing right through it transversely. The sponge's flagella would serve only to create local water movement in the vicinity of the collar cells according to Bidder.

It is true that hexactinellids have a very open, porous structure, with flagellated chambers laid out upon a simple syconoid plan and that instead of well defined canals they have a system of lacunae traversed by cobweb-like strands of the trabecular net; but reference to the older histological descriptions also shows that within the trabecular net there is still a perfect and complete separation of incurrent and excurrent waterways, passage from one to the other being possible only through perforations (prosopyles) in the walls of the flagellated chambers. There is thus no reason to doubt, *a priori*, that water flow is directional as in other sponges.

That it is indeed directional was first noted by Mr G. Silver, who made the first observations on feeding and water flow in hexactinellids. By using dyes and a flowmeter he was able to demonstrate these currents in intact sponges in their natural habitat. Later, it became possible to study these processes in animals maintained in tanks in the laboratory. The history of these studies, which are directly antecedent to the work reported here, is summarized by Mackie (1979). We now know that, not only is there a conventional feeding current, but the sponge can halt it when stimulated, and that these arrests are coordinated throughout the whole sponge by a conduction system having a precise excitability threshold (Lawn *et al.* 1981).

Far from it being the case then that hexactinellids show 'no hydraulic evolution or hydraulic efficiency' (Bidder 1923) they are probably among the most advanced of any sponges in terms of their ability to regulate their feeding current.

The present paper represents an attempt to define the characteristics of the arrest response and to examine the properties of the conduction system. We have used specimens of *Rhabdocalyptus dawsoni* for these investigations. It has now been determined (by Dr W. C. Austin, personal communication) that the specimens used in our previous work from the Bamfield region are conspecific with those obtained from Saanich Inlet, and belong to *Rhabdocalyptus*, not *Staurocalyptus* as originally suggested (Mackie 1979). *Staurocalyptus dowlingi* does occur locally, but in a different habitat.

The work reported by Lawn *et al.* (1981) was done with a flowmeter sensitive enough to record changes in the oscular currents of small sponges, but not sensitive enough to record currents passing directly in or out through the openings in the body wall. An improved flowmeter is now available that allows us to measure changes in rate of flow of water entering and leaving pores in the body wall and thus permits experimentation on excised pieces of body wall as well as on whole sponges. It has been possible to repeat, verify and extend the original findings and these results form the basis of the present paper.

The discovery that these sponges have a conduction system showing excitability properties resembling those of nerve and muscle revived the old question of whether sponges have a nervous system. Mackie (1979) reviewed the literature in this field, specifically that part of it dealing with propagated contractions, control of flagellar beating and the conduction mechanisms, both nervous and non-nervous, that might be responsible. He concluded that there is no good evidence for the existence of nerves in sponges and that conduction in hexactinellids is probably non-nervous. This is also the conclusion of Lawn (1982). Detailed study of the fine structure of *Rhabdocalyptus* by optical and electron microscopy (Mackie & Singla 1983) confirms that nerves are absent, and suggests that the conducting tissue must be the trabecular net itself, as the only tissue distributed throughout the sponge in all regions where conduction occurs. Experiments to be reported here point to the same conclusion, but direct recordings of the electrical signals presumed to be propagated through the tissue have not yet been achieved, and so a part of the present report is devoted to a consideration of the various alternatives.

2. Material and methods

Specimens of *Rhabdocalyptus* were collected by divers in the vicinity of the Bamfield Marine Station and were kept at the station in slowly running sea water in large darkened tanks in the basement of the building. Specimens whose atrial linings were clean and free of sediments were selected for the experiments. They were put in a 2 l Plexiglass tank with sea water running through it and were allowed to remain undisturbed for several hours before being used. As soon as they were found capable of responding to stimulation by arresting the feeding current they were considered ready to be used for experiments. It was found best to keep the water flowing very slowly during experiments, even though this leads to small fluctuations in the baseline of the flow record. If the water becomes stale or warm, the sponge rapidly ceases to respond. Excessive mechanical disturbance has the same effect, necessitating lengthy intervals between experiments in some cases.

A circuit diagram of the flowmeter used in these experiments is shown in figure 1. The

cessation of water flow during pumping arrest causes self-heating and consequent reduction of resistance in the sensing thermistor, T_s. The resulting bridge imbalance is recorded as a voltage change. T_r is remote from the recording site and compensates for changes in the temperature of the recording bath. T_s is a glass bead and T_r a glass probe thermistor, types GB32J2 and GB32P2 respectively, from Fenwall Electronics (Framingham, Massachusetts 01701, U.S.A.).
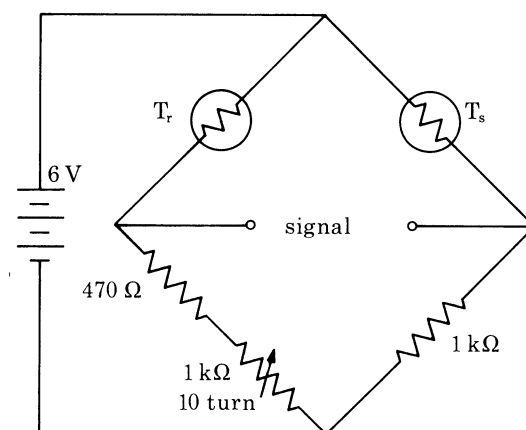

FIGURE 1. Flowmeter circuit diagram. A decrease in the rate of water flow past the sensor probe (T_s) causes it to self-heat and its resistance to change. The resulting bridge imbalance is recorded as a voltage change. T_r is remote from the recording site and compensates for changes in the temperature of the water bath.

The output from the flowmeter, after amplification, was displayed simultaneously on a Gould-Brush 220 chart recorder and on a Tektronix 5111 storage oscilloscope. Oscilloscope traces were recorded on Polaroid film. Polarity was arranged so that arrests are downward deflexions in all the illustrations.

For electrical stimulation paired chloride-coated silver wires or platinum wires were used. The wires were insulated to their tips with a flexible resin which allowed the tips to be bent to fit different preparations. A gap of 3–5 mm between the tips was usually found to be suitable. During experiments, mechanical jarring or vibration must be avoided as the sponge is responsive to such stimuli. Shock strengths in the range 5–20 V and durations of 20–50 ms were usually sufficient to evoke a response.

