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ON THE SPICULE-FORMATION OF *SPONGILLA LACUSTRIS* (L.)

1. THE DEPENDENCE OF THE SPICULE-FORMATION ON THE CONTENT OF DISSOLVED AND SOLID SILICIC ACID OF THE MILIEU

BY

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INTRODUCTION[1]

In the fresh-water sponge, *Spongilla lacustris*, the spicules belong to two classes, viz. flesh-spicules, microscleres, which seem to be scattered about loosely in the sponge tissue, and skeletal spicules, megascleres, which go to form the main skeleton of the sponge.

The formation of spicules in the siliceous sponges has been the subject of many investigations. MINCHIN (1909) has given an excellent review of older works. Recent investigations on fresh-water sponges have been carried out by SCHULZE (1923), WIERZEJSKI (1935) and SCHRÖDER (1936). The accounts by different authors of the origin of the spicules contain considerable contradictions; among the views expressed with regard to *Spongilla* the following is the best-founded and agrees with my own observations. The spicules are laid down in a mother-cell as an organic axial thread, the nature of which is rather unknown. The axial thread is stated to be formed by quite small granules forming a line and fusing into a fine thread (SCHRÖDER 1936). Upon this thread silica secreted by the mother-cell is deposited. There is still some disagreement as to whether the mother-cell alone is able to complete the formation of microscleres while, on the other hand, the megascleres evidently need the aid of several secondary silicoblasts for the completion of their formation.

Microscleres and megascleres are said to be laid down in cells of different types. The skeletal spicules arise in "undifferentiated" archaeocytes with nucleolus containing nucleus,

[1] The present paper forms part of a series of investigations into the developmental physiology of Spongillidae, planned by the laboratory. Work already published is: H. V. BRØNDSTED: Formbildungsprozesse bei einem sehr primitiven Metazoon, dem Süsswasserschwamm *Spongilla lacustris* (L). I. Die Entstehung der äusseren Form. "Protoplasma" 1943, Bd. XXXVII, Heft 2.

while the microscleres originate from "specialized" cells with nuclei rich in granulae and without nucleous. (EVANS 1899). WIERZEJSKI states, however, that he has observed the formation of microscleres in cells of a type which is generally ascribed to megascleroblasts.

As regards the chemical compositions the spicules of the siliceous sponges consist mainly of SiO_2 (ca. 92 %). The greater portion of the remainder is water (ca. 7 %), but some MgO, K_2O and Na_2O also occurs. C and N do not appear in such quantities as to be demonstrated (BÜTSCHLI 1901). SOLLAS (1888) and other authors have carried out investigations into the quantity of water contained in dried spicules. Excepting the fact that THOULET (1884) found the water percentage of the spicules to be 13, all other analyses have shown a water percentage varying from 6 to 7 and but rarely a little more. It is difficult to say to what extent water is chemically bound, but from the fact that the presence of submicroscopic watery vacuities has been proved in the inner layers of relatively larger spicules (SCHMIDT 1926), it can at any rate be inferred that water may occur also in other ways.

Below special questions regarding the formation of spicules will be discussed, particularly the formation of microscleres, which has been investigated on gemmula sponges kept in water with varying contents of silica. It has been my special endeavour to clear up a possible state of inter-dependence between the silicic acid content of the surrounding medium and the content of spicules, their form and size, in the gemmula sponges. Finally the question has been examined, whether the sponges themselves are able to dissolve solid silicates, or whether their formation of spicules depends exclusively on the presence of dissolved silicic acid. Before mentioning the experimental investigations a brief account will be given of the first development of the gemmula sponge from germination until differentiation of the sponge tissue has commenced.

Germination, Spreading and First Differentiation.

The content of the gemmulae of *Spongilla lacustris* consists only of one single type of cells, viz. the undifferentiated two-nucleused archaeocytes (WIERZEJSKI 1935). After germination

the latter are modified into all the various cell-components of fully developed sponge. The cell material leaves the gemmulae through the preformed porus, which in the intact gemmula is covered with a thin lid. When leaving the gemmula the cell material is normally quite undifferentiated, but an initiatory differentiation of the cell-components in the ungerminated gemmula has, however, been recorded, which can proceed so far that the formation of canals, flagellated chambers and spicules can be observed (WIERZEJSKI 1935). I never noticed this myself. In the experiments described below the cells which have crept out of the gemmulae have, at any rate, always been undifferentiated and sharply limited.

While passing out of the gemmula, the cell material will gradually cover the shell of the gemmula and spread on the underlayer. The size of the area covered by a young gemmula sponge depends upon both the number of cells and the nature of the underlayer (BRØNDSTED 1943). Germination and spreading of the content of a single gemmula will last about 24 hours at room temperature, but by the end of that time the shell of the gemmula is not necessarily empty; some of the content may still be left.

In the course of the first 24 hours I did not observe any development of specific tissues, but an initiatory formation of syncyties does take place, especially at the borders of the young gemmula sponge spread on the underlayer. Smooth transitions can be noted between the isolated, centrally lying archaeocytes, which have just broken out from the gemmula, and the strongly distended syncytieforming cells in the peripheric parts of the sponge.

