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THE REACTIONS OF SPONGES, WITH A CONSIDERATION OF THE ORIGIN OF THE NERVOUS SYSTEM

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WITH THREE FIGURES

I. INTRODUCTION

Previous attempts at the discovery of the nervous system of sponges have been made almost exclusively from an anatomical standpoint and with such negative results that Vosmaer and Pekelharing ('98, p. 18) believed themselves justified in declaring that the cells of sponges "are not connected in a way so as to enable them to conduct stimuli from one cell to another" and that these animals are therefore "destitue of the principle, the significance of which culminates in nervous tissue." It was the chief purpose of the investigations recorded in the present paper to ascertain whether there was any *physiological* ground for the assumption that sponges possess a nervous system, or whether from the standpoint of their activities, as well as of their structure, they showed no evidence of nervous organs. The general inertness of sponges has doubtless long deterred investigators from attempting a study of their reactions, and it must be confessed that even on close examination they show only a few form of inconspicuous response These few types of movement, however, are of considerable interest, for, as the following account will show, they throw considerable light not only on the question of the nervous system, in sponges but also on the still more fundamental problem of the origin of the nervous system in general.

The species on which my work was done was Stylotella heliophila Wilson, a monaxonid demosponge belonging to the order Helichondrina. This species will be described in a monograph on the sponges of Beaufort, N. C., soon to be published by Dr. H. V. Wilson, and I am indebted to Dr. Wilson for having called my attention to this sponge, which in all respects was extremely satisfactory for the work I had planned. My investigations were carried out in June and July at the Beaufort Laboratory of the United States Bureau of Fisheries, and I am under obligations to Commissioner G. M. Bowers for the privilege of working at this laboratory and to its director, Mr. H. D. Aller, for generous provision during my stay there.

2. STYLOTELLA UNDER NATURAL CONDITIONS

Stylotella heliophila is found in great abundance in the shallow water near the Beaufort Laboratory. It grows in masses about as large as a double fist and is attached to stones, oyster shells, and like materials. It is dirty orange-yellow or greenish yellow in color and, though sometimes simply massive in habit, it generally rises in finger-like processes from an incrusting base (Fig. 1). It is found near low-water level, and some colonies are so situated that at the spring tides they may be continuously exposed to the air for as long as four hours. As a rule the massive form is characteristic of those colonies which from time to time are exposed to the air; the long-fingered type is limited almost exclusively to such as are never uncovered by the sea even at the lowest tides. A Stylotella grows on the upper surfaces of stones, etc., in shallow water, it is often in strong sunlight for the greater part of the day and in fact when uncovered by the tide it may also be exposed to the extreme heat of the sun for hours at a time. That it not only survives under such conditions but seems even to court sunshine has doubtless given occasion to its specific name *heliophila*. The water in which it thrives is often deeply laden wuth sediment and this at times may shield it partly from the sun's rays, but when the water is clear or the sponge is exposed to the air, it receives

Fig. 1. Side view of a colony of Stylotella heliophila Wilson, about natural size. From a photograph taken by Dr. H. V. Wilson.

the full force of these rays yet without any apparently disastrous effects.

If a colony of Stylotella in natural position in quiet, clear seawater is closely examined, its numerous oscula, which occupy either the tips of the fingers or slignt elevations on the surface of its body, will be found as a rule to be widely open, so that an observer can look far down into the interior of the animal and see much of the branched gastral cavity and the excurrent canals leading into it. Although the fingers of the sponge are generally not much over seven to eight millimeters in diameter, the oscula may measure as much as four and a half millimeters in width when fully expanded thus giving a considerable view of the internal cavities. If such a colony is suddenly lifted out of the seawater into the air, the water rapidly drains from it and the air rushes through its oscula into its internal chambers. On returning such a sponge to the sea, the air thus introduced is with difficulty dislodged and may eventually as large bubbles distort and deform the sponge. Sponges that are exposed to the air on the beach by the natural fall of the tide show no such inclusions of air, and an examination of them in seawater brings to light the fact that their oscula are all firmly closed thus preventing the entrance of air. The steps of this closure can be easily followed by watching a sponge that is gradually becoming exposed to air when, in a quiet sea, the tide is falling. Under such circumstance the oscula remain open till they come into direct contact with the air when, with about three minutes, they close. If now the sponge colony is moved into deeper water, theoscula will reopen in from seven to ten minutes. If oscula at different levels on the same sponge are watched, those that come in contact with the air first, close first and those that are situated at a deeper level do not close until they in turn have been exposed to the air. These conditions are easily reproduced in the laboratory. Thus if a colony of fresh sponge is carried into the laboratory and placed in a glass vessel in which a current of seawater in kept running, on exposing the tip of any finger to air its osculum will close in a few minutes to reopen after it has been reimmersed in seawater for about ten minutes. On quickly removing a sponge from the sea the chimney-like membranes around the oscula very generally collapse showing that they are delicate structures. That the normal closure of the osculum is not a purely mechanical collapse of this kind is seen from the fact that when a sponge in which the closure has taken place by gradual exposure to air is returned to sea-water, its oscula do not flap open but can be seen to expand only gradually as by the relaxation of a sphincter. It is quite clear that the closure of the osculum is a definite response, which, among other things, prevents the entrance of air into the cavities of the sponge when, by a fall of the tide, the sponge becomes exposed to the air.

Another response which can be observed in Stylotella in its natural state is seen on comparing specimens that have been exposed for some time to the air on the beach with specimens still in the water. The latter as a rule have a plump appearance and a relatively smooth surface, whereas those that have been in the air look somewhat shriveled, and their surfaces are roughened as though their flesh had shrunken down on a rather resistant skeleton. At first sight it would seem that the sponge had shriveled simply because under action of gravity the water had been drained from it, but that this shriveling is probably not thus produced, but is dependent upon a positive contraction of the flesh of the sponge, is seen from the fact that the same shriveled, rugose appearance can be assumed by a sponge *in seawater* under conditions to be described later. These two reactions, the closing and opening of the oscula, and the shriveling and filling out of the common flesh of the body, are the most obvious natural responses exhibited by Stylotella.

3. STRUCTURE OF STYLOTELLA

Stylotella is an encrusting sponge that usually throws up longer or shorter fingers (Fig. 1.) These fingers, which represent the individuals in the colony, may attain a length of four centimeters and each one carries near its distal end usually one, sometimes two or more oscula. When fully expanded the oscula are roundish openings in a dome-shaped elevation or at the end of a more chimney-like projection. When contracted they are completely closed and the delicate tissue about them is puckered into a slight spine-like elevation, the point of which represents the real position of the osculum. The largest oscula when fully open measure, as already stated, about four and a half millimeters in diameter. From each osculum a branched gastral cavity extends through the substance of the sponge either down the length of a finger or into the massive body, depending upon the position of the osculum. In the fingered forms the gastral cavities lie either near the axis of the fingers, and are thus buried in the substance of the sponge, or on the surface of the finger, in which case they can be traced on the outside as translucent-walled canals well down the length of the finger. Excepting the regions where the gastral cavities show from the outside, the whole external surface of the sponge is faintly rugose and of a dirty-yellow color.

Fig. 2. Radial portion of a transverse section of Stylotella; the flesh of the sponge is tinted, the cavities are untinted; on the extreme left is the dermal membrane pierced by two ostia that lead into a large subdermal cavity, from which incurrent canals lead to the flagellated chambers, which in turn open by an excurrent canal into the gastral cavity at the extreme right. × 25.

The internal structure of the sponge is well seen in a transverse section of a finger. On the outside of such a section (Fig. 2) is a well defined membrane pierced in many places by dermal pores or ostia. These openings are roundish or oval in outline and, as seen in living bits of membrane torn from the outer surface of the sponge, they measure from ten to twenty micra in diameter. The ostia lead into relatively large sub-dermal cavities, which often form a definite layer around the whole finger diectly under the dermal membrane From these sub-dermal cavities pass off the incurrent canals, which lead centrally into the flagellated chambers. These chambers are usually spherical in form, measuring about twenty to forty micra in diameter and forming a more or less compact layer surrounding the gastral cavity; they connect with this cavity by short, irregularly branched, excurrent canals. Where the gastral cavity is close to the external surface of the sponge, there are apparently few or no ostia, sub-dermal cavities, or flagellated chambers, but the dermal membrane and the lining of the gastral cavity coalesce to form the translucent wall already mentioned. In the living condition of the sponge the layer of flagellated chambers is orange in color while the other parts of the animals are mostly dirty-yellow in tint.

