The Effect of reversing the Internal Water-Current on the Spicule Orientation in *Leucosolenia variabilis* and *L. complicata*

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With two plates (figs. 2 and 3)

SUMMARY

1. Specimens grown in quiet water in an aquarium tank produce the same pattern of spicules as do those occurring naturally. Woodland's hypothesis, that the orientation arises from turbulence in the environment, is thereby disproved.

2. Reversing the internal water-current in excised oscular tubes of *Leucosolenia* has shown that no direct causal relationship exists between the direction of the current and the orientation of the spicules.

3. The spicule arrangement is modifiable by experiment; the orientation is not dependent on the existence of static structural features in the wall of the sponge.

4. Removal of the oscular rim has little effect on the orientation of the spicules subsequently developing in the tube behind.

5. Diverticula develop from bulges, formed largely by a reshaping process. The arrangement of the associated small spicules is essentially the same as for *L. lieberkühnii*.

6. A mechanical hypothesis explaining the orientation of the spicules is briefly described. The formative cell sextet is believed to be oriented by a movement of the mesogloea over the epithelium to which the sextet is contiguous.

INTRODUCTION

A STRONG correlation between the orientation of the spicules in calcareous sponges and the direction of the water-current in the spongocoel was noticed by Haeckel and commented upon by v. Ebner (1887), Minchin (1908), Urban (1906), and Maas (1907): the basal rays of tri- and quadriradiates usually point in the opposite direction to the water-current. Probably the relationship is not a direct one, because at the distal ends of the closed diverticula of *Leucosolenia lieberkühnii* the newly developing spicules can be oriented towards the distal tip before the end has formed an osculum, and consequently before the current has reversed its direction (Minchin, 1908). However, the relationship seemed sufficiently close to justify an experimental investigation, and the following account describes the effect of reversing the internal current in oscular tubes of *L. complicata* (Montagu) and *L. variabilis* (Haeckel) (Minchin, 1904). The method adopted consisted of excising the tubes and mounting them on the tips of fine glass jets through which a current of sea-water was allowed to siphon. This procedure also enabled the effect of removing the oscular rim to be investigated.

A survey of the literature on spicule development has revealed only one

detailed attempt to explain the orientation of calcareous sponge spicules, namely that of Woodland (1905). He believed that the spicule primordia were initially oriented at random, and that the pattern of the fully grown spicules was to be attributed to the turbulence caused by wave action in the sea-water around the sponge. This turbulence was assumed to invaginate parts of the sponge wall, or to flex the oscular tubes, thereby causing the developing spicules to rotate into an equilibrium orientation. The hypothesis can be strongly criticized on theoretical grounds (Jones, 1952), but there is no need here to give a detailed criticism, because Woodland was wrong in assuming that the youngest spicules are haphazardly oriented; in fact the smallest spicules have the same orientation as their fully grown neighbours (Minchin, 1908). Moreover, the spicule pattern found when specimens of *L. complicata* or *variabilis* are grown in quiet water in an aquarium tank is, if anything, more perfect than that on specimens taken directly from the shore. Woodland's complementary orientating factor, the influence of gravity, also need not be seriously considered, since the oscular tubes may be found growing at all inclinations to the vertical, without any disturbance of the spicule pattern.

MATERIAL AND METHODS

The experiments were carried out with oscular tubes of *L. variabilis* at the Marine Biological Laboratory, Plymouth, from mid-August to mid-September during the four years from 1949 to 1952, and with tubes of *L. complicata* from mid-April to mid-June in 1955 and 1956, at the Marine Biology Station, Menai Bridge. The latter species proved the more satisfactory; its larger oscular tubes were less prone to produce diverticula and could be left on the jet for a longer period. The tubes were kept in place by means of a cotton ligature. The wall beneath the ligature tended to disorganize, allowing the tube to slip off the jet. The maximum period of mounting for tubes of *L. complicata* was 3 days, whereas the tubes of the other species could only be retained for a longer period than 46 h if an additional ligature was tied just before the first became ineffective. Longer periods were, however, unnecessary.

Fully expanded oscular tubes were used, since new spicules are not produced by contracted tubes, and in contracting material they may be dislodged from the choanoderm by the crowding together of the fully grown spicules. One cause of contraction is a change in temperature of a few degrees, so that the experiment should be carried out with sea-water at the same temperature as that at which the sponge has been growing. The specimens were therefore left in the aquarium in running sea-water for at least several days after collection in order to adapt them to the temperature of the experiment. The experimental temperature was between $8 \cdot 5°$ and $13°$ C when using *L. complicata*, and $16 \cdot 5°$ to $21°$ C when using *L. variabilis*, taking all the experiments into consideration.