About 48 hours after the cells have broken out the formation of the spicules of the young gemmula sponges usually commences, it being possible to see quite thin spicules, less than 1 μ thick, scattered about in the sponge tissue. The megasclerformation seems to be more vigorous than that of the microscleres at the outset, but the former is soon out-distanced by the latter. At first the formation of spicules shows a distinct tendency to localization since the sponge hat many patches with numerous developing spicules and great intermediate areas of tissue without spicules or with a few developing ones only.

In the course of the second 24 hours the formation of the dermal epithelium will also begin; after three days it can be fully developed, covering the strongly developed subdermal room. At the same time the epithelium covered canals are laid down in the sponge tissue and the differentiation of the flagellated chambers and their functional union takes place. Simultaneously the epidermis is perforated for the formation

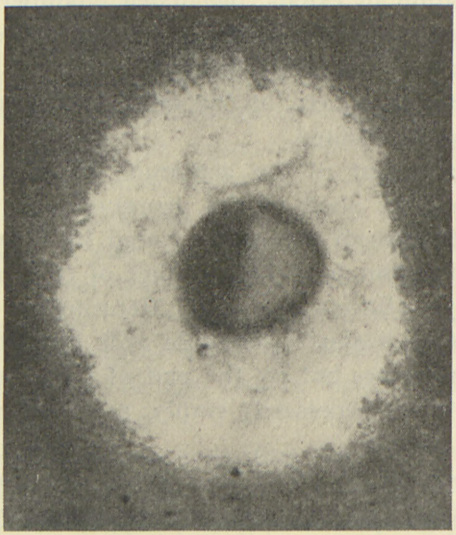

Fig. 1 a. Gemmula completely spread.

of osties, one or a few in each sponge, and also the oscular tubes are developed, likewise one or a few in each gemmula sponge, which thus passes into the functional stage (fig. 1 b). The diameters of the osties vary rather considerably. Three measurements showed the following lengths: 6, 20 and 40 μ. In spite of the fact that carmin granules have been observed to flow vigorously through the osties after carmin suspension has been added to the sponge cultures, I have never seen openings in the oscula on my own sponges. The easiest way of discovering the osties, which may otherwise be difficult to detect, is to add such suspensions. Outlets on such functional sponges have never been found in spite of numerous attempts.

The carmin granules are accumulated in the phagocytic cells of the nearest flagellate chambers, whereto they are carried

through a system of canals connected with the subdermal room. Carmin granules, however, are not found in the peripherical, flattened out portions of the sponge, where the flagellated chambers are primarily absent, and neither are they in the epidermis proper. Thus the cells of the epidermis do not, apparently, take up formed constituents although they are able to form pseudopodies; in other words, there seems to be no phagocytic action from the surface of the sponge.

Primarily, at any rate, the formation of microscleres takes place in the interior of the sponge, and during the first days

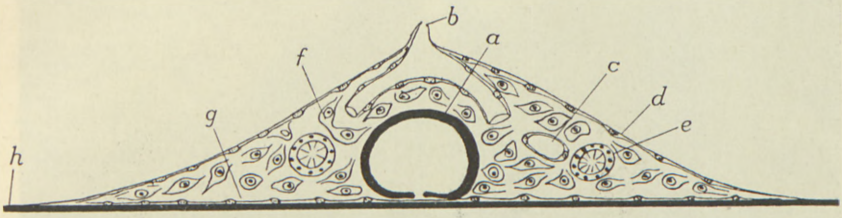

Fig. 1 b. Diagrammatic vertical section of a germinated gemmula.

after their appearance the microscleres hardly ever occur in the epidermis. But as early as two or three days after the first ones have been laid down the microscleres are carried to the surface of the sponge, where they are gradually placed in great numbers in the epidermis proper. Many are also placed in the epithelium covered walls of the subdermal room and canals. It is not only the fully formed spicules, which move peripherically, but spicules at every stage of development are met with in the epidermis at this juncture, all enclosed in their respective spiculiblasts. The larger spicules, however, prevail, while quite young ones are rare.

The development of the megascleres is not exactly known. They arise in the same places as do the microscleres. At their first appearance no definite arrangement or orientation in the sponge tissue can be detected; but gradually, often in the course of a week, they can be seen lying separately or in bundles of 5 to 6 spicules, placed more or less at a right angle to the surface of the sponge, their free ends projecting. The microscleres of the epidermis are, on the contrary, always placed on a level with it (fig. 2).