3. THE EFFECTOR RESPONSE

(a) Visual observations

With lighting arranged to give a dark field effect the oscular current can be monitored by watching the movement of particles in the water stream. Arrests are seen as a slowing down of particle movement, usually to the point of complete arrest. After a variable delay, pumping starts again, slowly at first and then more rapidly. In normally pumping sponges the flow rate shows only very minor fluctuations. Similar observations have been made with the help of dyes such as fluorescein injected into the sponge through the body wall.

Direct visual observations provide a useful check on the validity of the recordings produced by the flowmeter, but are hard to translate into quantitative terms. Visual observation of flow is only possible with intact sponges where there is a substantial oscular current. Water flow

across isolated pieces of body wall is too weak to be detected visually. Thus we came to rely heavily on the flowmeter for all precise current measurements.

(b) Waveform of the response recorded by thermistor flowmeter

The flowmeter is sufficiently sensitive to record changes in the rate of water flow across isolated portions of the body wall. The probe may be placed on the outside or the inside of the body wall. Because of the thickness of the ectosomal spiculation, the most accurate recordings are those obtained on the atrial side at points where the stream emerges from the excurrent lacunae.

Figure 2 shows arrest–recovery responses photographed from the oscilloscope screen. The duration of these events increased from about 1 min to about 2 min during the series shown in figure 2b. Arrests lasting up to 3 min have been observed in other preparations.
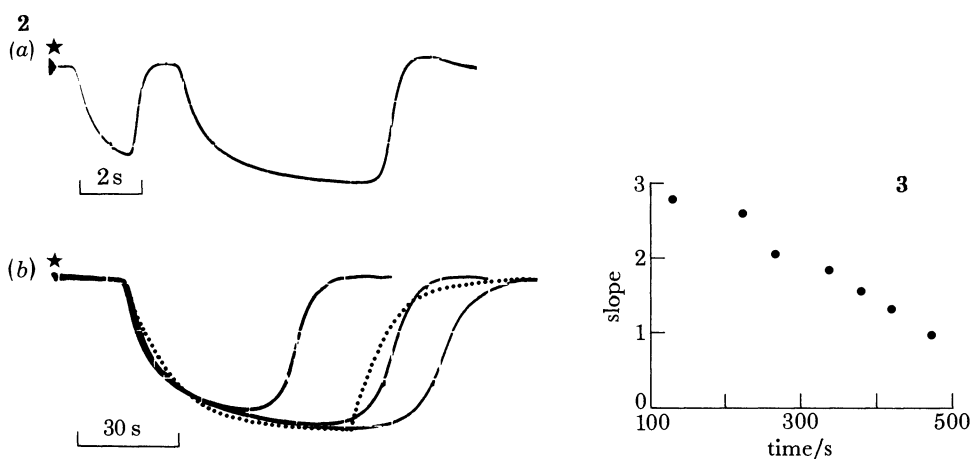

FIGURE 2. Arrest–recovery cycles, as recorded with a thermistor flowmeter across isolated slabs of sponge body wall. In (a), a single shock (*) evoked an arrest of brief duration, followed by a second, spontaneous arrest of longer duration. In (b), three successive responses, all evoked by shocks, are shown superimposed. Inter-shock intervals were 100 and 30 s. The dotted line is a theoretical curve which assumes that pumping stops and starts on an all-or-none, off–on basis.

FIGURE 3. Evidence of effector fatigue. The slope of seven successive recovery curves, which is a measure of recovery rate, is shown to decline linearly with time since start of arrest, which increases with each successive response in a series.

The dotted line in figure 2b shows a calculated arrest–recovery curve, derived from the equation for velocity distribution for accelerating flow in a smooth-walled pipe (Batchelor 1967) plotted over the same time course as the third in the series of recorded responses. This equation assumes that the driving force, or pump, stops and starts abruptly, rather than gradually slowing down or starting up. When the pump stops, flow rate does not immediately drop to zero, because of the momentum of the water moving along the tube. Similarly, when it starts again, the inertia of the water mass delays attainment of the full flow rate.

Comparison of the actual and theoretical curves shows that the arrest phases follow a similar course, which indicates that the sponge body wall can be satisfactorily modelled as a tube through which water flows, and that the actual 'pump', like that in the model, ceases activity abruptly at the start of an arrest rather than slowing down gradually.

Resumption of pumping during the recovery phase does not, however, closely follow the predicted curve. The 'pump' appears to start at a low output value and to build up gradually

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to full power (represented by the steepest part of the recovery slope), over some 30 s in the example shown.

If it is assumed that the 'pump' is the sum total of the active flagella in the body wall segment under study, it would follow that arrest of the current is due to sudden cessation of flagellar beating, and that when the flagella start to beat again they do so weakly at first, or that some start to beat earlier than others. We return to this question below (§3 d).

(c) Physiological properties of the effector response

(i) Fatigue

After a period of about 15 min without stimulation during which the sponge has been left to pump at a steady rate, a stimulus will cause an arrest which follows the usual slope, but is terminated after a relatively short period by resumption of pumping. The first response in figure 2 a is such a case. It might at first sight appear that this is an 'incomplete' response and that, with successive stimuli, responses build up to full 'amplitude' by a process analogous to neuromuscular facilitation. This would be a misconception. Although the three responses shown in figure 2 b show a progression in the degree to which they bring about arrest of the water current, they all represent complete stoppage of the pumping mechanism. The difference lies simply in the length of time between start of arrest and resumption of pumping. In the first example, pumping started again well before passive water movement had ceased. In the later curves, water movement had nearly stopped before pumping recommenced, giving a deeper arrest curve. The essential process is not one of facilitation but of fatigue, in the sense that pumping takes longer to start again with each successive response.

Fatigue is manifested not only in the increasing delay before pumping begins but in sluggishness of the recovery response. The slope of the recovery curve provides a measure of recovery rate. Plotting the slopes of a family of seven curves against time from start of arrest suggests that the two are linearly related (figure 3).