The apparatus employed is shown in the diagram (fig. 1) and requires little explanation. A special clamp made of paxolin is used to support a series of 5 siphon-tubes, each bearing a finely drawn jet. The tubes were made from

glass tubing 4 mm thick and of 2·3 mm bore. The jets were about 3 cm long with, at the tip, an internal diameter of 150–350 μ and an external diameter of 250–550 μ. Usually two clamps were used, allowing 10 oscular tubes to be mounted during the same experiment. The two clamps were either set on opposite sides of the same basin, or on the opposite sides of the same reservoir,
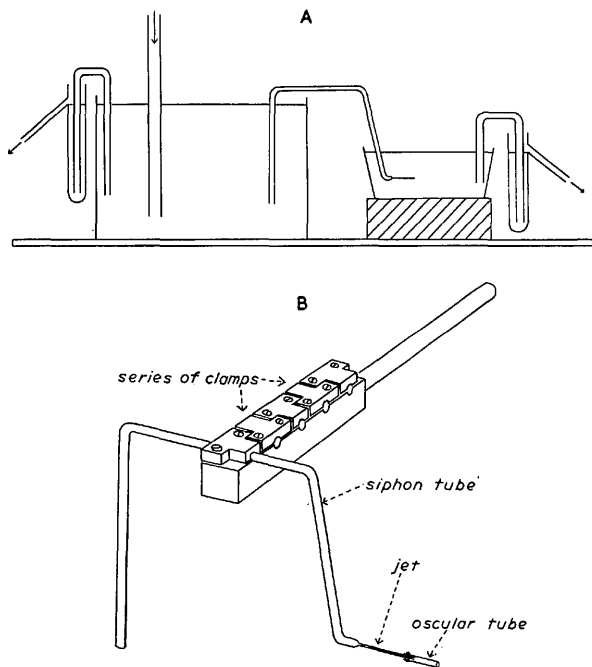

FIG. 1. A, the apparatus used in the jet experiments. Sea-water passes from a reservoir into a basin through a series of siphon-tubes. Each tube has a finely drawn jet at the end, upon which is mounted an oscular tube. Constant levels of sea-water are maintained in the vessels by means of the overflow tubes alongside. B, a siphon-tube in position in one of the clamps.

two receiving basins then being employed. A pressure head of 5–6 cm of water was found to give satisfactory results for both species. Fresh sea-water was used in order to avoid blockage of the oscular tubes by bacterial filaments.

The method for mounting the oscular tubes was as follows. Some siphon tubes of varying diameter of jet were completely filled with sea-water and laid on a support in a large dish of sea-water, so that the jets were clear of the bottom, but submerged. An expanded oscular tube was excised from the

specimen in the dish and the cut end opened by gently squeezing with forceps. If required, the oscular rim was also removed at this stage, the end being cut at a distance from the edge roughly equal to the diameter of the tube, or at twice this distance. The tube was then pushed over the edge of a jet of suitable size and tied on by means of single-stranded thread, prepared by untwining ordinary 6-core cotton.

With a finger over the open end the siphon-tube was next conveyed to the clamp and clamped in position. The oscular tube was taken lengthwise through the air-water interfaces to prevent flexion. At the end of the experiment the siphon-tube was removed from the clamp, again with a finger over the free end, and the oscular tube was dipped into 90% alcohol. It was taken off the jet by sliding the ligature along with forceps.

The tubes may be examined in 90% alcohol, but it was found better to dehydrate, clear, and mount in balsam under a raised coverslip, for then the cells become nearly indistinct and the spicules can be seen clearly. The spicule pattern is much the same all round the tube, unless diverticula are present, so that one need only concentrate on the upper surface. The position, orientation, and size of the small spicules were plotted on graph paper with the aid of a squared eyepiece-micrometer, by the use of a 16-mm objective and a × 10 eyepiece. Occasionally the use of polarized light, or of a higher-powered objective, was necessary in order to determine the orientation of a spicule.

Accurate measurements of the size of the tubes could not be made during these experiments, but sketches were drawn of the shape of many of the tubes and notes were made concerning their transparency, which gives a rough guide to their state of expansion.

In all, 34 tubes of *L. variabilis*, 23 of *L. complicata*, and 2 of *L. botryoides* have been accurately mapped, while many more were mounted and gave similar results, although some did not produce crops of small spicules.

There is little point in reversing the direction of the water-current by adjusting the levels so that water flows into the unattached end of the oscular tube, for experiment has shown that the tube simply contracts under such conditions, probably as a result of the decrease in the internal pressure. No small spicules, or few of them, can then be discerned amongst the crowded, fully grown spicules.

RESULTS

In the account which follows the tubes which were mounted at their oscular ends will be referred to as 'reversed' tubes, whereas those mounted