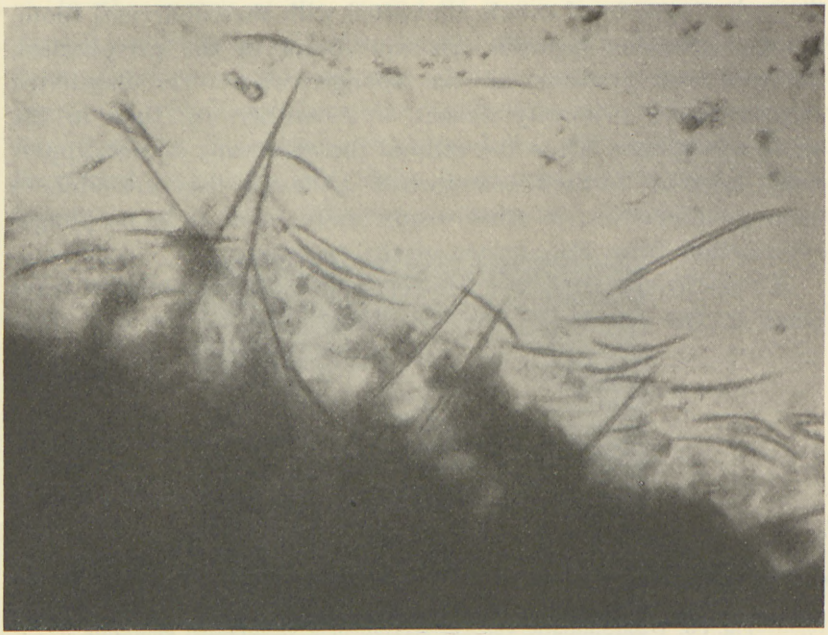

Fig. 2. The edge of a gemmula sponge 11 days old from a culture with 0.32 mM SiO_2. The megascleres are placed at a right angle to the surface of the sponge, while the microscleres lie in the epidermis proper. The subdermal room is easily distinguished above the deeper lying more compact sponge tissue. (× 190).

As regards the rate of growth no systematic experiments have been undertaken, but it may to some extent be estimated. In gemmula sponges kept in tap water, containing sufficient silicic acid as to permit a strong production of spicules, the first scattered microscleres, less than 1 μ thick, were discovered about 24 hours after germination and spreading. On examination 40 hours later the sponges showed the presence of numerous microscleres of 3 to 4 μ thick. 24 hours later a still greater number of microscleres had attained these dimensions, but none exceeded them. That is to say that the spicules can attain their full size, in this case 3—4 μ, in less than 40 hours at the temperature, silica concentration and in the experimental conditions used. Since it must be supposed that the rate of growth depends on many various factors e. g. the silicic acid content of the medium, a thorough investigation on this problem is strongly required.

Material and Technique.

The gemmula which were isolated for the experiments were as far as possible cleansed of adhering skeletal remains and loose spicules. The germination and development took place in paraffined *petri* vessels with 9 cm.'s openings containing about 50 ml double-destilled water, to which was added the necessary salts together with silica in the form of Na_2SiO_3, 8 H_2O in varying quantities. The second destillation was carried out in a glass-retort, and the water destilled was collected in a paraffined flask. The composition of the salt solution without silica is given in table I.

Table I.

	Cl^-	SO_4^{--}	Na^+	K^+	Ca^{++}	Mg^{++}
Milliequivalents	0.86	0.43	0.36—0.52 (2.56)	0.04	0.68	0.16

Each culture contained about 50 gemmulae, which during the germination partly gave rise to separate sponges, and partly constituted colonies containing varying numbers of gemmulae. The p_H values of the cultures were about 7 to 7.5 during the experiment excluding only the glass vessel with the greatest content of SiO_2 (1.28 mM) where p_H was nearer 8.

The formation of spicules commenced immediately after germination and spreading had finished. When the young sponges were 4 days old—that means about 4 days after germination and spreading, which lasted about 24 hours in the case of all gemmulae—the experiment was interrupted, and the sponges were fixed in 70 % alcohol.

The formation of spicules was estimated by counting and measuring the formed spicules, which were cleansed in the following manner. The young sponges were placed in a hollow-ground slide and covered with a few drops of 70 % lactic acid, which was carefully heated over a small gas-flame until it started boiling. In the course of a few minutes the soft bodies had entirely dissolved. Thereupon the solution was diluted with water and counts and measurements were carried out on the preparation treated in this manner, where the loss of spicules

was completely avoided. Lengths and thicknesses of the spicules were measured by means of a measuring ocular. In the following the measurements of the spicules are given in units of measurements at the degree of magnification used, unless otherwise started. 1 unit = 0.206 μ.

The method of measurement was quite simply as follows. All the spicules, which entered the field of vision as the preparation was carried before the objective, were measured. A cross-table served to conduct the object. In this way any special selection of spicules was avoided. As a rule, the thicknesses of the spicules have only been measured on the regularly formed spicules without middlethickenings and outgrowths, and the curves for the variation of the thickness of the spicules were based exclusively on slender regular spicules.