I have not studied with any fulness the histology of stylotella but in good osmic-acid preparations cut into sections ten micra thick and stained in picrocarmine much of the cellular structure of this animal can be made out. The outer surface of Stylotella is covered with a dermal epithelium composed of polygonal cells that are usually extremely thin, though at places they show a condition approximating that of a cuboidal epithelium. The sub-dermal cavities, incurrent and excurrent canals, and gastral cavity are lined with a very flat epithelium, whose presence is difficult to demonstrate unless it is well preserved and cut at a favorable angle. The flagellated chambers are of course lined with a layer of relatively large choanocytes. In some places the dermal membrane seems to be made up of nothing but the dermal epithelium and the lining epithelium of the sub-dermal cavity and is therefore extremely thin. In most regions, however, it contains several other kinds of cells. Of these one set is represented by elongated, spindle-shaped cells, the so-called myocytes, and these are arranged like irregular sphincters around the ostia. They also surround abundantly the sub-dermal cavities, gastral cavity, osculum, etc. Structurally they have the appearance of a primitive kind of smooth muscle-fiber. In some places in my preparations they seem to lie directly on the exposed surfaces of the canals and cavities that they bound as though they were merely elongated epithelial cells, as in fact they are believed to be in many other sponges (Minchin, '00, p. 46; Schneider, '02, p. 260). Even admitting, however, that in Stylotella they are in all cases covered by an epithelium, the epithelium is certainly in most instances so extremely thin that these cells are almost in contact with the sea-water passing through the cavities that they surround.

In the region of the osculum the myocytes are especially numerous and form a conspicuous sphincter on the inner face of the oscular collar and internal to the mass of longitudinally arranged spicules which surround this opening. As a result the contraction of the osculum is accomplished without much folding of the surface of the sponge next the oscular cavity, for this surface is immediately in contact with the contractile material, if in fact it is not contractile itself. On the other hand the outer substance of the oscular membrane and the palisade of spicules are thrown into many folds in contraction as though they passively followed the constricting ring of myocytes to the outside of which they are attached (Fig. 3). This palisade-like arrangement of the rigid siliceous spicules is the only one that would allow an easy contraction and expansion of the osculum and it is in strong contrast with that of the spicules in the rest of the sponge, in which these bodies show no such grouping.

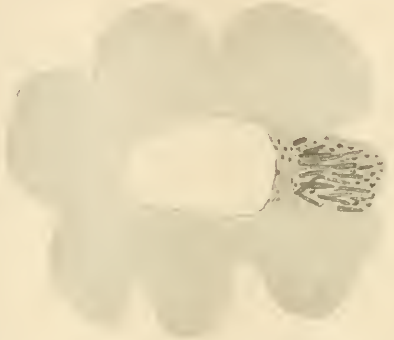

Fig. 3. Transverse section of the base of an oscular collar of Stylotella. The central cavity is the osculum, which, as is shown on the right is directly surrounded by a sphincter of myocytes, external to which is the tissue containing the spicules. × 35.

Although the larger apertures, including the osculum, possess well defined sphincters by which they are closed, I have never been able to find in Stylotella systems of radiating fibers by which they might be opened. Now and then I have seen what seemed to be slight radiating systems, but they were always associated with closed or partly closed openings and might perfectly well have owed their origin to the mechanical stretching of the elastic tissue in the neighborhood of a sphincter. I am inclined to believe therefore that the myocytic sphincters in Stylotella work against the general elasticity of the body tissue, which may have a slight radial arrangement in their neighborhood, rather than that they oppose a well defined system of radial myocytes. The absence of radial fibers in many sponges in which sphincters occur has been noted by Minchin ('00, p. 46).

4. REACTIONS OF STYLOTELLA

A. Movements of the Oscula

The opening and closing of the oscula in Stylotella, as already mentioned is the most obvious of the responses of this sponge. If a colony under ordinary conditions is examined, some of the oscula will almost certainly be found closed, though the majority will be widely open. If a small colony is closely inspected under a low power of the microscope, the open oscula will be seen to emit a large number of minute particles indicating that a current is setting out through these openings. In what seem to be closed oscula a minute but otherwise similar current can often be detected showing that they are really not closed. Some oscula, however, show absolutely no current, though I have invariably found that when in such cases the oscular tip was cut off, the current almost instantly could be seen, and I believe, therefore, that the oscula do close completely and thus check absolutely the current that ordinarily passes through them. In order to get some idea of the natural movements of the oscula, a vigorous colony of Stylotella was isolated and three of its oscula were kept under approximately hourly observation for three days. The results of these observations are summarized in Table I.

TABLE 1

Times in hours and minutes during which in the course of three days oscula 1,2, and 3 were open or closed

NUMBER OF THE OSCULUM	TIME IN HOURS AND MINUTES OF EACH SUCCESSIVE PERIOD OF OPEN OR CLOSED STATE								TOTAL TIMES IN 72 HOURS	
	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed
Osculum 1.........	0.45	2.00	19.05	3.20	20.15	7.50	2.35	16.10	42.40	29.20
Osculum 2.........	21.50	3.20	24.20	1.30	21.00				67.10	4.50
Osculum 3........	0.45	0.25	21.40	2.35	23.50	0.15	23.10		68.45	3.15

Since the three oscula whose conditions are recorded in Table 1 were on the same colony and near together and were exposed to almost identical surroundings, the fact that osculum 1 was closed on the average one hour in every two and a half, while oscula 2 and 3 were closed only one hour in every eighteen, must be attributed to the difference in constitution of osculum 1 as contrasted with that of the other two. The condition of general openness as exemplified by oscula 2 and 3 is doubtless typical for these organs. At least in any vigorous sponge under normal conditions, the majority of the oscula will be found open much of the time. When an osculum opens or closes, it does so in response to some stimulus. To ascertain what the effective stimuli are in this form of response, I have studied the oscular reaction in relation to mechanical and chemical stimulation and to heat and light.

a. Mechanical Stimulation

When at low tide a specimen of Stylotella was transferred from the shallow water of the outside to the laboratory tank, an operation that required about ten minutes, it was found that the animal that in the outside water had most of its oscula open usually had the majority of them closed when it had arrived in the laboratory, notwithstanding the fact that it had not once been exposed to the air during this transfer. At first it was suspected that this closure of the oscula was due to the disturbance caused in loosening the sponge from the bottom, etc., but it was found that this was not so, for, if a sponge after it has been dislodged, is left in the outside seawater, its oscula remain open. Change in illumination was also suspected of being the cause of the contraction. But if a sponge in its natural situation in full sunlight is suddenly shaded to an extent not unlike the diffuse daylight of the laboratory, the oscula still remain open. The reduction in the intensity of the light, then, is not the cause of the contraction. After this the effects of currents was tried, for, so far as could be judged, the chief difference between the condition of the sponge in its usual habitat and in the laboratory, aside from recent disturbance and illumination, was that in the first situation it was in moving seawater and in the second it was in the same water standing still. An aquarium with a free circulation of water was set up and sponges were placed in this in such situations that they caught the full effects of the current. The results were very uniform. Sponges from the exterior often arrived in the laboratory with many of their oscula closed. On putting these specimens into the aquarium under a strong current of seawater they almost invariably opened their oscula within ten minutes. The following record from my laboratory note-book will give a good idea of the character of these changes.

12:40 p.m. A sponge brought directly from the outside was placed in the aquarium with a strong circulation of seawater. Many of the oscula were closed.

12:45. Oscula began opening.

1:10. All oscula have been widely open for some time. Seawater current is now cut off.

1:12. Many oscula are closing.

1:14. Most oscula are closed. Seawater current is now turned on again.

1:18. Oscula have begun opening.

1:25. Most oscula are open.

1:39. Oscula remain open.