FIG. 2 (plate). A, the distal end of a reversed tube of *L. complicata*, showing the crowded spicules at the edge. On the right close to the edge, is a medium-size T-spicule. The end has partially healed over, the opening now being restricted to the left side. At the middle of the edge can be seen the healing membrane which has drawn the cut edges together on the right side. B, the distal part of the same tube at a different level of focus. Note the many small reversed spicules. The tube was mounted for 47¾ hours. The line measures 100 μ.
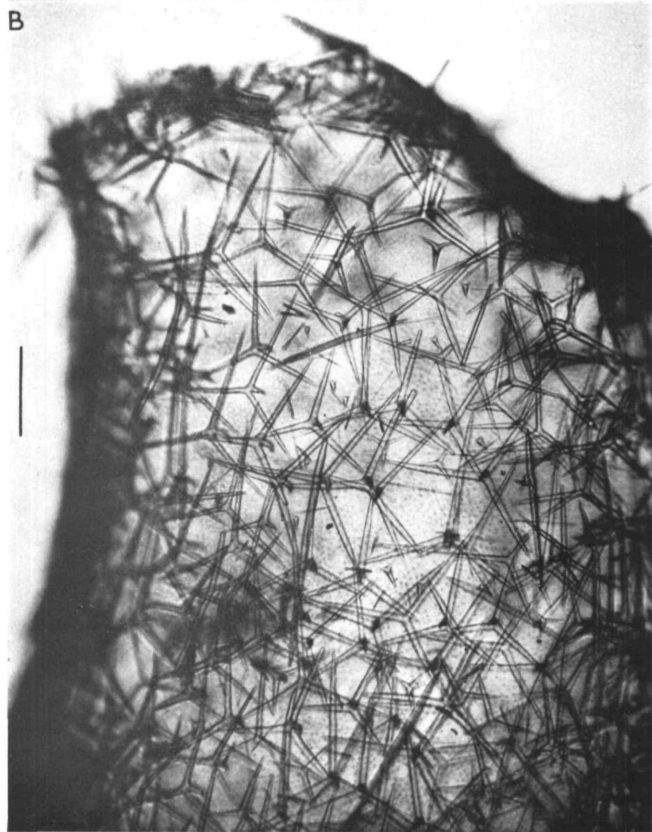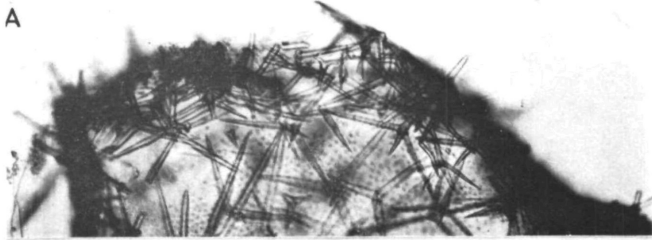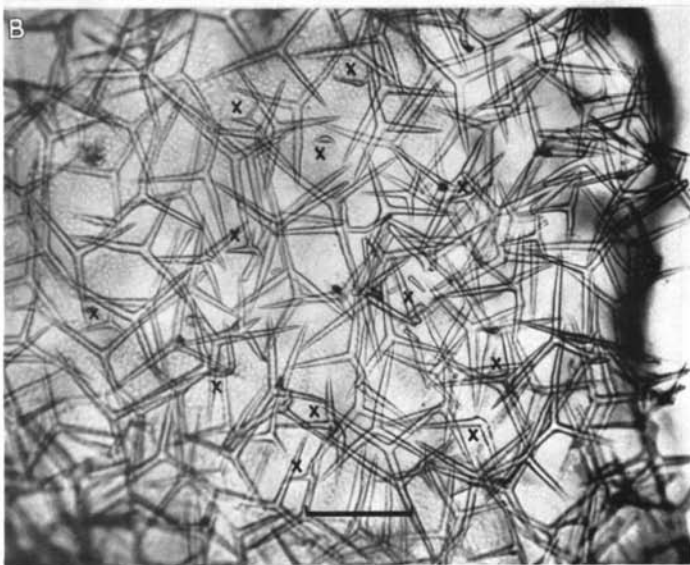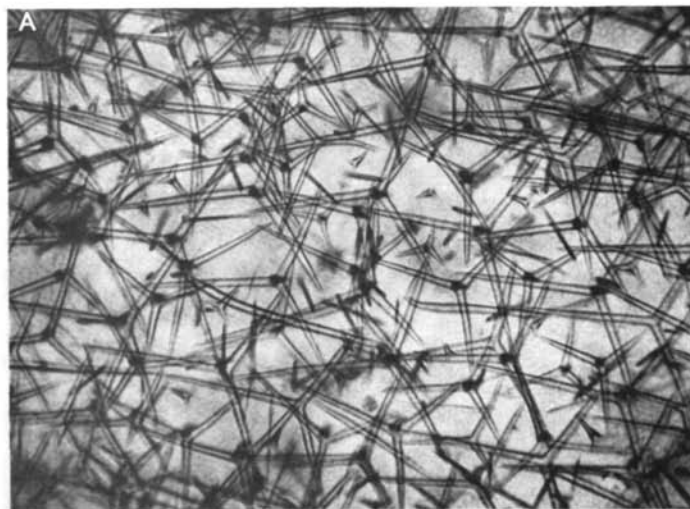
A

B

FIG. 2

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FIG. 3

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by their basal ends will be termed 'control' tubes. Of the 34 tubes of *L. variabilis* that were accurately mapped, 22 were 'reversed' and 12 were 'control' tubes, while 5 of the former and 9 of the latter had had their oscular rim removed before mounting. Of the 23 tubes of *L. complicata*, 14 were 'reversed' and 9 were 'control', of which respectively 2 and 4 were without their original oscular rim.

Both types of tube undergo changes in shape while they are mounted, though the changes may be small for the control tubes. Invariably the tubes constrict near the ligature, so as to embrace the jet more tightly, while the reversed tubes also tend to constrict at their distal end. Normally the tubes taper from near the base up to the oscular rim, so that when they are mounted in reverse the end with the smaller diameter will contain water at the higher pressure. Adaptation will therefore result in an expansion near the middle and a constriction at the free end of the tube. This end may even close up with the spread of a healing membrane (Jones, 1957) if the internal pressure is inadequate. The distal ends of the control tubes, however, remain open, but may constrict or expand while the tube is adapting itself to the new conditions. In one case the oscular rim constricted just behind the edge at 11 h after mounting, then slowly recovered and expanded widely during the period from 52 to 72 h.