As regards the counting of the total number of spicules, which was made by means of a net ocular, all spicules were counted in one single case, but this method was found to be too laborious. In all other cases the spicules were only counted on a rectangular area, the breadth of which was equal to the side of the biggest square of the net ocular, which could be seen in the field of vision without the corners cut off and the length of which was equal to the diameter of the preparation (about 20 times the breadth). The counting was most frequently carried out along two diametres at right angles to each other, and in such cases about $^1/_8$ of the whole area containing spicules has been counted. Owing to the hollow grinding of the underlayer the spicules are inclined to concentrate towards the middle of the preparation, and the total number of spicules has therefore been calculated from the formula $2\pi \sum r \cdot n$. r is the distance from the centre of the preparation, using the side of the net ocular as a unit of measurement, and n is the number of spicules in the square at such a distance. In this way two or four values for the total number of spicules were obtained, according as the countings had been carried out along one or two diameters respectively. On the basis of the greatest and smallest number of spicules found and the volume of gemmulae, which gave rise to the spicules, the number of spicules per volume unit of gemmulae was calculated. The numbers of spicules thus obtained may directly be compared.

Formation of Spicules at Various Silicic Acid Concentrations.

At the outset the silicic acid content may be considered to influence the following factors during the formation of spicules, viz. the number, length and thickness of the spicules. In order to examine if such a relation does actually exist gemmulae were kept in cultures with increasing silica content. The composition of the culture medium without silicic acid has been referred to on pag. 9. To each of the six paraffined *petri* vessels containing 50 ml of this weak salt solution sodium silicate was added in such quantities that the following concentrations were obtained (table II).

Table II.

Culture	Quantity added of Na_2SiO_3, 84_20 in mg	SiO_2 in mM
Si 0	0	0
Si 2	0.53	0.02
Si 4	1.06	0.04
Si 8	2.12	0.08
Si 16	4.25	0.16
Si 128	34.00	1.28

Thus the various cultures differed both as to the content of silica and sodium. It is, however, supposed that the variations in the sodium content are without influence on the sponges and thereby also on the formation of spicules. As matter of fact only about 2.5 mM Na^+ is found in culture Si 128, while *S. lacustris* has been found in nature in localities, which can, at any rate have a salt promille of 1.6 = ca. 27.5 mM Na.

Just after the silicate had been added, the p_H values of the cultures with a fairly great silicate content were rather strongly displaced in the basic direction, but in the course of a few days the values were 7.0 to 7.5 in all the vessels, excluding that with 1.28 mM SiO_2, where the p_H value was nearer 8.

When the reaction proved stable, 50 to 60 newly gathered gemmulae were placed in each vessel (17th April 1942). As early as the next day the germination of some gemmulae had started in most of the vessels. On the 21st all gemmulae had germinated and spread, and there was a strong formation of

spicules. On the 23rd the experiment was interrupted, and the sponges were fixed in 70 % alcohol.

a) Number of Spicules.

The counting of the number of spicules was carried out exclusively on sponges, where the gemmulae as far as possible had been quite emptied, and with empty gemmula shells, which had still kept their globular shape, thus making it easy to calculate their volume. Each culture contained only a few sponges complying with these demands. The result is given in tables III and IV.

The results of the counts of the number of microscleres are shown in table III. The table also comprises the number of spicules in sponges from experiments, which will later on be referred to (pag. 39). In the latter the gemmulae were kept in cultures of various substratum, as e.g. the spicules of the siliceous sponge Jophon sp. and pulverized ortoclas. These series of experiment swere carried out simultaneously, the gemmulae used in both series originating from the same sponge. The figures can accordingly be directly compared. The lines 1—2 give the smallest and greatest total number calculated on the basis of the counts made along a radius. 3 denotes the number of radia along which the counting has been undertaken. 4 shows the volume of the gemmulae giving rise to the sponge, from which the spicules have been counted. 5 denotes the number of gemmulae composing the sponge, and 6—7 give the number of spicules formed per 0.01 mm^3 gemmulae, calculated on the basis of the total numbers from line 1 (6) and from line 2 (7).

The number of megascleres for the same sponges, whose microscleres were counted, are shown in table IV.

As the two types of spicules in question behave somewhat differently under the influence of silicic acid content of the water, each type will be separately considered, and the conditions of the microscleres will first be dealt with.

It may be seen from the table that the counting of the number of spicules is most uncertain, owing to the difficulties in distributing the spicules evenly in the preparation. It appears, however, from the calculated number spicules formed per volume unit of gemmula in various cultures, that the number of spicules

Table III.

Nr.	Si O	Si 2	Si 4	Si 8	Si 16	Si 128_1	Si 128_2	Iophon par a	Iophon par b	Ortoclas par a	Ortoclas par b	
1	<10	3100	4300	2300	1600	2600	1700	3300	2900	6200	3500	Total number of spicules (lowest and highest number calculated).
2		6200	7200	2500	2800	4600		5500	3800	8800	4700	
3		2	4	2	4	4	Directly counted	4	4	4	4	Number of radies counted.
4		0.277	0.250	0.155	0.125	0.212	0.125	0.176	0.209	0.414	0.212	Volumen of gemmulae in mm³.
5		2	2	2	1	2	1	2	3	4	1	Number of gemmulae.
6		140	170	150	130	120	140	190	140	150	160	Lowest and highest number of spicules per 0.01 mm³ gemmulae.
7		270	290	160	220	220		310	180	210	220	

Table IV.