(ii) Response latency

In any excitable system involving conduction pathways and effectors, total response latency must be made up of (a) time to achieve threshold excitability in the conducting system, (b) conduction time to the effector and (c) effector response time. It is not possible at this stage to provide a quantitative breakdown of response latency in terms of these components. However, by minimizing conduction time by stimulating the preparation close to the recording point, it is possible to obtain overall response latencies as low as 1–2 s. This delay must also include the interval before the recording device starts to record a change in flow rate. The time constant of the thermistor used was about 1 s. It would appear therefore that threshold excitability is rapidly reached in the conduction system and that the effector response occurs immediately on arrival of the conducted signal. The longer delays recorded when the stimulating electrodes are placed further away can therefore be safely attributed to conduction time through the intervening tissues.

(iii) Refractory period

Responsive preparations can exhibit long series of arrest–recovery cycles when stimulated every 3–5 min, without any noticeable change in the form of the response. If the interval between shocks is reduced so that the second shock falls within the recovery period following the

first evoked arrest, the arrest is prolonged for at least the duration of a second complete arrest (figure 4a). It is therefore easy to distinguish normal (single) arrest events from double responses even when there is no marked inflexion showing where the second response begins (figure 4b). Intermediate responses are not seen. By reducing the interval between the two shocks progressively until only a single response is consistently obtained, we can obtain a value for the absolute refractory period of the preparation. This value generally lies between 28 and 33 s, with a mean of 30 s.

We assume here that we are dealing with refractoriness in the effector mechanism rather than in the conduction system. If the conduction system had a longer refractory period than the effector, there would be a period after full or partial recovery of pumping during which the preparation was not susceptible to further arrests. This is not observed. It may be assumed
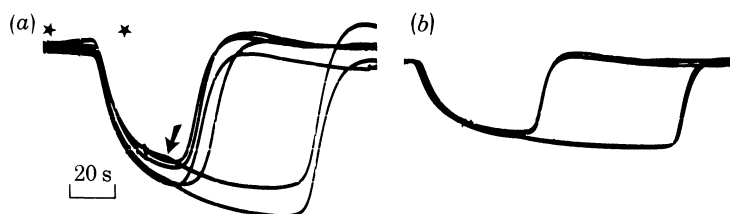

FIGURE 4. Refractory period. A single shock or pair of shocks up to 28 s apart produce one family of curves. Where the inter-shock interval is longer than about 30 s a second arrest is evoked. Its onset is shown in (a) as an inflexion (arrow) in the arrest curve at a point where recovery had just begun. In another preparation (b) no inflexion is apparent, as recovery had not begun.

then that the conduction system has an absolute refractory period equal to or less than that of the effector system. If the conducted signal is indeed an action potential, as we argue below, it is unlikely to have an absolute refractory period of more than a few milliseconds. This cannot be decided until ways are found of recording directly from the conducting tissues. It is, however, generally true that conducting tissues have much shorter refractory periods than effectors.

(d) Mechanism of current arrest

It has been seen above that the form of the arrest response is indicative of a sudden, complete stoppage of the pumping mechanism, rather than of some more gradual process. This immediately suggests flagellar arrest as the most likely mechanism. It is well known that sudden coordinated ciliary arrests occur in many invertebrate groups (Aiello 1974). In *Rhabdocalyptus* we have only been able to observe flagellar activity in small wafers of tissue or in dissociated fragments. Such pieces do not respond to stimulation by arresting their flagella. This is in keeping with the general observation that badly damaged tissues pump continuously and fail to give arrests in response to stimulation (§4 (a) (i)). Thus it has not yet been possible to observe flagellar arrests or to demonstrate directly that they are responsible for the cessation of water flow.

The only other way in which we could envisage current arrests being brought about is by the contraction of pores or internal passages in the sponge. The rigidity of the hexactinellid skeleton precludes any possibility of oscular closure, or of general contraction of large areas of body wall. Direct observation of the atrial wall during arrests shows that the atrial pores remain wide open. If contractility is involved, it must be on a microscopic scale, the regions in which it would occur being the pores in the dermal membrane or the prosopyles in the walls

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of the flagellated chambers. For a number of reasons it seems extremely unlikely that contractility is responsible:

(1) It is scarcely conceivable that the response could occur quickly or completely enough to account for arrests of the type observed. The dermal pores in some demosponges are contractile and can close completely, but the opening and closing take many minutes to become apparent and some 30 min to be complete (Weissenfels 1980). As noted above, arrests in *Rhabdocalyptus* are sudden, all-or-none events, the fact that flow does not immediately cease being attributable to the momentum of the water mass.

(2) Examination of the dermal pores and prosopyles by electron microscopy has failed to reveal any aggregations of microscopic fibrils or filaments which might be responsible for closure. Hexactinellids lack specialized contractile cells (Mackie & Singla 1983).

(3) If closure of dermal or atrial pores were responsible for current arrest, removal of the dermal and atrial membranes would be expected to produce a preparation incapable of arrest. Experiments have been conducted that show that this is not the case: normal arrests are exhibited following removal of both dermal and atrial layers.

(4) Visual observation of living dermal membranes suggests that the pores are incapable of contraction. A preparation in which the dermal pores were readily visible was set up in a perfusion chamber. Photographs were taken at intervals. No changes in pore diameters were observed over a period of 4 h. In other preparations, chemicals were added to the preparation, for instance dilute acetic acid, formalin, ammonium hydroxide and magnesium chloride. Photographs made before and after these treatments showed no differences in pore diameters. After fixation for electron microscopy both prosopyles and dermal pores remained open.

In view of the extreme improbability of contractile events being responsible for current arrest, it may be confidently assumed that a change in the beating of the flagella is the causative mechanism.

Until some way has been found of observing or monitoring flagellar activity directly in physiologically normal preparations, some doubt must remain as to the exact nature of the flagellar response. It is conceivable that, rather than undergoing simultaneous arrest, the flagella change the direction of their power strokes, beating in random directions and so cancelling each other out. There are no precedents for such a process in the literature on ciliary control. There are, on the other hand, numerous precedents for unified ciliary arrests or reversals.