The control tubes without their original oscular rim behave much like those with, except that there is a tendency for the spicules at the cut edge and just behind it to become crowded together. This tendency is much more noticeable in the reversed tubes, in which quite a thick band of closely packed, partly broken spicules may be found at the distal edge (fig. 2, A). This band can be explained by the occurrence of longitudinal tension in the surface epithelia coupled with a removal of mesogloea from in between the spicules near the edge, for the act of cutting squashes the surface epithelia together and healing over the edge is probably a rapid affair. If the cut edge is uneven, one will get local patches of crowded spicules as the edge becomes more uniform.

More extreme changes of shape may become apparent, particularly when tubes of *L. variabilis* are used. Quite often they constrict in some regions, or bulge in others, and the bulges may be transformed into diverticula.

The pattern of small spicules arising in the wall of reversed tubes

Reversed tubes which were mounted for more than 17 h (*L. variabilis*) or 35 h (*L. complicata*) were found to have small spicules in the distal part of the tube which were oriented towards the distal edge; that is, their basal rays pointed towards the attached end (fig. 2, B). Fig. 4, A is an example from

FIG. 3 (plate). A, a group of irregularly oriented small spicules in part of the wall in between the oppositely directed orientation zones of a reversed tube of *L. complicata*, mounted for 47¾ hours. B, the distal end of a reversed tube of *L. variabilis* which was mounted for 80 h. Most of the small reversed spicules present are aberrant in form (indicated by *X*), some being deficient in a ray and others asymmetrical owing to a rotation of the spicule primordium about the axis of the basal ray. The line measures 100 μ.

L. complicata. At the distal end there is a zone of reversed orientation of small and medium-sized spicules extending for nearly one-third of the length of the tube; then a zone in which almost all the small spicules are reversed whereas the larger ones are directed roughly towards the proximal end of the tube; then a zone in which the arrangement of the smallest spicules is confused; and finally a zone extending for nearly half the length of the tube, in
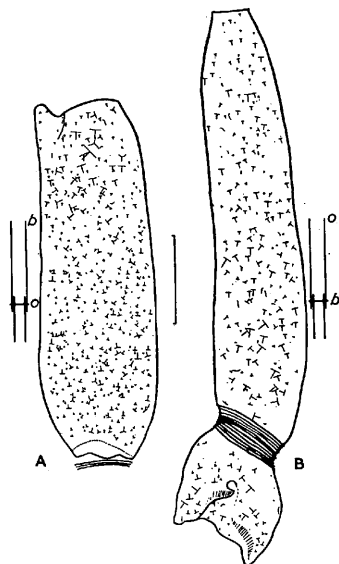

FIG. 4. The pattern of small spicules produced in two tubes of *L. complicata*. For convenience the spicules (except for obvious aberrants) in this and subsequent diagrams are drawn as T-shaped, the stem indicating the length and orientation of the basal ray. No attempt is made to give an accurate representation of their form. A, a reversed tube mounted for 47¾ h (description in the text). The broken line indicates the limit of the choanoderm. B, a control tube mounted for 53¼ hours. The shading indicates areas of crowded spicules. *o*, osculum; *b*, basal end. The line measures 1 mm.

which nearly all the spicules point towards the original oscular end. The area of 'random' orientation separating the reversed zone and the proximal unreversed zone of another reversed tube is shown in fig. 3, A.

The tube depicted in fig. 4, A was mounted for 47¾ h and comparison with other reversed tubes of the same species suggests that with longer periods the reversed zones tend to become more extensive. In fig. 5, A the fraction z.r.o./length is plotted against the period mounted for all the reversed tubes in which small spicules were found at the distal end. There is considerable

variation, due partly to the arbitrary nature of the measurement, but the reversed zones seem to have been established after roughly 30 h. In no case did the reversed zone exceed half the length of the tube.

A tendency for the reversed zone to spread from the edge was also occasionally shown by the difference in extent of the zones for spicules of different sizes. In 3 tubes the zone for the smallest spicules extended farther from the
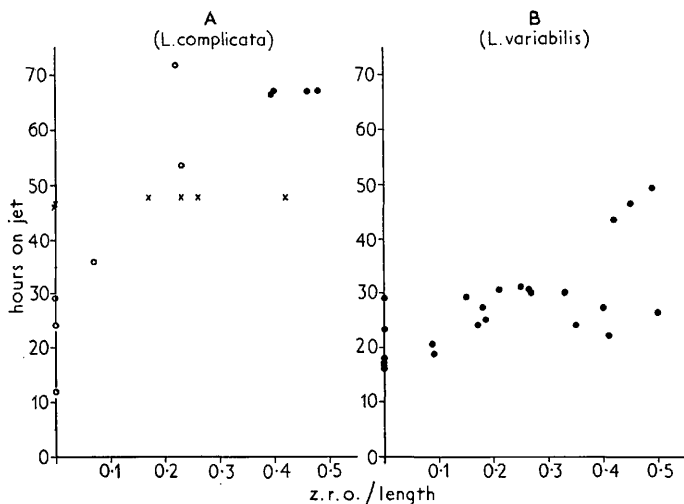