Nr.	Si 2	Si 4	Si 8	Si 16	Si 128_1	Si 128_2	Iophon par a	Iophon par b	Ortoclas par a	Ortoclas par b	
1	640	800	130	410	280	Directly counted 80	400	320	760	320	Total number of spicules (lowest and highest number calculated).
2	1020	1320		630	390		950	480	1300	580	
3	28	32	8	33	13	6	23	15	18	15	Lowest and highest number of spicules per 0.01 mm³ gemmulae.
4	45	53		50	18		54	23	31	17	

for sponges from all the cultures fall within nearly the same interval of spreading—that is to say, are of the same size. Apparently, the number of spicules do not in the least tend to increase at higher concentrations of silicic acid. Sponges from the culture Si 2 have shown the same intensity of spicule-formation within the variations occurring in the counts as have the sponges from the culture Si 128. This applies also to the sponges from the culture with ortoclas compared, for instance, with those from the Jophon cultures, in spite of the fact that the silicic acid content of the latter, as will appear later (pag. 42) has been considerably higher than the concentration of the culture where ortoclas is the source of the silicic acid. On the basis of the results given in table III we are, however, certainly justified in saying that if the size of production of spicules increases somewhat with the increasing silica content of the culture medium, viz. from 0.02 to 1.28 mM SiO_2, this effect must be considered rather slight, at any rate, when compared whit the rise in intensity of production due to a change in the silica content from 0 to about 0.02 mM SiO_2. As shown in the table hardly any microscleres are formed in the culture, which is (approximately) without silicic acid. The quite few spicules per gemmula were also very thin (hardly 1 μ thick).

The above mentioned facts lead to the conclusion that the formation of microscleres apparently reaches its maximum rate already at rather low silicic acid concentrations, that is to say, the laying down of microscleres does not increase, even when greater quantities of silica are placed at the disposal of the sponge.

A microscopic investigation of the gemmula sponges from the culture Si 2 showed that 0.02 mM SiO_2 evidently comes near to the lowest limit at which the formation of microscleres may take place to its full extent at the temperature used (room temperature) and in the period that the experiment lasted. The fact is that in some of the sponges the formation of microscleres was found to be defective, varying from hardly any production through medium stages to a normal number of spicules, as represented in the culture Si 2 recorded in table III. (A defective formation of microscleres has not been observed in healthy sponges belonging to any of the other cultures with greater silica content.)

Unfortunately the number of spicules has not been counted on sponges with a reduced production of spicules in the culture Si 2, but the fact that the number of microscleres may be reduced appears indirectly in one case from an estimation of the relation between the numbers of microscleres and megascleres. As mentioned in the following, the megascleres make about 10

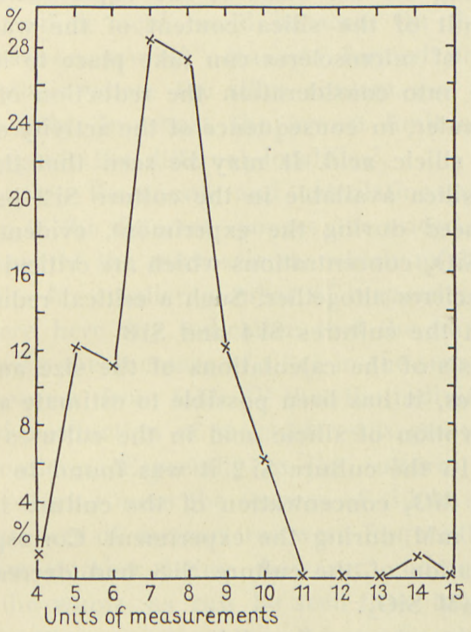

Fig. 3.

to 25 % of the total number of spicules formed, when the content of silica is sufficient to allow a formation of microscleres to its full extent. At silica concentrations, which are too low for the development of the highest number of microsleres during the period of the experiment, the megascler-formation has, apparently, not yet been appreciably retarded. Even with the extremely small quantities of silicic acid, which may have been contained in the culture Si 0, the production of megascleres is rather considerable. As, therefore, it was found that the megascleres made 46 % of the total number of spicules in the preparation $Si\,2_5$ (table V), and that all microscleres were rather thin (curve $Si\,2_5$ fig. 3), this must mean that the formation of

microscleres has been reduced in this sponge on account of too low silica concentrations. As a matter of fact it must be considered extremely unlikely that the number of megascleres should absolutely have been far higher in this preparation than is otherwise the case; neither is this indicated by microscopic observations.

In estimating on the basis of the experiments in question the lowest limit of the silica content of the water, at which the formation of microscleres can take place to its full extent, we must take into consideration the reduction of silica in the surrounding water, in consequence of the activity of the sponges in taking up silicic acid. It may be seen that the quite small quantities of silica available in the culture Si 2 have been considerably reduced during the experiment, evidently they have decreased to SiO_2 concentrations which are critical to the formation of microscleres altogether. Such a critical reduction has not taken place in the cultures Si 4 and Si 8.