This and many other similar experiments pointed to the importance of currents in keeping the oscula open, but this form of experiment did not show what particular aspect of the current caused the osculum to open or to remain open. Did the sponge give out excretions which in quiet water gathered to such an extent in its immediate neighborhood as to cause its oscula to close and only on the removal of these by a current of water would the oscula open, or did the current carry oxygen to the sponge or act in a purely mechanical way to induce the opening of the oscula? To test these matters the following simple apparatus was constructed. Three cylindrical glass aquaria of considerable size were placed at three levels so that the water from the uppermost aquarium could be siphoned freely into the intermediate one from which the water overflowed into the third. Having filled the apparatus with seawater, it was possible to keep it running continuously with the *same* seawater by returning that which collected in the third or lowest aquarium to the uppermost one. If now the current of seawater carried away excretions from the sponge or brought oxygen to it and these operations had anything to do with the opening of the oscula, the use of the same water over and over again ought soon to bring on a condition that would no longer cause the oscula to open. But sponges placed in the current of the middle aquarium remained with their oscula open for hours in seawater that had been used many times over. Moreover the oscula closed quickly when the current was cut off and reopened soon after it was started again. I therefore believe that the mechanical stimulation of a current of water is an effective means of opening or keeping open the oscula Stylotella.

These first experiments were made on whole colonies of Stylotella and only the general condition of their oscula was recorded. I next turned to the individual sponges, the so-called fingers, to ascertain what parts of the finger must be exposed to the current to induce an opening of the osculum or the reverse. To test this question I placed a colony of Stylotella in a strong current of seawater and, when the oscula were well opened, I lowered a glass tube over a vertical finger so that the tube protected the whole length of the finger from the laterally impinging current but was at no place in contact with the finger. The water in this tube on examination was found to be for the most part quiet; its condition, however, did not interfere with the slight currents produced by the sponge itself. Although the osculum of the finger under examination was fully open when the tube was lowered over the finger, it closed in seven minutes after the tube was in position and remained so for a quarter of an hour. I now inserted a small tube into the upper end of the large tube and ran a gentle current of seawater down into the end of the large tube where the finger was situated. Thus the sponge was again in a current of seawater and in fourteen minutes its osculum was fully open. On cutting off this current, the osculum closed in six minutes. From these experiments it is quite evident that when no current of seawater impinges on a finger, its osculum closes and when such currents do strike the finger the osculum opens. It was noteworthy that during the time of these experiments the oscula in the immediate neighborhood of the one tested showed no changes in reference to those observed in the individual within the tube, but they remained for the most part persistently open in the general current of seawater.

The next question that naturally suggested itself was how much of a finger must be exposed to a current of seawater to induce the opening of its osculum. To test this, I placed the glass tube over the distal half of a finger leaving the proximal half exposed to the general current. I found, however, that the current eddied up into the tube and thus impinged on a part of the sponge supposed to be protected from it. To check this I inserted a small ring of cotton-wool between the free end of the tube and the sponge. Under these conditions the osculum closed in eight minutes even though the lower half of its finger was in a strong current of seawater. This form of experiment was repeated with only the distal fourth of the finger protected from the current, and again the osculum closed in seven minutes. Thus it is only necessary to have quiet water around the outermost fourth of a finger to cause its osculum to close, and a strong current on the proximal three-fourths of the finger will not induce the osculum to open.

I next reversed these experiments and attempted to ascertain how much of the distal tip of a finger must be exposed to a current to induce the opening of its osculum. In making these trials, a piece of light-weight brass-tubing was cut to such a length that when it was slipped down over a vertical finger of the sponge, it covered the finger all but the tip. The space between the oscular tip and the tube was filled with cotton-wool and the whole allowed to stand in quiet seawater. After the osculum had been closed for about a quarter of an hour, a gentle current was started across the end of the tube so that it impinged on only the oscular membrane. In three minutes the osculum showed signs of opening and in eight minutes it was fully open. This form of experiment was many times repeated with essentially similar results. It is therefore necessary for the current to impinge on only the oscular tip of the finger in order that the osculum shall open. The closing of the osculum in quiet water and its opening in a current of water are then both very local reactions and cannot be induced from points on the finger a quarter of its length (about half a centimeter) from the osculum.

If the oscula Stylotella close simply because the water in their immediate vicinity ceases to move and not in consequence of the acculumation of waste products or lack of oxygen, they probably close in the air on a falling tide because of the same mechanical conditions. If in the laboratory an inverted test-tube full of air is lowered over a finger whose osculum is open till the oscular membrane just comes in contact with the air, the osculum closes in about three minutes. The same result can be obtained when the test-tube contains washed hydrogen in place of air. Hence this reaction is not due to the oxygen of the air, but is very probably induced by a purely mechanical condition of quiescence into which the tip of the sponge finger passes in going from the water into the gas.

If an osculum opens to the mechanical stimulation of a current and closes in its absence, it is reasonable to suppose that it might respond to the stimulation produced by touching it with a bristle or stroking it with a fine brush, but my attempts in these directions were not conclusive. Touching or stroking an oscular membrane inside or outside when the osculum was open and in a current of seawater never resulted, as might have been expected, in a contraction of the osculum. Similar attempts on the outside of a closed osculum in quiet seawater occasionally resulted in a partial opening of the aperture, but these occurrences were so irregular and at such lengthy periods after the application of the stimulus that no reliance could be placed on them.

So far as my observations on mechanical stimulation go and they are full only in reference to currents, it is quite clear that an osculum closes quickly (in from about three to eight minutes) in quiet seawater or air, and opens more slowly (in from about seven to fourteen minutes) in a current of seawater. The fact that the oscular closure is quick and its opening relatively slow supports the view that I have already advocated from the standpoint of the structure of these parts, namely, that the sphincters are myocytic and work against the general elasticity of the surrounding tissues. Hence closure might be expected to be rapid and expansion relatively slow.

b. Injury

In making preparations of Stylotella for physiological tests it became quite apparent that the closing of the osculum was a common accompaniment of cutting the sponge. If a finger of Stylotella is cut off about a centimeter from the osculum, that aperture even in a current of seawater is likely to close within a short time and to remain closed for an hour or more. The occurrence of an oscular closure is much less likely, if the finger is cut off at two centimeters from the osculum than at one centimeter. If the cut is made at half a centimeter from the osculum that opening closes very quickly and may remain so for as much as a day.

If a finger instead of being cut off, is only cut into one on side, there is less likelihood of contraction than in the preceding cases. A finger cut into on one side at one centimeter from the osculum retained an open osculum, and the same was true when the cut was half a centimeter from the osculum. But when a cut was made three millimeters from the osculum, this aperture closed in nine minutes and remained so over a quarter of an hour.

If a pin is stuck into a finger of Stylotella, its influence on the osculum depends on the distance it is from that aperture. At one and a half centimeters no certain response was observed, but at half a centimeter the osculum closed in about ten minutes and remained so several hours, though it eventually opened. This observation is in accord with what Merejkowsky ('78, p. 13) found to be true of Rinalda; if the oscular edge of the sponge is struck several times with a needle, the osculum quickly contracts and remains so several minutes, after which it more slowly opens. The same is said by Merejkowsky (p. 14) to occur in Suberites.

As might be inferred from the statements already made, the injuries done to one finger of Stylotella have no influence on the condition of the oscula of neighboring fingers, nor do injuries inflicted on the common flesh of the colony between fingers influence the oscula of these fingers.

The nature of the stimulus produced by cutting the flesh of a sponge seems to be rather mechanical than otherwise. Such an injury besides mechanically disrupting tissues does little more than liberate juices from the substance of the sponge. These juices, however, when collected and discharged artificially and with great freedom in a normal sponge with open oscula, do not cause the oscula to close. I am therefore led to believe that the closing of the osculum on injury to an adjacent part of the sponge is due to the mechanical disrupting of tissues rather than to the effects of the juices that are liberated. If, however, the stimulus from the injury is chiefly mechanical, it results in a very different form of response from that due to currents, for the latter cause an opening of the osculum while the former induce its closure.

c. Chemical Stimulation

Since the oscular sphincter of Stylotella is made up of tissue that has a striking resemblance to smooth muscle, I tried the effects of a number of drugs on this sphincter to ascertain whether or not they influenced this organ as they did the smooth muscle of the higher animals. The drugs used were ether, chloroform, strychnine, cocaine, and atropin dissolved in seawater. This water was then used in the circulating apparatus already described (p. 12), so that sponges could be exposed to it in currents. I also tested in the same apparatus the effects of diluted seawater, of freshwater, and of seawater deprived of its oxygen by boiling.