FIG. 5. Graphs showing the relation between the hours mounted and the extent of the zone of reversed orientation divided by the length of the tube (z.r.o./length). A, *L. complicata*: the tubes mounted simultaneously in the separate experiments are indicated by the same mark. B, *L. variabilis*: the points are derived from tubes used in a number of separate experiments. Small spicules were present in all the tubes plotted.

edge than that for the medium-sized spicules (for example, fig. 4, A), indicating a spread of the 'reversed orientation field'. In one tube, however, which was mounted for 72 h, the reversed zone for the smallest spicules was slightly less extensive than for the larger spicules.

With tubes of *L. variabilis* the results (fig. 5, B) were also variable, but again there may have been a spread of the reversed orientation field from the free edge, especially as in 6 tubes the reversed zones had their largest spicules fairly close to the edge and the smallest extending the zone to its farthest distance. In other tubes the zone would appear to have either spread rapidly, or been established simultaneously all over the distal part of the tube.

No appreciable difference was detected between the reversed tubes with and those without their original oscular rim. In both types the original orientation was maintained in the proximal portion of the tube.

In fig. 6 the length of the basal ray of the largest reversed spicule in a zone of reversed orientation is plotted against the period for which the tube was mounted (*L. variabilis*). The length tends to increase with longer periods, so that there can be little doubt that the spicules were growing during the experimental period. Again there is much variation, owing to a combination of several factors, such as variation in the rate of growth of the spicules,
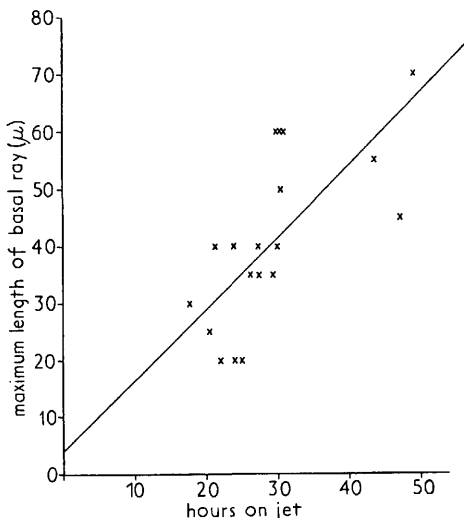

FIG. 6. Graph showing the relation between the hours mounted and the maximum length reached by the basal rays of reversed spicules in the distal part of the reversed tubes of *L. variabilis*. The regression line (correlation coefficient 0·703) cuts the ordinate at 4 μ and has a slope indicating an average growth rate of 1·2 μ per hour.

differences in the relative time of establishment of the reversed orientation field, and a possible limitation in spicule size due to a delay in the onset of production of new spicules. For these reasons the graph in fig. 6 is of little use for calculating the rate of growth of the basal ray accurately, and the results for *L. complicata* were even less satisfactory, partly because less points could be plotted and because some of the reversed spicules had reached the fully grown condition at an indeterminable time before the tube was fixed. However, a regression line (correlation coefficient 0·703) has been calculated for the points in fig. 6 and the slope of this line gives the average growth rate as 1·2 μ per h, which is in surprisingly good agreement with the figures of 1·0–1·8 μ per h obtained by photographically recording spicule growth in *L. variabilis* (Jones, 1952; to be published).

The regression line cuts the ordinate at 4μ, from which one would infer that some of the spicules which began growing before the tube was mounted have suffered reorientation. This is a possibility that must not be neglected, though it would seem improbable except in the case of very small spicule primordia. Certainly the fully grown spicules do not suffer rotation, and the graph indicates that medium-sized spicules (basal ray about 40μ or more) were not rotated after periods of up to 20 h on the jet. It is likely that the mesogloea is only fluid in the layer close to the choanoderm (Jones, 1956), and that once the spicules have reached a certain size, the thickness of the rays brings the spicule into contact with the outer, firmer layer so that rotation is hindered. The form of the larger spicules (a low tripod) also would entail a considerable resistance to rotation over the curved surface of the choanoderm.

Another possibility is that some reversed spicules were already present at the time of mounting. Even in healthy oscular tubes the orientation pattern may not be perfect, particularly at the base, where diverticula may be about to form. However, the possibility of spicule reorientation would make the calculation of the time of establishment of the reversed field uncertain, even if one knew accurately the rate of spicule growth.

For reversed zones of spicules of different sizes to be readily distinguishable in the same tube, the spread of the reversed orientation field would have to be slow. Assuming a constant rate of growth of $1\frac{1}{4}\mu$ per h during development, the basal ray would require 32 h to increase in length by 40μ, during which time the field would be required to spread from the boundary of one zone to that of the other, in practice a distance of less than half the length of the tube. As stated above, only 6 tubes out of 22 of *L. variabilis*, and only 3 out of 14 of *L. complicata*, showed this effect.