On the basis of the calculations of the size and number of the microscleres, it has been possible to estimate approximately the total absorption of silicic acid in the cultures Si 2 and Si 8 (see pag. 26). In the culture Si 2 it was found to be about 20 γ SiO_2, i. e. the SiO_2 concentration of the culture has decreased to about 0.01 mM during the experiment. Correspondingly the SiO_2 concentration of the culture Si 8 had decreased to about 0.06 to 0.05 mM SiO_2.

Germination and spreading did not occur quite simultaneously in all gemmulae, but the processes on the whole lasted about 24 hours that is to say that, at the time when the sponges first germinated have actually started the spicule-formation, the last ones are still germinating. Thus the formation of spicules of the latter will, as a rule, begin at a slightly lower SiO_2 concentration than that of the sponges first germinated. It is most probable, therefore, that it is just these gemmulae last germinated which exihibit the reduced numbers of microscleres. We may, therefore, conclude that the lowest limit for obtaining the full number of spicules is a little below 0.02 mM SiO_2. This low limit is, however, doubtless subject to individual and racial variations, and depends upon other factors in its surroundings.

This fact is evident from JEWELL's most interesting experi-

ments on the ecology of fresh-water sponges (JEWELL 1935). She has among other things examined the SiO_2 content of the water in a long series of localities in which *S. lacustris* was present, and she found that the typical form containing well-developed spicules only rarely occurred at a silicic acid content as low as 0.005 mM SiO_2. In a single case the sponge containing well-developed spicules were found in a locality, where only traces of silicic acid could be seen. On the other hand, the species had on several occasions formed no microscleres at all in localities where the silica content was between 0.005 and 0.01 mM SiO_2. In short, the lowest limit of silica is not constant as regards the different localities[1].

With regard to the counts and calculations of the number of megascleres in the different sponges, the uncertainty is greater than in the case of the microscleres, which might be expected, considering the far smaller number of megascleres formed; but neither is there here any indication that the number of megascleres rises with the increase of silica in the surrounding water. It appears, however, from table IV that the individual variations in the rate of production of megascleres is greater than in the case of microscleres. Especially in the cultures where *Jophon* spicules and ortoclas were the sources of silicic acid, comparatively low numbers of megascleres were frequently found, without these low number being, however, characteristic of the cultures on the whole, as can be seen from table V. Here the numbers of megascleres are expressed in a percentage of the total numbers of spicules, which have been directly counted, i. e. all the spicules of the preparation, which have entered the field of vision have been counted. In the tables the figures Si 2, Si 4, Si 16, Si 128_1 and 128_2, *Jophon* par a and b, ortoclas par a and b originate from counts, on the basis of which the absolute numbers of the table III and IV have been calculated. All the other megascler percentages originate from the counts which have been undertaken simultanously with the measurements of thick-

[1] In this connection another factor must likewise doubtless be taken into consideration viz. the state of the silicic acid in the surrounding water—that is, whether it appears in an ionized, molecular or more or less polymerized form, as it is reasonable to suppose that the absorption of silicic acid by the sponges depends on these conditions. Investigations dealing with this problem are being carried on.

Table V.

Culture- and preparation-marks	Total number of spicules	Number of megascleres	Megascleres expressed in % of total number
Si O	83	79	95
Si 2_5	156	72	46
Si 2	441	83	19
Si 4	1302	346	25
(Si 8	138	8	6)
Si 16	521	124	24
Si 128_1	1117	78	7
Si 128_2	1780	80	4
Si 128_3	466	24	5
Si 128_4	1660	115	7
Iophon par a	586	79	14
» » 3	255	55	22
» » b	515	57	11
» needles 2	254	44	17
Ortoclas par a	1185	162	14
» » b	647	80	12

ness and length of the spicules. Table V shows that although the percentage of megascleres undergoes rather considerable variations, it has, however, only once exceeded 25 % at silica concentrations of between 0.02 and 0.16 mM SiO_2, namely in the culture Si 2_5, as has been explained above, and the percentage does not drop lower than 10. As mentioned above, the fluctuations, if not altogether due to the uncertainty of the counts, are most likely due to individual variations of the number of megascleres.

If we descend to quite low values of silica content—at 0.02 mM SiO_2 we were alredy near the limit during our investigations —it was seen that the formation of megascleres was far less retarded than was the formation of microscleres. Even sponges from SiO contained a fairly large number of megascleres, all of which were, however, quite thin, about 1 μ thick. As the silicic acid content of this culture can only have been extremely low, we are almost forced to suppose that the spicules were

formed from silica which was present in the gemmulae before these were placed in the water free of silicic acid. This view is also strengthened by records from other investigators (see WIERZEJSKI 1935) regarding the fact that the formation of spicules may arise in the gemmula before germination has commenced. As spicules do not enter the gemmula at its formation, they have consequently been formed either from the stores of silicic acid in the gemmula, which were present at its formation, or—and this is perhaps most likely—they have been formed from silica absorbed by the gemmula from the surrounding water.