When a sponge whose oscula were open in a current of pure seawater suddenly had this changed for a current of seawater containing a half per cent of ether, the oscula closed in from three to three and a half minutes. When in place of pure seawater, seawater containing a half per cent chloroform was used the oscula closed in a minute and a half to two minutes. The sponges treated with ether-water reopened their oscula in about two hours; those that had been subjected to chloroform-water did not reopen in less than four hours and some never recovered. Since both ether and chloroform-water induce a closure of the oscula, even when they are applied to the sponge in the form of a current, I regard these drugs as vigorous stimulants and of the two, chloroform is the more effective and, as in so many other cases, the more harmful.

When a current of seawater containing one part of strychnine to fifteen thousand parts of seawater was substituted for a current of pure seawater, the sponges closed their oscula in from eight to twelve minutes. Thus strychnine must be regarded as a stimulant to contraction.

Sponges whose oscula had remained open for some time in a current of seawater, were subjected to a current containing one part in a thousand of cocaine, whereupon their oscula closed in from seven to ten minutes. All such sponges reopened their oscula after having been in a current of pure seawater for about half an hour. Since the sponges closed even in a current, cocaine at the strength used must be regarded as a vigorous stimulant to closure.

In a solution of one part of cocaine in ten thousand parts of seawater, the oscula remained open as in pure seawater, but, as the following observations show, this drug was not without its effect. A particular osculum was found on several trials in pure seawater to close in from four to five minutes after the current had ceased. On subjecting this osculum for some fifteen minutes to a current of cocaine in seawater, one to ten thousand, it was found that on the cessation of the current the osculum closed in from eight to nine minutes. After an hour in a current of pure seawater the rate of four to five minutes was reëstablished. A weak solution of cocaine, then, inhibits slightly the closure of the osculum.

A cocaine solution of one part in fifty thousand of seawater could not be distinguished in its action on the osculum from pure seawater.

A solution of one part of atropin to one thousand parts of seawater checked the rapidity of oscular contraction much as the stronger of the two effective solutions of cocaine did, and a solution of one part of atropin in ten thousand parts of seawater could not be distinguished from pure seawater.

Although the observations on the actions of these various drugs as given in the preceding paragraphs are insufficient to admit of any detailed analysis, the results are in agreement with what is known of the action of these materials on smooth muscle. To this type of muscular tissue chloroform is more destructive than ether, strychnine renders it especially contractile, and cocaine and atropin inhibit this property somewhat (Grötzner, '04, p. 65). This evidence, therefore, supports the view that the sphincter myocytes of sponges are in the nature of primitive smooth muscle fibers.

The effects of dilute seawater and of freshwater itself on the oscular mechanism were tried in the circulating apparatus. If a sponge whose oscula have been open in a current of seawater for over an hour is flooded with a current of water composed of one-fourth fresh water and three-fourths sea-water, the majority of the oscula contract somewhat in twenty minutes, after which they remain partly open. In a mixture of half freshwater and half seawater the oscula contract but do not close completely. In three-fourths freshwater and one-fourth seawater, the oscula contract in about seven minutes but do not close. In pure freshwater, they remain expanded as though dead, but even after having been twenty-four minutes in fresh water, such sponges will revive in running seawater, though their oscular collars are seriously damaged and are regenerated only after several days. As Stylotella inhabits the shallow waters near the shore, it must often be subjected after heavy rains to the effects of diluted seawater, but, as the observations recorded above indicate, it would not be seriously damaged by these changes and would probably protect itself against them by oscular constriction.

To prepare seawater free from oxygen a large volume was boiled vigorously for some time to discharge the contained gas and after this had been accomplished the water was set aside in a tightly stoppered vessel to cool. Sponges in a current of normal seawater and with open oscula were suddenly subjected to a current of seawater thus deoxygenated drawn with as little exposure to the atmosphere as possible from the storage vessel. Their oscula closed in from ten to twelve minutes. On returning them to a current of ordinary seawater, they reopened their oscula in from fifteen to twenty-five minutes. Lack of oxygen will therefore cause the oscula to close.

d. Heat and Cold

The seawater in which Stylotella was found living in the neighborhood of the laboratory in June and July had a temperature of about 25° to 28° C. In a current of this water the oscula of Stylotella will often remain open many hours together. If the temperature of the current was changed to about 35° C., the oscula often constricted slightly, and the same was true at 40° C. At 45° C. the oscula in five or six minutes went into a state of flabby contraction, and if this temperature was maintained for a considerable time much of the sponge died. At temperatures lower than the normal, from 25° to 9° C., the oscula remained open and outward currents could be demonstrated. Thus low temperatures were apparently without effect on the oscula and high temperatures called forth a partial contraction.

e. Light

Sudden changes from the most intense sunlight to the most complete darkness were not followed by any observable movement of the oscula in Stylotella, which in this respect follows the general statement made by Minchin ('00, p. 89), that adult sponges are not sensitive to light.

B. Movements of the Dermal Pores or Ostia

The movements of the dermal pores or ostia in Stylotella were not so easily demonstrated as those of the oscula were. The small size of the ostia makes a direct determination of their condition almost impossible and consequently the presence or absence of a current of water through them was taken as an indication of their state. The demonstration of this current has been accomplished from the earliest times (Carter, '56, Lieberkühn, '56, Bowerbank, '58) by the addition to the water of some such substances as carmine, starch, or indigo, whose particles could then be followed as they were carried in the moving water. Latterly this method has been severely criticised by von Lendenfeld ('89, p. 592), who claims that even these small suspended particles mechanically stimulate the sponge and cause it to close its ostia. Von Lendenfeld has used milk as an indicator and has found no objection to it. With Stylotella it is easy to demonstrate the ostial currents with carmine, etc., and so far as I could discern this material could be used without causing partial closure of these apertures. In fact I must agree with Bidder ('96, p. 32) that the carmine particles seemed to have no effect whatever on the ostia, but were swept into the interior of the sponge with great freedom for hours at a time. It must, however, be confessed that not only carmine but even milk is an unnatural substance for a sponge and as Stylotella lives in water that ordinarily contains much fine suspended material, I found it necessary only to watch this substance to gain all the information that was needed as to the direction of ostial currents, their strength, etc.

In testing the ostia I usually pinned a finger of sponge under the microscope in a small glass aquarium so arranged that a continuous current of seawater could be kept running through it, and by watching the suspended particles along the sides of such a preparation under a magnification of about ninety diameters, it was comparatively easy to ascertain whether the ostial currents were running or not. As a rule the objective of the microscope was used as an immersion lens and plunged under the surface of the seawater. In making these observations it was, however, necessary for the time being to stop the current of seawater that was running through the small reservoir, otherwise the movement of the suspended particles over the surface of the sponge was so rapid that it was impossible to tell whether they entered an ostium or glided by it. When this current was shut off the osculum often closed and under such circumstances, as might have been expected, the ostial currents ceased. To be certain that the cessation of these currents was due to the closure of the outlet, I cut off a closed osculum and found that the ostial current almost immediately began again. Moreover when I ligated the cut oscular end of a finger on which ostial currents could be easily seen these currents ceased at once and on the removal of the ligature the currents recommenced. From these observations it is quite clear that the osculum controls in a purely mechanical way the current within the sponge. When the osculum is open this current may run; when it is closed the current ceases even though the ostia are open and the choanocytes continue to beat. In view of these facts I regularly removed the oscular ends from fingers of Stylotella on which I wished to test the ostia.