Flagellar arrests have not previously been implicated as a means of current control in the Porifera. Changes in water flow rates in Calcarea and Demospongiae are generally best attributed to contractile processes (see review by Mackie 1979). Thus we conclude that *Rhabdocalyptus*, and presumably the Hexactinellida generally, differ from other sponges in being able to arrest their flagella in a coordinated way, and so to control water flow through their body walls on a simple on–of basis. This statement requires qualification only in so far as the sponge goes through a transitional phase when starting to pump after a period of arrest. As noted above, flow rate is initially low, and the full rate is not established for several seconds. This would presumably mean that the flagella when they start to beat again beat weakly at first. This is also true of the stigmatal cilia in the ascidian branchial sac (Mackie *et al.* 1974).

In the following pages and elsewhere, when we discuss current arrests, it will be assumed that flagellar inhibition is the causative agency.

4. The conduction system

(a) General physiological properties

(i) *Sensitivity to stimulation*

Tactile stimulation of the sponge at any point will cause arrest of the oscular current. All regions appear equally sensitive. Pinching, jabbing and scraping are effective stimuli. Sensitive preparations respond to mechanical disturbance of the external spicule mass. Twanging a single spicule may even be effective. Vibrations in the preparation area sometimes cause arrests. This may be attributable to mechanical movement of the spicules against the surface on which the sponge rests and is not necessarily an indication of sensitivity to water-borne vibrations in Nature. Observations in the natural environment suggest that arrests occur when excessive amounts of particulate matter are present in the incurrent water supply (G. Silver, personal communication). Mr Silver also noted that sponges sometimes stopped pumping when divers were in the area. Turbulence due to divers or to some other cause might result in sedimentary matter becoming dislodged from the ectosomal spicule mass and entering the incurrent stream and this might be the effective stimulus. The sensitivity of specimens in the laboratory to such stimuli has not yet been systematically investigated, and we know little regarding the effects of variations in the physicochemical environment. The sponge shows no photosensitivity but becomes unresponsive in temperatures above 15 °C. It can be assumed that in Nature mechanical agitation of any sort, and perhaps the presence of excessive sedimentary matter in the incoming water, will cause arrests. The arrests studied in this investigation are unified events which must involve flagellar inhibition throughout the whole sponge and, as such, are coordinated by signals propagated in a conduction system. Sensitivity to stimulation would therefore appear to be a property of the conducting system. This is not to say that local, non-propagated responses within individual effector units cannot also be directly evoked, but we have no evidence for such events.

In the laboratory, electrical stimulation was used for most experiments. Electrical stimuli can be exactly controlled and repeated in a way impossible with mechanical stimulation. The excitability threshold to electrical stimuli varies considerably in different preparations. This variation is probably largely due to differences in electrode placement. The body wall of the sponge is so porous that it is hard to get a good contact between electrode tip and tissue surface. Much current is shunted through the sea water. Shocks as high as 20 V, 100 ms are sometimes needed to excite the sponge, though under optimal conditions stimuli of strengths that would be regarded as normal for other physiological preparations in sea water may be effective (§4 (a) (ii)).

Sponges respond best after a period of several hours without disturbance in gently flowing water. If the water is turned off they become progressively less responsive and eventually become incapable of arrests. Increasing the shock strength may serve to excite unresponsive specimens and normal responses then occur, which suggests that the insensitivity observed represents reduced excitability in the conduction system rather than effector disability.

Following severe mechanical disturbance or damage, including surgical operations, the sponge may be unresponsive for hours, pumping continuously at a steady rate. When responsiveness returns, normal arrests are immediately exhibited.

The failure of the sponge to show arrests when overstimulated may be adaptive. In certain locations in the natural habitat *Rhabdocalyptus* is exposed to considerable disturbance owing

to tidal currents, and prolonged arrest under these conditions would be nutritionally disadvantageous.

(ii) *All-or-none nature of the response*

Subthreshold stimuli cause no detectable response. Shocks stronger than the threshold value produce no increase in response amplitude or duration. The stimulus strength–duration curve (Lawn *et al.* 1981) shows a similar form to curves for nerve and muscle. Although the sponge is less excitable in absolute terms (chronaxie 38 ms), this may be attributable in part to the shunting of stimulating current already referred to. Responses have been consistently obtained with stimulating strengths below 5 V. One responsive preparation consistently arrested in response to shocks of 2.7 V, but not to 2.6 V, which shows how precise the excitability threshold can be.
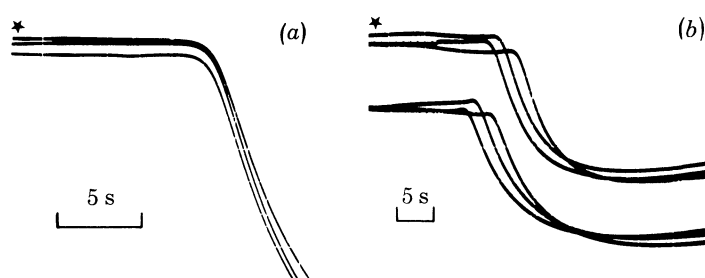

FIGURE 5. Constancy of conduction velocity in strip preparations. (*a*) Three superimposed arrest curves from a 15 mm wide strip of intact body wall. (*b*) Three superimposed arrests recorded in two places on the body wall following stimulation on a flap of dermal membrane, like that in figure 7*c*. Conduction time was less variable in the intact body wall than in the two-dimensional sheet of tissue.

(iii) *Conduction velocity*

Combining data from experiments on several different preparations gives a mean value for conduction velocity of 0.26 ± 0.07 cm s^{-1} at 11 °C for pieces of body wall. No significant differences have been observed between tissues from large and small sponges. This compares with a value of 0.22 cm s^{-1} determined by Lawn *et al.* (1981) for whole sponges. In any given recording position, conduction velocity remains remarkably constant over long periods of stimulation (figure 5*a*). The only evidence for differential conduction velocities in different parts of the sponge comes from experiments with a flap of dermal membrane dissected away from the body wall, remaining attached at one end. Conduction along this flap took place with a velocity of 0.13 ± 0.02 cm s^{-1}, which is both slower and more variable than the equivalent value for intact body wall (figure 5*b*).