Another possibility is that the quite thin spicules which were found in the sponges of the culture SiO consist mainly or completely of organic substance—in fact, we are dealing with the axial thread.

The fact that the formation of megascleres can take place with lower quantities of silicic acid in the water than that of the microscleres, indicates that there is a physiological difference between the respective spicule-forming cells, and thus supports EWAN's statements that both microscleroblasts and megascleroblasts are present. Observations on the formation of megascleres at the highest silica concentration in our experiments likewise confirm this theory.

The number of megascleres is comparatively small in the sponges of the culture Si 128. It appears from table V that the percentage of megascleres is below 10 in all cases examined, and if we compare the tables III and IV, it is seen that the drop is absolute. There is no sign to indicate an increased production of microscleres, while the number of megascleres is, apparently, lower than that of the sponges examined from the other cultures. (Exluding only the sponge examined from Si 8, which is, however, no true representative of the formation of spicules of this culture. As the number of microscleres seems to have been very small, the sponge is considered to have been defective. Unfortunately there are no other counts available of the number of spicules of this culture). Accordingly it appears that the megascler- and microscler-forming cells have reacted differently in the culture with a high silica content.

It is difficult to say if the drop in the number of megascleres indicates an injury to the spicule-forming cells, due directly

to the high silicic acid concentration. It must, however, be mentioned that the outgrowths on the spicules occurred far more frequently in this culture than in those with lower SiO_2 contents (table VII). As the outgrowths are considered an abnormity, this phenomenon suggests that the large silica content has an injurious effect on the sponge tissue, and that the megasclerforming cells are affected much more by the high silica content of the water than are the microscler-forming cells. As previously

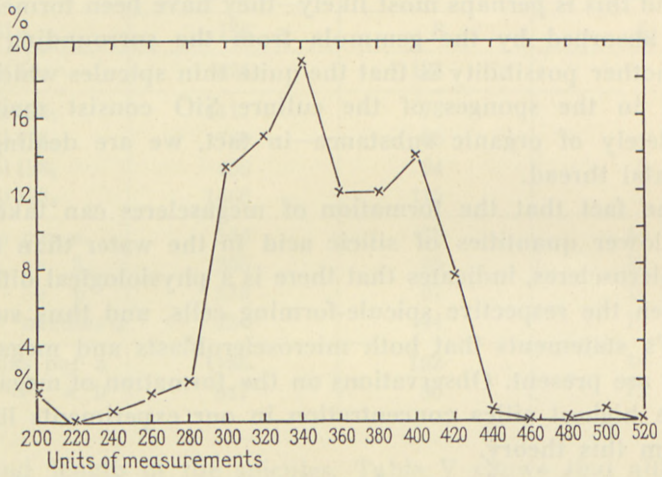

Fig. 4.

mentioned it is unlikely that a higher Na^+ content should bring about any reaction on the part of the sponge, nor should it be responsible for the formation of outgrowths. Also the somewhat higher p_H value might be left out of consideration. JEWELL (1935) has found normal *Spongilla lacustris* in water-courses with a p_H value of 8.6.

b) The Length of the Spicules.

The length of the microscleres of the young gemmula sponges varies considerably. Values ranging right from about 40 μ to about 110 μ have been found, but by far the most are between 60 and 80 μ. A typical distribution of the spicules according to length is given in fig. 4, which shows the measurements from $Si\,8_5$. Almost all the short spicules under 50 μ will prove to be quite young and newly laid down, but among the quite short

Table VI.

Si 0		Si 2		Si 4		Si 8		Si 128		Adult sponge	
Average length in μ	Number	Average length in μ	Number	Average length in μ	Number	Average length in μ	Number	Average length in μ	Number	Average length in μ	Number
290	4	330	100	350	200	350	175	355	100	340	100
..	..	330	100	..	..	365	200	..	..	..	..
..	..	340	300	..	..	350	286	..	..	..	..

spicules we may also find thicker ones with a more finished appearance. On the whole, there is only a slight correlation between the length of the spicules and their thickness, i. e. their age, as can be seen from fig. 5. The spicules are here laid down in a co-ordinate system, where the abscissae are the thicknesses of the spicules, and the ordinates are the length of the same. Only this single example has been recorded, as all the other cases are quite similar. It will be noted that the final length of a spicule is usually far longer than that of the newly laid down axial thread and spicule, but the increase in length taking place during the growth of the spicules, varies considerably, and no length seems to be especially preferred within the interval of 60 to 80 μ. This also applies to all the sponge preparations examined, no matter to what culture they belonged. If the silicic acid content of its surroundings has any influence on the length of the spicule, it must be very slight.

The average length of the microscleres from the various cultures are shown in table VI. The somewhat lower average lengths of Si 0 and Si 2 are due to the higher percentage of quite young spicules present in these cultures. The table also includes the average length of the spicules from an adult *Spongilla*, which was taken from the same locality as the gemmulae used in the experiments.