The concentration of small spicules varies considerably. In some the small spicules are abundantly distributed in all regions, whereas in others they may be localized, usually in places where one would expect there to be active growth. Thus the oscular regions and diverticula are good sources of small spicules, while sometimes the distal end has a greater concentration than the middle of the tube. Some tubes, however, seem to be perfectly expanded and yet do not possess small spicules at all. Thus the production of spicules is not necessarily a continuous process at any particular level of the tube. With *L. complicata* a tube with an abundant crop may average 100 small spicules of various sizes per square mm, with the concentration as high as 3 per $10^4\mu^2$ in some places. With *L. variabilis* one tube had a crop averaging 190 small spicules per square millimetre at the oscular end and 160 per square millimetre at the distal end, with local patches containing as many as 4 small spicules of various sizes per $10^4\mu^2$. Contracted regions of the wall are poor areas for small spicules.

Aberrant spicules are commonly found in the reversed zones, and fig. 3, B shows a tube in which there is a large proportion of aberrants. There is no need to explain their form in detail here, but in general it is the result of the optic axis not lying in an axial plane of the tube (Jones, 1955). This could

arise from a rotation of the spicule primordium about the axis of the basal ray, the sextet presumably being tilted laterally, or dislodged, by a laterally directed shear of the mesogloea. In fact many aberrants are probably the result of a clumsy reorientation of small spicules. Some have a ray missing, either because a calcoblast has become separated from the sextet, or because its secretory activity has been inhibited through the cell being squashed against the epithelium as a result of the abnormal setting of the primordium.

Tubes of *L. complicata* which were left unmounted on the bottom of the basin for 2 days constricted and closed up at their basal end without the
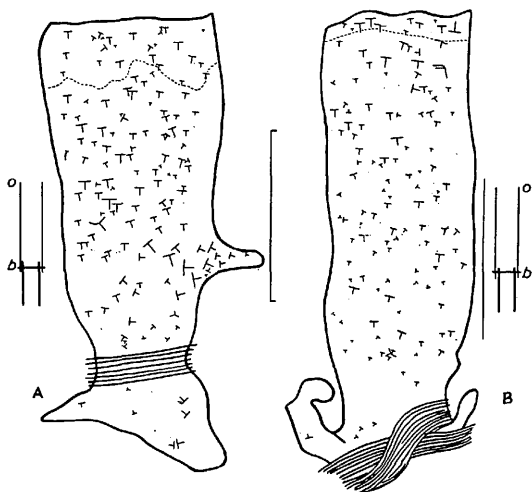

Fig. 7. Patterns of small spicules produced in two control tubes of *L. variabilis*. A, with the original oscular rim. B, without the original oscular rim. Note the reversed spicule at the edge on the right side in B (explanation in the text). The line measures 1 mm.

formation of a reversed orientation zone (fig. 4, B). From the behaviour of similar tubes it is known that a healing membrane (Jones, 1957) forms at the basal edge towards the end of the first day, and this rapidly spreads across the gap, drawing the cut edge inwards. In the mounted tubes the establishment of a reversed zone at the distal end thus probably depends upon the end remaining open. Presumably the spread of a healing membrane is inhibited by the vigorous internal water-current.

The pattern of small spicules in the control tubes

In the control tubes of *L. variabilis* the spicules were mainly directed towards the oscular end, with perhaps some of the small spicules directed obliquely or laterally, even at sites near the edge. An example is shown in

fig. 7, A. The tube was mounted for only $17\frac{1}{4}$ h and the basal rays of the laterally directed spicules are $25\,\mu$ or less in length. Thus they probably commenced development at about the time the tube was mounted or later. The pattern is not much different from that shown in fig. 7, B, a tube from which the original oscular rim was removed just after mounting. One or two of the small spicules are laterally directed, or reversed, but by far the majority maintain the orientation towards the oscular end of the tube. Again there can be little doubt that the smallest spicules began development during the experimental period (the tube was mounted for $23\frac{1}{4}$ h), so that the removal of the oscular rim has interfered but little with the orientation field.

Near the distal edge of the tube in fig. 7, B is a reversed spicule with a basal ray of length $45\,\mu$. Assuming a constant rate of growth of about $1\frac{1}{4}$ per hour, this spicule would have been about $16\,\mu$ long at the time of mounting, and it is probable that this spicule was reversed by the act of cutting, which must introduce a considerable amount of mechanical distortion near the edge. Two other control tubes similarly had one reversed spicule each close to the cut edge, whose sizes at the time of excision were calculated to be respectively $25\,\mu$ and $28\,\mu$.

At the proximal ends of the control tubes one often finds a confused pattern of small spicules, and sometimes, when the tube has been mounted for a considerable time, a zone of reversed orientation. With *L. variabilis* 3 tubes mounted for respectively 46, $46\frac{2}{3}$, and 91 h had a reversed zone at the proximal ends, whereas 15 tubes mounted for $16\frac{3}{4}$ to 40 h either had no reversed spicules, or had their small spicules arranged more or less at random in the proximal region. With *L. complicata* a reversed zone was present on 4 tubes mounted for respectively $53\frac{1}{4}$, $53\frac{1}{3}$, 67, and 72 h (fig. 4, B), but not on 4 tubes mounted for 46, 48, $53\frac{1}{4}$, and 72 h. The latter tubes displayed a confused pattern at the attached end, whereas a tube mounted for $43\frac{1}{2}$ h displayed no reversed spicules at all. Thus the establishment of a reversed zone occurs much later in the control tubes than in the reversed tubes. In the former the cut edge is some distance behind the ligature, while a free edge only appears at the ligature (by the disorganization of the tissue beneath) some considerable time after mounting.