(iv) *Diffuseness of conduction*

All parts of the sponge respond to stimulation and conduct. Conduction velocity measured in the longitudinal direction as in figure 6 is the same as in the circular direction. Strips of body wall incised to form Z-shaped pathways conduct (figure 7*a*), as do other more elaborate shapes. Conduction can occur along strips 1.5 mm wide and 20 mm long (figure 7*b*). Splitting the body wall horizontally so as to divide the tissues lying on the atrial side from those on the dermal gives two layers that both conduct normally. A partially isolated strip of dermal

membrane (figure 7c) also conducts, as noted above. Impulses generated in such pieces invade the whole of the sponge and spread through it causing normal arrests.
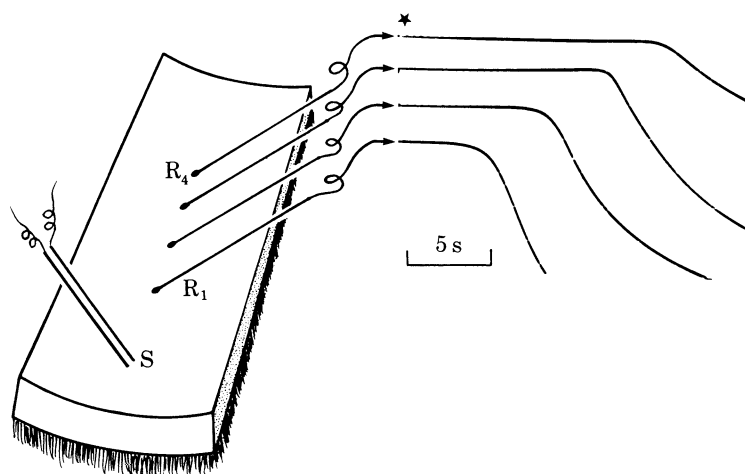

FIGURE 6. Arrests recorded at four points, R_1–R_4, at increasing distances along a slab of body wall following stimulation at S. In this experiment, conduction velocity was calculated to be 0.25 cm s^{-1}. The same value was obtained for conduction in the transverse direction.
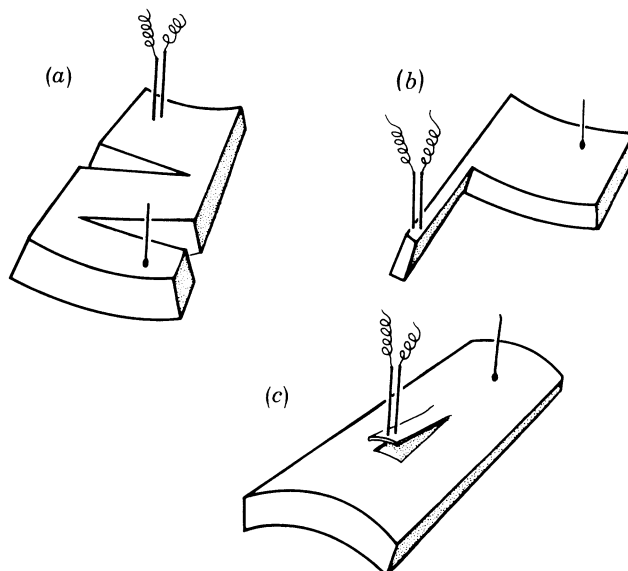

FIGURE 7. Preparations that through-conduct impulses: (a) a zig-zag preparation; (b) a strip 1.5 mm wide; (c) a flap of dermal membrane.

(v) *Spontaneity*

Specimens left in slowly moving water for long periods show occasional arrests. There is no diurnal rhythm and no consistent pattern in the occurrence of these events, and it seems probable that they represent responses to exogenous stimuli of unknown provenance. Specimens that have been subjected to a fair amount of stress or have been kept for some time in still water sometimes exhibit repetitive arrests when stimulated. A single evoked arrest may be followed by one or more spontaneous arrests. Some cyclical re-excitation process seems to be involved, because the peak-to-peak intervals between the spontaneous arrests are remarkably

constant (151 ± 7 s in the example shown in figure 8). The tendency of *Rhabdocalyptus* to execute a spontaneous, rhythmic arrest pattern when water conditions are unsatisfactory is remarkably reminiscent of ciliary arrest behaviour in ascidians (Mackie *et al.* 1974). In both cases the behaviour can be exhibited by excised portions, and so pacemaker capability must be widespread. Spontaneous arrest sequences have also been seen in young specimens of *Rhabdocalyptus* attached to one another by parental tissue (figure 9). In these bud-colony preparations, if one sponge arrests, the other always does so as well, even though the atrial cavities are not confluent. When spontaneous arrests occur, sometimes one sponge leads, sometimes the other. These relationships can change during the course of a series of spontaneous arrests, again indicating the existence of dispersed pacemakers.
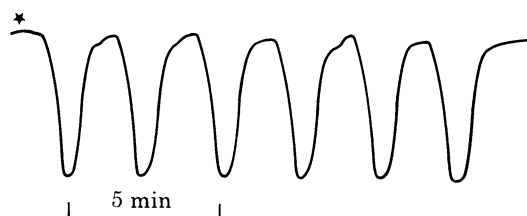