Although the presence of an ostial current is conclusive evidence of the openness of the ostia, its absence is not proof that the ostia are closed even supposing that the oscular end is cut off, for it is conceivable that the choanocytes may cease to beat, in which case the cessation of the current would be misleading as to the condition of the ostia. For some time I was puzzled as to a means of meeting this difficulty, but a simple method finally suggested itself and was adopted. If the oscular end of a finger of Stylotella is cut off at some distance from the osculum, the cut face includes not only the gastral cavity and some of the flagellated chambers, but also the sub-dermal cavities. An examination of the currents from such a cut end will show a large, slow, central current emerging from the gastral cavity and a considerable number of smaller more rapid currents entering the surrounding sub-dermal cavities. These cavities form a set of intercommunicating spaces over the whole surface of the sponge, and the currents that set into them at the cut end depend purely upon the action of the choanocytes. If, now, no inward currents can be detected at the ostia but currents can still be seen to enter the sub-dermal cavities at their cut ends, it is clear that the absence of lateral currents is due to the closure of the ostia and not to the cessation of the choanacytes. In this way, then, I used the presence of ostial currents to indicat that the ostia were open and their absence, when coupled with the presence of sub-dermal currents, to indicate that these apertures were closed. Most of the tests that were carried out on the oscula were repeated on the ostia and the results will be stated briefly in the following paragraphs.

a. Mechanical Stimulation

A finger of Stylotella from which the oscular end has been cut off may be kept a long time in running seawater in apparently normal condition. When the current of seawater is temporarily shut off, small suspended particles can be seen to drift slowly up to the surface of the finger and disappear by suddenly darting into the ostia. In this way the ostia can be demonstrated to be open. Many particles are too large to enter these apertures and they will accumulate on the surface of the animal in quiet water. When, however, the general current is set going again, it sweeps these larger particles away and leaves the surface of the sponge relatively clean. If after the ostia have been demonstrated to remain open for some time in running seawater, this current is permanently shut off so as to leave the sponge in quiet water, the ostia may continue open for many hours during which the surface of the sponge often becomes deeply buried under an accumulation of particles most of which are too large to enter the ostia. In no case has a cessation of the seawater current been followed by a closure of the ostia as with the oscula. The ostia, then, differ from the oscula in that they remain open in both quiet and circulating seawater.

Prepared fingers of Stylotella in which strong sub-dermal and oscular currents could be seen but no ostial currents were persent, were kept in some instances in running seawater, in others in quiet seawater without, however, yielding any evidence to show that either state of the seawater caused the opening of the ostia. Flowing seawater and quiet seawater both seem to have no effect on the opening or closing of the ostia in Stylotella.

Even when Stylotella is covered with a deep layer of silt, its ostia can often be demonstrated to be open. Under ordinary circumstances, however, it is not usual to find this sponge thus covered. Its natural habitat is in a current of seawater and though this water may often be heavily loaded with suspended matter, its current seems to be sufficient, as already noted, to remove many particles which, from their size, accumulate on the surface of the sponge. But this is not the only means for the removal of these larger particles. A close inspection of the outer surface of Stylotella will show that it is regularly inhabited by several animals; chief among these are young ophiurans, caprellas, and a species of copepod. All these animals, and especially the copepods, keep up an incessant movement over the surface and loosen and dislodge much of the accumulated drift. The copepods and probably the other forms find much to feed on in this omnium-gatherum, and their relation to the Stylotella seems to be of a symbiotic character. These organisms together with sea currents are responsible for the generally clear character of the surface of the sponge. But even when this sediment is abundantly present on Stylotella its ostia remain open. I have also failed to find any evidence in favor of the view that when the ostia are closed the accumulation of silt on the surface of the sponge will cause them to open.

Exposure to air likewise seems to have no effect on the ostia. A finger of Stylotella on which the ostia were freely open was exposed to air for about a quarter of an hour. Upon reimmersing it in seawater the ostial current could be seen at once. It was again put in the air, this time for three-quarters of an hour, whereupon it was reimmersed and the ostia again gave evidence of being freely open though the finger as a whole had the shriveled appearance characteristic of sponges that have been exposed sometime to the air.

Stroking the surface of Stylotella seems neither to bring about a closure nor an opening of the ostia. In this respect they are as irresponsive as the oscula.

So far as mechanical stimulation is concerned, the ostia are very unlike the oscula. The oscula are responsive to water currents and their absence and the mechanical effects of exposure to the air; the ostia are uninfluenced by any of these changes and are apparently also undisturbed by sediment.

b. Injury

The great majority of fingers cut from Stylotella, if put directly under the microscope, show no ostial currents. As a rule these currents begin to appear in from ten to fifteen minutes after the finger has been cut off. When a finger that has established its ostial currents is cut in two, these currents often cease in the two parts though sub-dermal and oscular currents can be easily demonstrated. After a quarter of an hour the ostial currents can usually be seen again in such pieces. Not all specimens show these conditions, but they are of common enough occurrence to justify the conclusion that a considerable incision in Stylotella produces in its neighborhood a temporary closure of the ostia. In this respect the ostia resemble the oscula.

c. Chemical Stimulation

To seawater containing ether ($\frac{1}{2}$ per cent) or chloroform ($\frac{1}{2}$ per cent) the ostia closed even more quickly than the oscula did, and on fingers whose ostia were closed, the presence of ether or chloroform in the surrounding water did not induce these apertures to open. Strychnine, one part in fifteen thousand of seawater, was followed by a gradual closing of the ostia, a condition already observed by von Lendenfeld ('89, p. 608) in other sponges. To one part of cocaine in a thousand parts of seawater open ostia closed in about ten minutes and closed ostia remained closed. To one part of cocaine in ten-thousand parts of seawater the ostia remained open or if closed in the beginning, they open in about eight minutes. Of this drug von Lendenfeld ('89, p. 640) states that the stronger solutions cause a contraction of the ostia, which is true, and that the weaker solutions leave these apertures unchanged, which is probably not wholly correct, for they inhibit to some extent the contractibility of the sphincters. To atropin, one part in a thousand of seawater, the open ostia remained open and the closed ones opened in about nine minutes. Thus atropin probably also inhibits the action of the sphincter. As the actions of these various drugs on the ostial sphincters is very similar to their action on smooth muscle, it is probable that the ostial myocytes, like those of the osculum, are cells not inappropriately described as primitive smooth muscle-fibers.

In water composed of three-quarters seawater to one-quarter freshwater, the ostia remained open, and a strong ostial current could be seen at the end of twenty minutes. When the ostia were closed the effect of this mixture was to induce an opening of the ostia in about a quarter of an hour, after which a strong ostial current continued to flow. To mixtures of half seawater and half freshwater oscular, and sub-dermal currents as well as ostial currents ceased in about ten to twelve minutes, showing that though the ostia probably remained open, the currents ceased because of the collapse of the choanocytes. Closed ostia in this mixture opened slightly and then all currents ceased in from nine to ten minutes. In fresh water all currents ceased immediately. These mixtures of seawater and freshwater so far as their effects can be seen, influenced the ostia much as they did the oscula, in that they induce a partial but imperfect contraction.

In seawater rendered free from oxygen by boiling and subsequently cooled to 28° C., the ostia remained open or, if closed, they opened in from seven to ten minutes. This reaction was precisely the reverse of that of the osculum and to make close comparisons of the two I prepared several fingers of Stylotella by cutting them off rather short at the proximal ends thus permanently opening the gastral cavity and by leaving the oscular end intact. These preparations were placed one after another under the microscope in pure running seawater and after the oscula were freely open the current of pure seawater was changed for one of deoxygenated seawater. Under these conditions all the oscula closed in about ten minutes, if not completely at least nearly so, and the ostia remained open, their currents now discharging chiefly through the cut end of the gastral cavity. Deoxygenated water, then, is a means of closing the oscula and opening or leaving open the ostia.

To seawater containing juice expressed from an oyster, either fresh or foul, the open ostia remained open and their currents seemed at times to increase. I was never able to demonstrate with certainty that to these materials the closed ostia would open, though occasionally they seemed to. Like the oscula, the ostia were indifferent to seawater containing juice from the body of Stylotella itself.

d. Heat and Cold

Prepared fingers of Stylotella in which the ostial currents were running vigorously continued to exhibit these currents after the temperature of the seawater had been changed from 28° C. to 36° C., and fingers in which the ostia were closed at 28° C. opened them after the sponge had been a few minutes in water at 35° C. At 40° C. all currents, sub-dermal and oscular as well as ostial, became rapidly feeble and then stopped, and at 45° C. these currents ceased abruptly as though the heat had caused the choanocytes to stop beating. This view is supported by the fact that few fingers of Stylotella ever recovered after having been subjected to seawater at 45° C. for any length of time. Cold water at 9.5° C. caused all currents to run more slowly, but did not bring about a closure of the ostia. In fingers in which the ostia were closed these organs did not open after having been a quarter of an hour in seawater at 9° C. In these specimens the subdermal and oscular currents became sluggish on reducing the temperature of the water, hence the effect of the low temperature was probably chiefly on the choanocytes.

e. Light

As in the case of the osculum, I have observed no effect from intense sunlight or shadow on the opening or closing of the ostia.