All 7 control tubes of *L. complicata* (3 with their original oscular rim) that were mounted for more than 53 h had an extensive confused zone. In fig. 4, B it extends for 2·79 mm between the distal zone of 1·2 mm and the proximal reversed zone of 0·66 mm length. In another tube the distal zone was 0·93 mm long and the confused zone continued along to the ligature, a distance of 5·18 mm. It would appear that in such regions of irregular orientation there had been no overriding orientation field.

The part of the tube behind the ligature is of little value for mapping the orientation of small spicules owing to its contracted condition and the occasional presence of diverticula. Also some tubes broke free from the ligature just before fixation and the part behind was not retained. However, in most of the cases mapped the small spicules were mainly directed towards the cut

edge, except in the vicinity of the ligature. The results seem to be consistent with those obtained at the distal ends of the reversed tubes, as would perhaps be expected since the presence of the glass jet prevents the closure of the cut end.

One control tube of *L. complicata* was mounted on a jet which was filled with air, so that no current could siphon through. After 46 h this tube was no longer cylindrical, but partially constricted at two levels besides the attached end. The resulting pattern of small spicules was irregular over most of the tube, with some reversed spicules here and there. The arrangement at the basal end was also confused. This tube indicates the importance of maintaining an adequate pressure inside the spongocoel.

The pattern of small spicules in bulges and diverticula

Diverticula were quite often produced on the experimental tubes and the pattern of their fully grown spicules reveals their mode of origin. The spicules on the surface facing the oscular rim are directed towards the base of the diverticulum, whereas those on the surface facing the base of the main tube are directed towards the apex. On the two sides the spicules are oriented across the diverticulum, in the direction roughly towards the osculum. The diverticulum has thus arisen from part of the wall of the main tube which has bulged outwards and then constricted at the perimeter of the bulge. The bulging is probably the result of a localized contraction of the internal epithelium, which has been shown to have contractile properties (Jones, 1957). However, amongst the fully grown spicules may be seen small ones which point away from the apex of the diverticulum regardless of their position in its wall (fig. 7, A). Thus the orientation pattern for the small spicules is the same as for the closed diverticula of *L. lieberkühnii* (Bidder, 1891; Minchin, 1908); and more so, because the small spicules may develop in the opposite orientation both before (at least in *L. complicata*) and after an osculum has appeared at the distal end (figs. 8, C, D).

The tendency for the small spicules to be directed away from the apex may also be seen in the radiating pattern on bulges of the wall (fig. 8, A), though the orientation is not always regular. Even the parts around the diverticulum may have small spicules directed away from it (figs. 8, B, 7, A). In fig. 8, A is shown a tube which had begun to produce diverticula before the start of the experiment. One diverticulum (depicted in the figure) is close to the proximal end, whereas two others are much nearer the distal end and sited, one on each side, on the opposite surface. Zones of reversed orientation extend from the distal edge up to each diverticulum, the one shown covering more than half the length of the tube. This was the only case in which a reversed zone reached beyond the half-way level, and, as indicated above, it did so on part of the surface only and its presence is to be correlated with the formation of the diverticulum. Many of the reversed spicules in this zone must have been present at the time the tube was mounted, judging from their size, for reversed spicules of $40\,\mu$ length occur, even though the tube was

mounted for only 21 h. This experiment indicates the importance of mounting tubes without diverticula when studying the establishment of a reversed zone at the distal end of the tube.

Occasionally the diverticula rounded off completely to form spheroidal objects at the tips of fine tubes (fig. 8, E). There can be little doubt that such
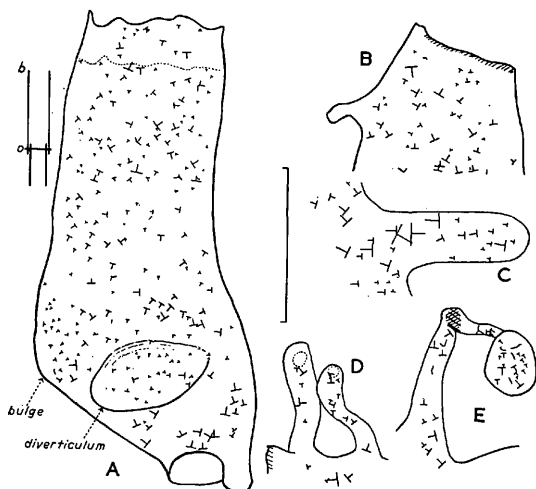

FIG. 8. A, reversed tube of *L. variabilis*, mounted for 21 h. Note the radiating pattern of small spicules in the bulge and diverticulum. B, distal end of a reversed tube of *L. variabilis*, mounted for only 17¾ h. A zone of reversed orientation has not been established at the distal edge. Small spicules radiate from the base of the diverticulum. C, a closed diverticulum from a tube of *L. complicata*; the small spicules are directed towards the tip. D, two diverticula from a tube of *L. variabilis*, each with an osculum at the tip. Some small spicules are oriented towards the oscula. E, spheroid formed at the distal end of a diverticulum (*L. variabilis*). The line measures 1 mm. Shading indicates areas where fully grown spicules are crowded together. The broken line indicates the edge of the choanoderm. *b*, basal end; *o*, osculum.

objects, broken away from the parent tube, would be capable of distributing the species. Vasseur (1879) has described this process of asexual reproduction in *L. botryoides*.