FIGURE 8. Chart recording of a series of spontaneous arrests exhibited by a whole sponge following an evoked arrest (the first).
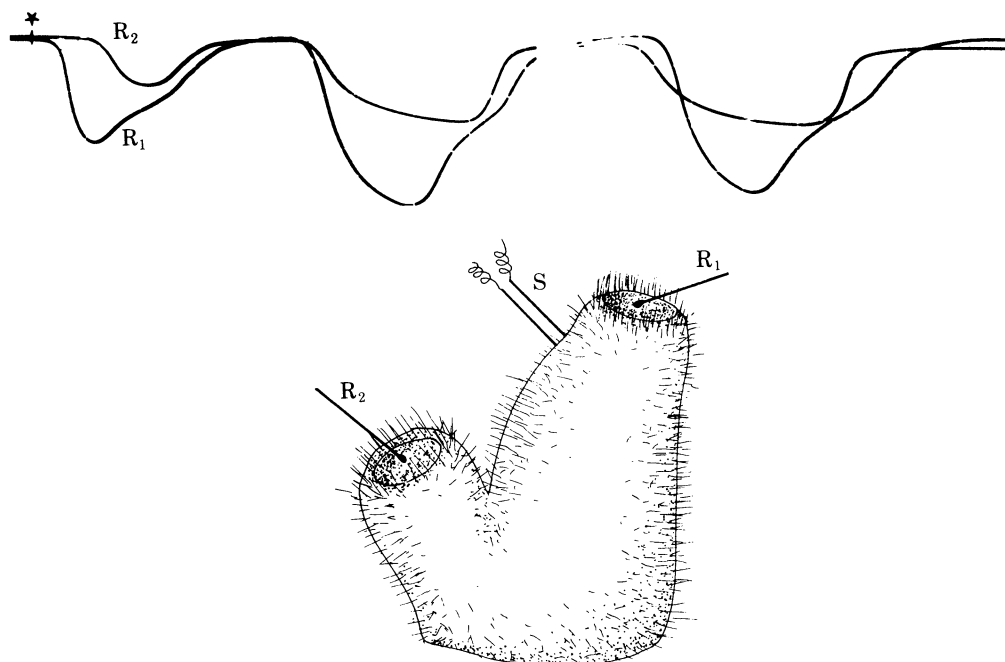

FIGURE 9. Evidence of dispersed pacemaker activity. An evoked arrest, followed by two spontaneous arrests, as recorded from two oscula in a bud-colony. Traces from the two recording probes are superimposed to show shift in onset time relations.

(b) Location and identity of conducting tissues

It is clear from the physiological experiments already described that conducting tissues are very widely distributed in the animal. All regions of the animal conduct, and conduction is unpolarized. Pieces of body wall incised with scissors to make narrower tongues of tissue conduct. Tongues only 1.5 mm wide conducted at normal velocity. Clearly, if the conduction system

were in the form of a network with meshes wider than 1.5 mm, conduction could not occur in such pieces. Pieces narrower than 1.5 mm failed to conduct but this may have been because excessive damage was caused in attempts to prepare them. The spicules tend to shatter when cut and lacerate the soft tissues. The thinner is the tongue, the worse is the damage.

Slitting the body wall horizontally into atrial and dermal halves gives two pieces that conduct, which shows that conduction is not regionalized to inner or outer layers. The best-localized specific conducting pathway has already been mentioned: the isolated dermal membrane.

Histological study of *Rhabdocalyptus* (Ijima 1904; Mackie & Singla 1983) has shown that the only tissue distributed uniformly throughout the whole animal is the general trabecular syncytium. This tissue is then, by default, the primary conducting tissue. In most regions it consists of a network of fine strands or loosely organized membranes which subdivide the internal waterways. It contains no nerves or nerve-like specializations. The dermal membrane is a specialized part of the general trabecular syncytium, consisting of a flat sheet with pores in it. We know that it conducts, albeit rather slowly. It is continuous with the deeper-lying trabecular tissue. Signals may be envisaged as propagating freely and in both directions between the dermal membrane and other parts of the trabecular syncytium. Extensions of the trabecular tissue penetrate the walls of the flagellated chambers, mingling with choanosome elements, including the flagellated collar bodies, the effectors presumed responsible for current arrest. Trabecular processes and collar bodies are interconnected by direct cytoplasmic connections. Intracellular plugs are lodged in some of these bridges. It can be assumed that the bridges can still function as communication pathways as no membrane barrier is involved, and the plugs contain visible pores. Signals would therefore be able to pass unimpeded between the trabecular conducting tissues and the choanosomal effectors.

(c) Possible conduction mechanisms

Various theoretical possibilities exist for transmission of signals and we have attempted to test these where possible. Conduction could be by a chemical, mechanical or electrical propagative process.

(i) Chemical mechanisms

It is conceivable that a chemical factor could be released by cells in the stimulated area and diffuse extracellularly causing arrest of the flagella. To be propagative, tissues would have to respond to the stimulating chemical by releasing more of it themselves, thus giving rise to a moving chemical wave. It is hard to see how such a process could work in a sponge as water flow occurs transversely across the body wall of the sponge. Any chemical produced would tend to be washed away sideways in the effluent currents. Two experiments were conducted to test the rather slim possibility that a diffusible metabolite was capable of causing arrests. In the first (figure 10a) oscular current from one sponge was directed upon the wall of a second sponge. Both had been tested for normal responsiveness. Arrests evoked in the first sponge failed to cause arrests in the second. In the second experiment (figure 10b), a piece of body wall that conducted normally was cut in half as delicately as possible and the two parts were left in close physical contact along the separation point. Arrests failed to spread from one half to the other.

Spread of a chemical intracellularly is scarcely a serious possibility. The fastest known intracellular transport process (fast axoplasmic transport) is three orders of magnitude slower (figure 11).

(ii) *Mechanical coordination*

It is conceivable that arrests could spread as a propagating mechanical wave across surfaces where the flagella were close enough together to affect each other's activity. This, however, could not account for the fact that conduction occurs in regions where there are no flagella, in particular the isolated dermal membrane. In any event it is very doubtful that densely flagellated surfaces extend continuously through the sponge. The flagellated chambers are clustered in systems around large excurrent water spaces and the tissues spanning the spaces between different systems are not flagellated. Finally, the experiment shown in figure 10*b* argues against flagellar interaction as a possible conduction mechanism.
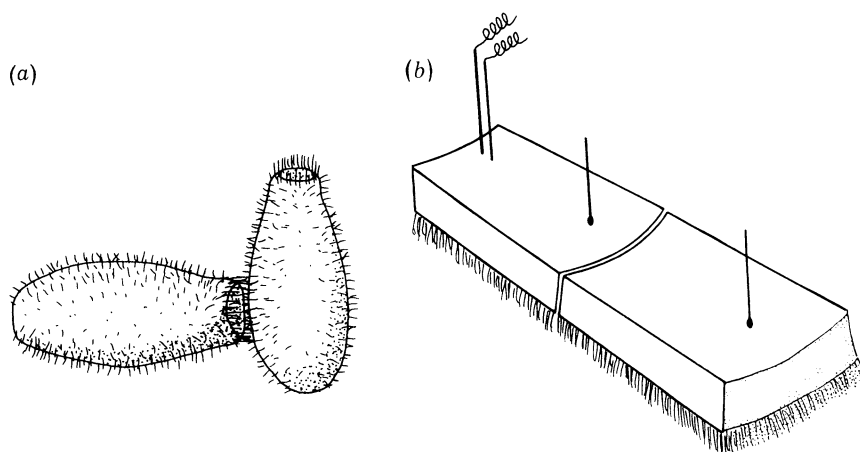