C. Movememts of the Body as a Whole

Aristotle in the fourteenth chapter of his fifth book on the history of animals makes the interesting statement that the sponge is supposed to possess sensation because it contracts if it perceives any movement to tear it up and it does the same when the winds and waves are so violent that they might loosen it from its attachment. He further adds in his characteristic way that the natives of Torona dispute this. The idea that the common flesh of the sponge is contractile is not without modern support. Merejkowsky ('78, p. 14) states that if Suberites is so placed that it is partly out of water, it will curve the body until it is under water as much as possible, and if the body is then covered with water, it will return to its former position.

It must be evident, from what has already been stated, that much of the common flesh of Stylotella is contractile. As already noted, specimens our of water quickly assume a shriveled and rugose appearance as though the flesh had contracted on a resistant skeleton, a condition which it also quickly assumes in quiet seawater. Moreover, if a sponge is placed partly in running seawater and partly in the air, the portion in the seawater remains smooth and that in the air becomes rugose. Specimens made rugose either in the air or in quiet water soon recover their smooth appearance on being placed in running water. Air or quiet water may then cause a contraction of the common flesh of Stylotella, a condition counteracted by running water.

The contraction of the common flesh can also be seen well around some of the larger cavities, such as the gastral cavity. If a long finger of Stylotella whose two ends have been cut off and whose gastral cavity extends along one of its sides is placed in quiet seawater, the gastral cavity is soon indicated by an external groove due to the apparent collapse of its wall. This groove, however, is caused not by collapse, but by the contraction of the common flesh which as partial partitions or even travecula is abundant about the sides of the gastral cavity. On returning the finger to running water the flesh relaxes and the groove mostly disappears.

Although the common flesh of Stylotella is unquestionably contractile, I have never observed that the body of this sponge as a whole moves in consequence of this contractility. Thus in no instance have I seen a partly immersed finger of Stylotella bend farther into the water, though I have let fingers stand in a position favorable for this for over a day. Nor have I ever observed fingers to turn in conformity to the direction of the current. Thus some fingers of Stylotella are not directed straight upward, but have their tips turned to one side or the other, so that the oscula open laterally. A number of these were set, some with oscula facing the current, some with these openings away from the current, and others sidewise to the current. After three days none of these had materially changed their directions, thus giving no evidence of a general movement of the body.

I also attempted to get evidence of the general movement of the body through geotropic responses. Stylotella ordinarily grows with its fingers and oscula directed upwards, as though it was negatively geotropic. A large colony was, therefore, kept inverted in an aquarium of circulating seawater for about a week on the assumption that the fingers might turn from this unusual position, but at the end of this period there was no apparent change of position. This observation, however, does not prove that Stylotella is not geotropic. Slight evidence of geotropism is to be found in its method of regenerating oscula. When a moderately long finger of Stylotella is cut off and the whole of its oscular end, removed, the cylindrical body thus resulting will under favorable conditions form a new osculum. Whether tnis regeneration will take place at the end nearer or farther from the former osculum seems to depend chiefly on the position of the piece of sponge in reference to gravity. If the end that was nearer the former osculum is uppermost, it always regenerates the new osculum; if it is down, the opposite end very generally regenerates the new organ. Thus in the regeneration of the osculum Stylotella shows some slight geotropic activity, and while it must be admitted that the common flesh of this sponge is contractile, this contractility does not seem to result in movements of the body as a whole such as might be looked for in geotropic and other like responses. It is possible that in this sponge the skeleton, which is well developed, is too resistant to allow the body as a whole to be bent, and that, therefore, the contractility of the common flesh can make itself manifest only in the local ways already mentioned.

D. Currents

The currents of sponges, which were supposed by many older naturalists to reverse in their direction from time to time and to depend upon a systole and diastole of the body of the sponge, have been generally acknowledged since the time of Grant ('25, '26, '27) to be uniform in their direction and to depend upon the action of cilia-like organs. Some years ago Miklucho-Maclay ('68) and Haeckel ('72) maintained that a reversal of the current could occur, but more recent observers have not confirmed this statement. In the thousands of living individuals of Stylotella that I have examined I have never seen an exception to the rule that water enters the ostia and sub-dermal cavities, when open, and makes its exit through the osculum. Moreover I have never found a living specimen of Stylotella in which currents could not be demonstrated. Even in those in which the ostia and oscula were closed and no external evidence of currents could be seen, the cutting off of the oscular end and the consequent exposure of the gastral and sub-dermal cavities always was followed by the appearance of characteristic currents. It seems to me probable that under nurmal conditions the choanocytes beat incessantly in Stylotella. The currents produced by them would then be controlled by the opening and closing of the ostia and the oscula. It must be borne in mind, however, that a continuous current does not necessarily mean that all flagellated chambers are continuously at work. Some may cease from time to time without causing the general current to cease. All that the presence of a continuous current really proves is that all flagellated chambers are not inactive at once. It would not be surprising to me, however, to find, if evidence could be obtained, that the action ofthe choanocytes is uninterrupted. The fact that a current could always be demonstrated in all fingers of Stylotella by cutting off the oscular ends leads to the conclusion that, aside from the ostia and the oscula, there is no other complete check on the current such as prosopylic or apopylic sphincters, etc.

If the ostial and oscular sphincters are the organs of control for the currents in a sponge, they must be strong enough to resist the pressure produced by these currents, and when these apertures are closed the tissues of the sponge must also withstand a certain strain produced by the working of the choanocytes. Doubt has been expressed by some writers as to the ability of the body of a sponge to meet these mechanical requirements, but as no one, so far as I am aware, has ever attempted to measure the pressures involved, it seems useless to urge such objections till actual measurement has been accomplished. It is comparatively easy to determine the pressure produced by the activity of the choanocytes of such a sponge as Stylotella. To make this measurement the following simple device was employed. A glass tube of about five millimeters bore was drawn out at one end to a diameter of about one millimeter, and fixed vertically at such a height that its pointed end was well under the surface of the sea-water in an aquarium. The water of course rose in this tube to the level of that in the aquarium. A long finger of Stylotella in which the currents were running well was ligated at its cut end so that no water could escape from this end, and the osculum was fitted over the small end of the glass tube and firmly tied there. The sponge continued to pump water in through the ostia, and this water naturally rose in the glass tube until the pressure of the column of water in the tube just neutralized the strength of current produced by the choanocytes. This position having been read on a scale attached to the glass tube, the finger of the sponge was then carefully cut off from the tube, whereupon the water fell in the tube almost to the level of that in the aquarium, the difference being due to the capillarity of the tube. This new level was then read and the difference between the two levels was taken to represent the pressure exerted by the current produced by the sponge. Ten such trials were made and in all cases the readings fell between 3.5 mm. and 4 mm. The current produced by Stylotella, then, has a maximum pressure equivalent to a column of water between 3.5 and 4 mm. high. This slight pressure is what must be resisted by the tissues, and particularly the sphincters of Stylotella when in a closed condition of the sponge the choanocytes continue to beat. To ascertain what the resistance of the sphincters was, I subjected them to a simple test. A finger of Stylotella in which the ostia were closed was tied as before to the small end of a glass tube which was bent in the form of a siphon and was so placed that the end carrying the sponge was in one vessel of water and the other end, quite free, was in another vessel of water. The water in these two vessels was kept at the same level. After the whole apparatus was set up the water in which the sponge rested was deeply colored with methyl green. The vessel with uncolored water was then lowered till the difference in level between the water contained in it and in the other vessel was sufficient to break through the ostial openings, a state of affairs that could be recognized by the passage of the deep bluish green water up one arm of the siphon. The difference in level was then measured and in the eight trials that were made it was found to be between ten and fifteen millimeters. Thus the actual amount of resistance in the closed ostia is much more than is necessary to hold in check a current whose maximum suction is represented by a pressure of not over four millimeters of water. I also attempted to get the resistance of a closed osculum. Oscular tips were tied to the small end of the siphon tube, which in this instance was made to carry colored water, and by raising the reservoir on the colored-water side a pressure was sought at which the osculum would open and discharge colored water. But my experiments failed mostly because of leakage, probably through the ostia near the osculum. They went far enough, however, to assure me that the resistance of the osculum was higher than that of the ostia. From these observations it is quite evident that the currents produced by the choanocytes of Stylotella are of such a strength that they can be readily held in check by the ostia and the oscula, and that there is no mechanical ground for suspecting that these currents could in any physical way endanger even the delicate structure of the sponge.