Formation of a new oscular rim at the cut end

As stated above, there was a tendency for the spicules to become crowded together at the cut edge. The boundary of the choanoderm, moreover, tended to separate from the edge, leaving a clear zone. This occurred in reversed tubes as well as in control tubes without their original oscular rim. The choanocyte boundary was often very uneven, but with *L. variabilis* in most cases an obvious clear zone seems to have been established by about

20 h. This zone is to be regarded as a new oscular rim, and the occurrence of developing T-shaped spicules near the cut edge confirms that the growth processes normally associated with an oscular rim had been in operation.

With tubes of *L. complicata* the tendency for a clear zone to form at the cut end was much less apparent. Even after 72 h on the jet a control tube still had no clear zone and the same was true for 2 other tubes mounted for about 53 h. Only in 3 reversed tubes, mounted for 36, 67, and 67 h, was there found a narrow zone extending for respectively $13–93\,\mu$, $13–66\,\mu$, and $40–55\,\mu$. Since a zone of reversed orientation was found in 5 tubes in which the collar-cells reached the free edge, while some T-spicules were present close to the edge on 2 reversed and 2 control tubes which did not have a clear zone, it may be concluded that the presence of the latter is not a prerequisite of the establishment of the growth processes controlling the form and orientation of spicules. There is evidence that the collar-cells rest upon a porocyte epithelium (Jones, 1957), so that the position of the collar-cell limit need not have significance in respect to the spicule pattern.

When the cut end closes up a clear zone is not to be seen. On the contrary the spread of the healing membrane across the gap would tend to draw the choanocyte-region in the direction of the cut edge.

DISCUSSION

Three conclusions concerning the mechanism of orientation may be derived from the above account.

(1) The reversed orientation of the small spicules at the distal ends of the reversed tubes, and the random orientation in various regions elsewhere prove that the mechanism of orientation is a dynamic one; the orientation at a particular site is not dependent upon the existence of static structural features in the wall, but is controlled by factors which are easily modifiable.

(2) The maintenance of the original orientation field at the proximal end of the reversed tubes, despite the reversal of the internal water-current, shows that there is no direct causal relationship between the direction of the current and the spicule orientation.

(3) Since the removal of the oscular rim does not appreciably disturb the orientation field, it is unlikely that the rim is controlling the spicule orientation. It might have been supposed that the calcoblasts were guided by a chemical or bio-electric gradient established by the metabolic activity of the oscular rim. However, this is unlikely because three apparently equal cells (Minchin, 1908) participate in the formation of a triradiate spicule and it is difficult to explain how the three similar cells could dispose themselves appropriately across an uniaxial gradient. Moreover, the spicule orientation is maintained right up to the oscular edge, where there is nothing of histological distinction to warrant the conclusion that it constituted an active source of bio-electric potential or of a chemical substance.

The production of aberrant spicules has been explained by a laterally or

obliquely directed shear of the ambient mesogloea, and the normal arrangement of a sextet containing a spicule primordium also suggests that it is the result of a shear of the mesogloea in the direction of the oscular edge (Jones, 1954). The latter shear could arise either through a greater expansion of mesogloea in the more basal regions of the tube, or through a greater growth of the epithelium (contiguous with the sextet) at the oscular end, or through a combination of these two factors. The hypothesis will be presented in more detail in a later paper, but for the present it can be stated that the results described above can all be explained in similar terms. Thus in reversed tubes the mesogloea can be assumed to spread into regions of extension at each end of the tube, while the shear distally would be aided by the process of spicule-crowding at the distal edge. The formation of a bulge is probably the result of a localized contraction of the internal epithelium, which, coupled with a secretion of mesogloea at the apex of the bulge, would result in a radiating pattern of shear. Subsequently the epithelium at the apex of the diverticulum would begin active cell-division, the secretion of mesogloea there would cease, and the direction of shear would be reversed. Eventually a new osculum would open at the tip and development would proceed as in the main oscular tube. With the unmounted tubes the spread of the healing membrane across the cut basal end would draw the epithelia towards the base and thus produce a shear which would maintain the orientation towards the oscular end.

No small spicules were produced in which the optic axis was perpendicular to the wall of the tube, even in regions of irregular orientation where there would possibly have been no shear (fig. 3, A). Perhaps the sextets only start to secrete their spicules when there is a movement of mesogloea around them, extremely localized movements being the cause of the irregular orientation in such regions. Alternatively the stiffness of the outer layer of the mesogloea may be responsible for the tilting of the sextet when there is no shear, there being insufficient room between this layer and the internal epithelium for the outer three cells to perch on top of the inner trio. If this be the case, the variation in inclination of the optic axis along the tube (Jones, 1954) may be determined by differences in the thickness of the softer mesogloeal layer. Whatever may be the explanation for the inclination of the optic axis, a process of shear is a likely cause of the orientation of the sextet.

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