FIGURE 10. Experiments to test the possibility of a chemical signal transmission mechanism. In (*a*), effluent water from one sponge was directed toward the wall of an adjacent sponge while the first was caused to arrest. In (*b*), a slab preparation was cut in half and the two pieces were pushed closely up against one another.
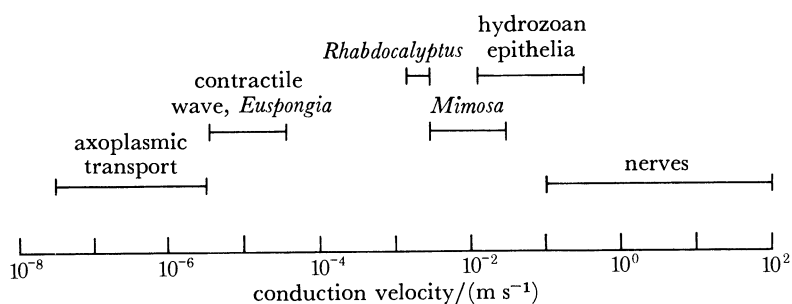

FIGURE 11. Conduction velocity ranges in various systems.

The possibility of a cytoplasmic contraction wave merits consideration, as propagated contractions have been reported in some other sponges (Pavans de Ceccatty 1969). Frame-by-frame analysis of moving picture sequences show waves passing at a velocity of 8–30 μm s^{-1} in *Euspongia*. The possibility that such a process is responsible for coordination in *Rhabdocalyptus* can safely be dismissed. First, conduction in *Rhabdocalyptus* is much too rapid. Secondly, hexactinellids show no evidence of contractility in the trabecular net. As already noted, no contractions are observed during arrests and there is no evidence of submicroscopic contractile filaments.

(iii) *Electrical impulses*

Action potentials are propagated not only in nerves and muscles but in some epithelia. First demonstrated in hydrozoans (Mackie 1965) and amphibian larvae (Roberts 1969), the pheno-menon of epithelial conduction is now known in some molluscs, tunicates and polychaetes (see review by Anderson 1980). Non-nervous conduction also occurs in plants (Sibaoka 1966). Conduction velocities vary greatly. The lowest values reported for hydrozoans and colonial ascidians are around 2.0 cm s^{-1}, ten times higher than in *Rhabdocalyptus*. However, in the sponge, impulses would have to pass through strands of the trabecular syncytium, which is organized in the form of an irregular network of slender filaments. Study of Ijima's drawings (Ijima 1904) suggests that the actual conduction distance through the trabecular net might be 1.5–2.0 times the distance as measured in a straight line, so that the true conduction velocity would be about 0.4–0.5 cm s^{-1}, that is to say, 25 % of the lowest coelenterate values, and equal to the lower range of values reported for plants like *Mimosa*.

Thus, the conduction velocity values for *Rhabdocalyptus*, though low, are not impossibly low for propagated action potentials, and the excitability properties of the preparation are such as would most readily be explicable in terms of an electrical signalling system.

Attempts to record electrically from *Rhabdocalyptus* have so far been unsuccessful. The various problems are discussed elsewhere (Mackie 1979). In brief, the trabecular net, as a presumed conducting tissue, might have been designed expressly to defeat the electrophysiologist. The thinness and fragility of the tissue strands, the shunting effect of the innumerable sea water spaces, the presence of spicules and the difficulty of visualizing what one is recording from are but the more obvious problems facing the investigator.

Despite persistent failure in attempts to demonstrate electrical activity in *Rhabdocalyptus* it seems extremely likely that action potentials are the signals responsible for coordination. There are no serious conceptual barriers to this explanation. The difficulties are technical, and will eventually be resolved by better techniques.

5. Discussion

The findings reported here go a long way toward confirming the conclusions reached in our previous papers: that *Rhabdocalyptus* (and presumably the other hexactinellids, for the group is a tightly knit one) are capable of propagative electrogenesis in their syncytial trabecular tissues, and that impulses are conducted by this system to the choanosome, entering the latter through cytoplasmic bridges and there causing the flagella which are responsible for produc-tion of the feeding current to stop beating. Other filter feeders, notably bivalve molluscs and ascidians, possess comparable ciliary arrest mechanisms in their gills (Aiello 1974). In these animals, the cilia constitute a major effector system, perhaps even more important in some ways than the muscles. In *Rhabdocalyptus*, the flagella are the *only* known effectors. If a hexacti-nellid is more than 'a mere living screen between the used half of the universe and the unused half' (Bidder 1923) the place to seek evidence of the animal's ability to regulate its environ-ment would be in the operation of these effectors, and here indeed we find such an ability to exist.