The experiments with various stimuli had in many cases little or no observable influence on the currents produced by the choanocytes. The mechanical stimulation of the exterior of the sponge had no effect on the current. In ether- and chloroform-water all currents ceased, as might be expected from the well-known inhibitory action of these drugs on cilia, etc. Strychnine apparently increased the vigor of the current, whereas cocaine and atropin seemed to have no effect upon it. Dilute seawater and fresh water brought the current quickly to a standstill. Lack of oxygen first accelerated and then retarded it. Cold caused it to become slow, and excessive heat brought it to a standstill.

Light, as might have been expected, did not alter it. So far as these various stimuli change the current at all, they do so as one would expect of them supposing that they acted directly on the choanocytes and not through any intermediate structure. Nevertheless it cannot be said from the evidence presented that the complete cessation of the current, as for instance in fresh water, may not be due to the contraction of sphincters other than the ostia and oscula rather than to direct action of the choanocytes.

E. Coördination of Reactions

A comparison of the reactions of the oscula, ostia, and choanocytes of Stylotella, as described in the preceding sections, can best be made through a summary such as is contained in Table 2.

The most marked feature brought out in Table 2 is the very striking independence of the several reactive organs. Thus the activity of the choanocytes, as indicated by the current they produce, is apparently quite independent of that of the oscula or the ostia and the only stimuli that have any effect on these cilia-like organs are such as would be expected to influence them directly. The oscula and ostia both possess sphincters that from a histological standpoint are much alike in that they probably are a primitive form of smooth muscle, a view supported by their reactions to drugs, and yet they are influenced in totally different ways by several stimuli, especially of a mechanical kind. This is so striking that under natural conditions one of these sets of apertures may be found open when the other is closed. The contraction of the common flesh is also quite independent of the ostia, for, though these apertures are in a measure imbedded in this flesh, the latter may be contracted when the ostia are open. The contraction of the flesh and the closure of the oscula always take place together when the sponge is in quiet water or exposed to the air, but that this is probably a coincidence rather than evidence of a real physiological interdependence is seen from the fact that the oscula close in ether- and chloroform-water, though the common flesh does not contract in these media. Thus the various motor elements in Stylotella seem to be as independent of one another as the several parts of a single animal can well be.

TABLE 2

Summary of the Reactions of the Oscula, Ostia, and Choanocytes of Stylotella to Various Stimuli

STIMULI	REACTION BY		
	Osculum	Ostium	Choanocyte
Mechanical Stimuli			
Seawater currents	Opens and remains open	No reaction	No reaction
Quiet seawater	Closes and remains closed	No reaction	No reaction
Brushing	No reaction?	No reaction	No reaction
Silt		No reaction	No reaction
Exposure to air	Closes and remains closed	No reaction	No reaction
Injury	Closes	Closes	No reaction
Chemical Stimuli			
0.5 per cent ether	Closes and remains closed	Closes quickly and remains closed	Currents soon cease
0.5 per cent chloroform	Closes and remains closed	Closes quickly and remains closed	Currents soon cease
1/15,000 strychnine	Closes and remains closed	Gradually closes	Currents strong
1/1,000 cocaine	Closes and remains closed	Closes and remains closed	No reaction
1/10,000 cocaine	Remains open; closure inhibited	Remains open or if closed, soon opens	No reaction
1/50,000 cocaine	Remains open		No reaction
1/1,000 atropine	Remains open; closure inhibited	Remains open or if closed, soon opens	No reaction
1/10,000 atropine	Remains open		No reaction
3/4 seawater + 1/4 freshwater	Closes slightly, then reopens	Remains open, or if closed, opens	No reaction
1/2 seawater + 1/2 freshwater	Contracts but does not close	Probably remains open; if closed, opens slighty	Currents soon cease
1/4 seawater + 3/4 freshwater	Contracts but does not close		
Freshwater	Remains open but inactive	?	Currents cease
Deoxygenated water	Closes	Opens and remains open	Currents strong, then cease
Thermal Stimuli			
9° to 10° C	No reaction	No reaction	Currents become slow
25° to 28° C	Normal	Normal	Normal
35° C	Slight constriction	Remains open or if closed opens	Normal
40° C	Slight constriction	?	Currents cease
45° C	Flabby contraction	?	Currents cease
Light	No reaction	No reaction	No reaction

This organic independence in Stylotella also appears in the almost complete absence of transmission from part to part. The opening and closing of an osculum on one finger in accordance with the condition of the surrounding water has no influence on adjacent oscula even though they be only a centimeter or so distant. Extensive wounds, which can be made with much local precision, influence oscula or ostia only within a very close range. Transmission at best cannot be over a much greater distance than a centimeter or so. Nor is the nature of this transmission at all nerve-like. A cut made about 3 mm. from an osculum was followed by the closure of the osculum only after eleven minutes, though this osculum had previously closed in quiet water in from four to five minutes. The form of reaction resembles that seen in the vertebrate iris, in which in response to a point of light the iris contracts locally, the contraction gradually spreading through the whole organ (Hertel, '07). In the sponge, as in the iris, we are probably dealing with the direct stimulation of smooth muscle, which when locally contracted stimulates by its contraction the adjoining resting muscle and thus a slow form of transmission is accomplished through the muscle substance itself.

These studies of the reactions of Stylotella support the conclusion arrived at from earlier anatomical investigations to the effect that sponges possess nothing that may with propriety be called nervous tissue. Their reactions, which have the general character of great simplicity and independence, are, I believe, entirely due to the direct stimulation of choanocytes or myocytes which are either on exposed surfaces or close to them and which, at least in the case of myocytes, exhibit a form of progressive stimulation that resembles sluggish transmission. Sponges are metazoans possessing muscular but not nervous tissue.

5. ORIGIN OF THE NERVOUS SYSTEM

In seeking evidence on the origin of the nervous system, investigators have naturally turned to primitive metazoans, and the cœlenterates have afforded the principal material for speculation on this subject. These speculations took their origin in the discovery by Kleinenberg ('72) of the so-called neuromuscular cells in hydra. These cells, which have since been found in great abundance in many other cœlenterates, were believed by Kleinenberg to contain the germ of the nervous and muscular systems of the higher metazoans. According to him the elongated basal process of the neuromuscular cell was the contractile or muscular element, and the cell-body that reached from the exterior to the muscular part was the receptive and transmitting or nervous part. In his opinion this cell became divided into two, one cell to become purely muscular, the other purely nervous, and these two cells, thus derived directly from the primitive neuromuscular cell, were supposed to be the forerunners of the muscular and nervous systems of the higher animals. Kleinenberg thus conceived muscular and nervous organs to have had a common origin and to have undergone a simultaneous differentiation. The neuromuscular-cell theory was favored by Van Beneden ('74), who claimed that in Hydractinia the intermediate condition between a neuromuscular cell and its two derivatives was to be seen, but Bergh ('78) showed this claim to be based on inaccurate observation.

The study of the nervous system and sense organs of marine cœlenterates led Oscar and Richard Hertwig ('78) to the conclusion that the so-called neuromuscular cells were not nervous but merely muscular, and they proposed for these elements the name epithelial muscle-cells. They also pointed out that the nervous system of the cœlenterates consisted of sense-cells and ganglion-cells and they believed that these two kinds of cells together with the epithelial muscle-cells were simultaneously differentiated from among the elements of the cœlenterate epithelium. Thus they did not trace the origin of nervous and muscular tissue to a single cell but to a layer of cells from which the three types just named were supposed to arise by simultaneous differentiation. This view, though slightly modified by such workers as Havet ('01), who declared that what the Hertwigs called ganglion-cells were more strictly speaking motor-cells, has been more or less tacitly accepted by most modern students of the neuromuscular mechanism of cœlenterates (Schaeppi, '04; Wolff, '04; Hadzi, '09; Groselj, '09).

The views of Kleinenberg and the Hertwigs, as this brief survey shows both contain the common element of simultaneous and inter-related differentiation of nervous and muscular elements. As contrasted with this aspect of the question Claus ('78) and Chun ('80) claimed an independent origin for these two types of tissue and that their connection was secondary. The ground for this opinion, at least as maintained by Chun, is chiefly the condition found in vertebrates where in ontogeny it is very probable that nerve and muscle are independently differentiated and secondarily united. Thus this opinion gets its support from highly specialized rather than from primitive metazoans.

The view as to the origin of the nervous system, or better of the neuromuscular mechanism, to which the study of the activities of sponges has led me, is in strong contrast with the opinions that have already been expressed. The fact that sponges have an organized musculature, though they show no evidence of nervous organs, leads me to the conclusion that nerve and muscle have not differentiated simultaneously, but that muscular tissue has preceded nervous tissue in order of evolution. The condition in sponges is absolutely contrary to the statement of Kleinenberg ('72, p. 23) that there are no animals with muscles and without nerves, nor is it consistent with the view of the Hertwigs ('78, p. 165) and their followers that these two kinds of tissue differentiate simultaneously. Muscular tissue unassociated with anything that can reasonably be called nervous tissue certainly occurs in these primitive metazoans, and muscle of this kind directly stimulated, i. e., without the necessary intervention of other cells, is in my opinion the initial stage in the growth of the neuromuscular mechanism. The next step in this process is, I believe, that realized in most cœlenterates, i. e., a muscular mechanism to which has been added certain receptive cells, sense cells, that serve as delicate organs for bringing the muscles into action. This step is the first step in the differentiation of true nervous tissue, though it is the second in the growth of the neuromuscular mechanism as a whole. At this point my view is in strong contrast with that of Claus and Chun who, as already stated, have maintained that nerve and muscle arose independently. This I do not believe possible, for I agree entirely with Samassa ('92) when he declares that a nervous mechanism without muscles or other effectors is inconceivable. In my opinion nervous tissue has differentiated not independently of muscle, as claimed by Claus and by Chun, but in most intimate relations with it and as a more effective means of bringing it into action than direct stimulation is. Primitive muscles, then, as independent effectors, were centers around which the beginnings of nervous differentiation probably occurred, in that certain peripheral cells came to be specialized as receptors for stimuli and excitors of muscular activity, a condition now realized in cœlenterates.

From this standpoint it must be clear that the histogenesis of primitive nervous tissue involves cells that are in contact with the exterior on the one hand and with muscular tissue on the other. The conditions realize almost perfectly the requirements of the well-known theory of neurogenesis advocated by Hensen ('64), and I, therefore, believe that this theory is a truthful portrayal of primitive neurogenesis. I do not admit, however, that it presents a correct picture of the histogenesis of vertebrate nerves. In this problem the evidence seems to me to be strongly in favor of the initial separateness of nerve and muscle and their secondary union, an operation which in my opinion is a cœnogenetic modification of the primitive process. But whether the axis-cylinders of vertebrate nerve-fibers are outgrowth of neuroblasts or not, is a question that has no direct bearing on the one herein discussed, the differentiation of the primitive nervous system. Such a primitive nervous system, essentially receptive in character, is, however, merely the beginning of that structure which in the higher metazoans is designated as nervous. This primitive nervous system is not in any appropriate sense to be called centralized. Its diffuse character, from an anatomical as well as from a physiological standpoint, is well known, and only after the nervous structures have become concentrated either in the periphera lepithelium, as in some worms, or on separation from this epithelium, as in the higher metazoans, is a condition arrived at which necessitates the formation of true nerves, and allows the establishment of common paths (Sherrington, '06), a condition which may be appropriately called centralized. When this concentration takes place it usually occurs near the chief group of sense organs and gives rise to what is conventionally called the brain. In nervous differentiation, then, the chief central organ or brain follows, in its early evolution, the lines of sensory differentiation. The differentiation of the complete neuromuscular mechanism as possessed by the higher animals, has occurred, I believe, in three successive steps; first, the formation of independent effectors, tor as seen in the muscles of sponges; secondly, the addition of receptors to such effectors, as seen in what I have elsewhere called the receptor-effector systems of the cœlenterates; and finally, the differentiation near the receptors of adjusters or central organs concerned primarily with easy transmission from receptors to effectors (Parker '09).

6. SUMMARY

1 Stylotella under natural conditions closes its oscula and contracts its flesh when at low tide it is exposed to the air.

2 Its outer surface is perforated by many ostia which lead to large subdermal cavities, these in turn connect through incurrent canals with the flagellated chambers from which excurrent canals pass to the gastral cavity and the osculum.

3 The flesh of Stylotella contains many myocytes, which are arranged as sphincters around the ostia, internal cavities, and osculum. These sphincters apparently work against the general elasticity of the flesh and not against radiating systems of myocytes.

4 The oscula close in quiet seawater, on exposure to air, on injury to neighboring parts, in solutions of ether (0.5 per cent), chloroform (0.5 per cent), strychnine ($\frac{1}{15000}$), cocaine ($\frac{1}{1000}$), and in deoxygenated seawater. They contract but do not close in diluted seawater and at temperatures higher than normal (35° to 45° C.). They remain open in currents of seawater, and their closure is inhibited by solutions of cocaine ($\frac{1}{10000}$) and of atropine ($\frac{1}{1000}$), and in fresh water. They are apparently uninfluenced by low temperatures, by weak solutions of cocaine ($\frac{1}{50000}$) and of atropine ($\frac{1}{10000}$) and by light.

5 The ostia close on injury to neighboring parts, in solutions of ether (0.5 per cent). chloroform (0.5 per cent), strychnine ($\frac{1}{15000}$), and cocaine ($\frac{1}{1000}$). They open in solutions of cocaine ($\frac{1}{10000}$). and of atropine ($\frac{1}{1000}$), in dilute seawater, deoxygenated seawater, and warm seawater (35° C.). They are apparently unaffected by mechanical stimulation, except injury, by low temperature, and by light.

6 The choanocyte currents cease in solutions of ether (0.5 per cent), and of chloroform (0.5 per cent), in diluted seawater and at high temperatures (40°–45° C.). They become slow at low temperatures (9° – 10° C.), and fast in solutions of strychnine ($\frac{1}{15000}$). In deoxygenated water they first become fast and then cease.

7 The flesh of Stylotella is capable of contraction, but such contractions give the sponge only a shrivelled appearance without changing its general form.

8 The currents in Stylotella are constant in direction and give no evidence of reversal. They are controlled by the ostial and oscular sphincters. They produce a pressure equivalent to 3.5 to 4 millimeters of water. The pressure necessary to break through the closed ostia is 10 to 15 millimeters of water and through the closed oscula somewhat more.

9 The reactive organs of Stylotella, the ostia, the oscula, the flesh, and the choanocytes, are all more or less independent of one another and their action is changed by direct stimulation. In the ostia, oscula, and flesh contraction is accomplished by spindle-shaped cells, the myocytes, which resemble primitive, smooth muscle-fibers.

10 The body of Stylotella is almost without transmission and such transmission as is present is so sluggish in character and so silght in range as to resemble transmission in muscles and not in nerves. It is probable that Stylotella possesses no organs that can reasonably be called nervous.

11 The nervous and muscular systems of metazoans were not differentiated simultaneously (Kleinenberg, O. and R. Hertwig) nor independently (Claus, Chun), but muscles, independent effectors, as represented by the sphincters of sponges, were the first of the neuromuscular organs to appear and these formed centers

around which the first truly nervous organs, receptors, in the form of sense-cells developed giving rise to a condition such as is seen in the cœlenterates today. To this receptor-effector system as seen in modern cœlenterates was added in the higher metazoans the adjuster or central organ, thus completing the essential parts of the neuromuscular mechanism as seen in the higher metazoans.

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