The biophysics of excitability, conduction and effector action in *Rhabdocalyptus* are speculative at the present time. We presume that a certain area of membrane must be depolarized for an

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impulse to be generated, and below that threshold no response, or only a local response, occurs. We assume that conduction involves passage of action potentials through the tissues via cytoplasmic bridges (as in plants); such bridges have been located in the appropriate places (Mackie & Singla 1983). We propose that flagellar inhibition is due to a change in the intracellular ionic environment of the choanosome as in the ciliary arrest or reversal responses of other animals and protozoa: Ca^{2+} ions would enter the cytoplasm when the cell membrane
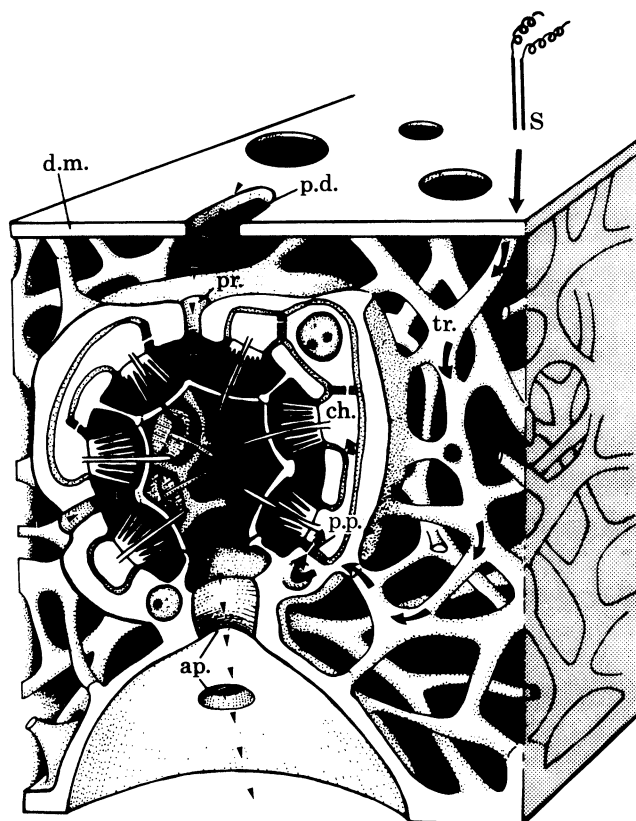

FIGURE 12. Water flow and impulse conduction pathways in the body wall of an idealized hexactinellid sponge. As shown by the arrowheads, water enters pores (p.d.) in the dermal membrane (d.m.), passes through incurrent lacunae in the trabecular net (tr.) and thence through prosopyles (pr.) into the flagellated chamber. Water leaves the chamber via apopyles (ap.), passing out into spacious excurrent lacunae which empty into the atrial cavity. Following electrical stimulation of the outer surface (S), impulses propagate through the dermal membrane and trabecular net as shown by the arrows. Entering trabecular tissues in the lining of the flagellated chamber, impulses then pass to the choanosyncytium (ch.) via pores in the plugged junctions (p.p., pore particle) and cause arrest of the flagella, thus halting water flow.

depolarizes and arrest would be due to elevation of the intracellular Ca^{2+} level. Active pumping would restore the transmembrane ionic balance and the flagella would start to beat again when this reached a certain point. In the ascidian *Corella*, the stigmatal cilia arrest in less than 1 s when the cell depolarizes. They stay motionless for a few seconds, then gradually straighten up and start beating again, feebly at first and then more strongly. The time course of these events has been closely studied (Mackie *et al.* 1974). The total time for an arrest varies over the range 11–22 s, with a mean of 15 s, measured up to the moment when full, metachronal beating is established. *Ciona* can maintain the cilia in a state of arrest for several minutes (Takahashi *et al.* 1973). These examples provide a conceptual model for what we think may

be happening in *Rhabdocalyptus* and it is quite possible that the responses of the sponge and the ascidian correspond very closely in terms of actual electro-ionic events. The time course of the recovery curve following an arrest in *Rhabdocalyptus* shows that pumping starts slowly and picks up gradually to full power. According to the ascidian model, the slow start would represent the initial, feeble beating of the flagella at a stage before the intracellular Ca^{2+} balance has been fully restored. The onset of arrest by contrast is abrupt, as would be predicted from the observations on ascidians. Confirmation of the validity of these suggestions must await development of techniques for observing the flagella directly during normal arrest sequences.

It would seem then that hexactinellids parallel ascidians in possessing a ciliary arrest mechanism coordinated by a through-conduction system located within the filtration organ. In the ascidian, nerves distribute arrest signals to scattered points in the gill epithelium, but from there on the spread of signals within the gill is thought to be non-nervous and to take place between the ciliated cells themselves, which are connected by gap junctions. Many stigmata are not innervated, but show arrests synchronously with those that are. In the hexactinellid, conduction is presumably non-nervous all the way, from receptor to effector, as in the protective involution response of some medusae, e.g. *Stomotoca* (Mackie 1975). Responses mediated by non-nervous conduction are typically associated with defensive, avoidance or escape behaviour of some sort. In *Rhabdocalyptus*, the arrest of the flagella probably helps to prevent entry of water containing excessive amounts of sediment. Any contact with the sponge tends to shake loose clouds of particulate matter lodged in the ectosomal spicule mass. The entry of such particles may indeed be one way in which the arrest response is triggered.

The evidence for behavioural responses in sponges other than hexactinellids is meagre. Oscular contractions occur in several groups, but the different oscula are not coordinated. Prosser *et al.* (1962) and Prosser (1967) found no evidence for propagation or electrical excitability, but propagation has been demonstrated by time lapse cinematography in *Euspongia* (Pavans de Ceccatty 1969), and the sponge *Tethya*, which shows generalized contractions, is responsive to electrical stimulation, and may propagate (Pavans de Ceccatty *et al.* 1960). There is at present insufficient experimental evidence to support speculation as to the mechanisms involved, but the extremely slow velocity of propagation (8–30 μm s^{-1} in *Euspongia*) make an electrical process seem most unlikely. Most sponges show no behaviour in the usual sense.

On the grounds of their lack of 'sentience', Linnaeus placed the sponges in the vegetable kingdom. Bidder (1937), while agreeing that the sponge is insensate, regarded it as 'an animal more perfect than man'. He added: 'That the sponge has no sensation follows from the fact that its cells are not protoplasmically connected'. This is still probably a fair statement as applied to the Calcarea and Demospongiae, where the cells do not appear to be interconnected by gap junctions. It would have seemed quite reasonable to Bidder that a syncytial sponge would be sensate, and capable of coordinating effectors throughout its body by signals propagating through the tissues via protoplasmic bridges.

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coordinates for the theoretical curve shown in figure 2b. We are also obliged to an unknown questioner in the audience at the Oxford meetings of the Society for Experimental Biology, December 1980, for suggesting the need for experiments similar to those illustrated in figure 10.

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