On the basis of the available material, nothing could be decided with certainty concerning the relation between the lengths of the megascleres and the silicic acid content of the water, because of the smaller number and greater variations in length of the spicules, but there is no sign to indicate that the megascleres and microscleres behave differently in this respect.

c) The Thickness and Volume of Spicules.

While it could not be demonstrated that the intensity in formation, the length of the spicules and the silicic acid content depended on each other, the average thickness of the spicule was found to increase with the increasing silica content of the liquid of the culture. Typical microscleres from two different

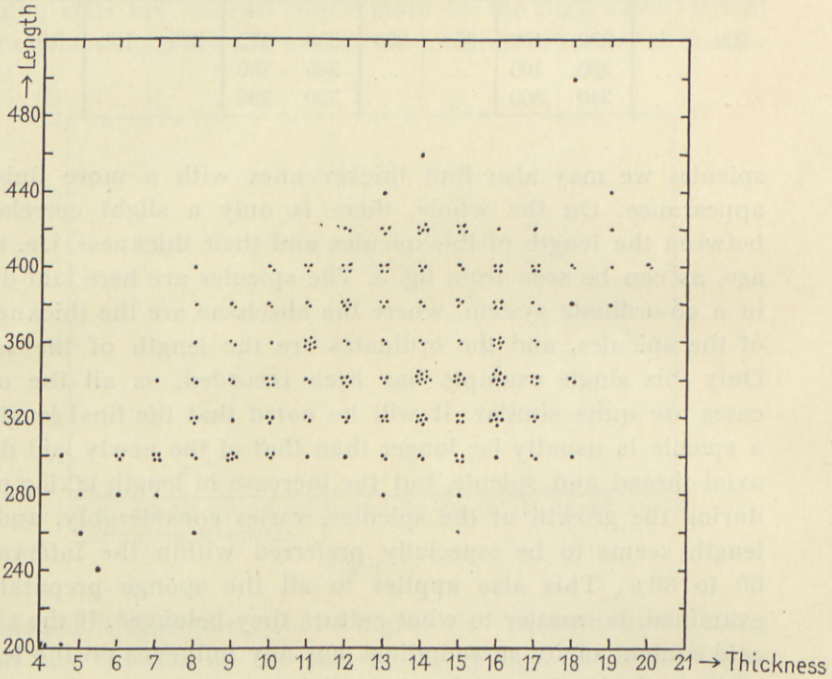

Fig. 5.

silica concentrations are shown in figs. 6 and 7. Figs. 8 and 9 show the distribution of the spicules according to the various thicknesses expressed in a percentage of the total number of microscleres measured from Si 2, Si 4, Si 8 and Si 128. Each of the curves represents the average of several gemmula sponges, each originating from one or more gemmulae. It is striking to note, how the number of microscleres, grouped round thicknesses of 8 to 10 measuring units decreases with the increase of SiO_2 content of the culture medium, while the number of microscleres, which reach the greatest thicknesses, is correspondingly increased. Of the 571 spicules measured, on the basis of which the curves

for Si 2 have been drawn, only about 20 percent exceeded 11 units, i. e. were c. 2 μ thick; of 569 spicules from the culture Si 4 about 35 % exceeded 11 units, of 557 spicules from the culture Si 8 about 65 %, and of 344 spicules from the culture Si 128 about 75 %. For comparison a curve is given (fig. 10) for the distribution of the thickness of the microscleres of an adult *Spongilla*. The individual, from which the spicules are derived, has been taken in the same locality as the gemmulae. The spicules show a distribution similar to a curve of normal distribution. We note, however, that at the same time a certain number of young thin spicules are present, which must be supposed to originate from the zone of growth in the sponge tissue. The curve is based on 400 measurements of thickness.

It is difficult to say, what the above mentioned curves for the distribution of the thickness of the microscleres signify, as their courses are due to an interplay between rather an unknown number of factors, the quantitative effect of which are practically unknown, Some of these factors may be mentioned: continuous but possibly varying new-formation of the spicules, decreasing silicic acid concentration in the culture medium during the experiment, the effect of which is presumably stronger, the lower the original concentration has been, rate of growth of the spicules, which is considered to rise with increasing supply of silica, individual variations and so on.

Hence it appears that we must deal with the curves most carefully, and not draw too many conclusions from their special courses, until we have a clear understanding of the importance which may be ascribed to the different factors, influencing the courses of the curves. But in any case, the thicknesses of the spicules and their distribution may even now lead to certain conclusions.

When the production of spicules has lasted 3 to 4 days, we may certainly conclude that the largest microscleres found in each of the different cultures have nearly attained those values for thickness which can on the whole be reached at the silica concentrations used, with the material employed and under the conditions of experiment in question[1]. As may be seen, these values

[1] This is doubtful only in a single case viz. the culture Si 2. As early as the day before the fixation, however, the thicknesses of some spicules in this culture were observed to be of the same size as the largest ones from the fixed sponges.

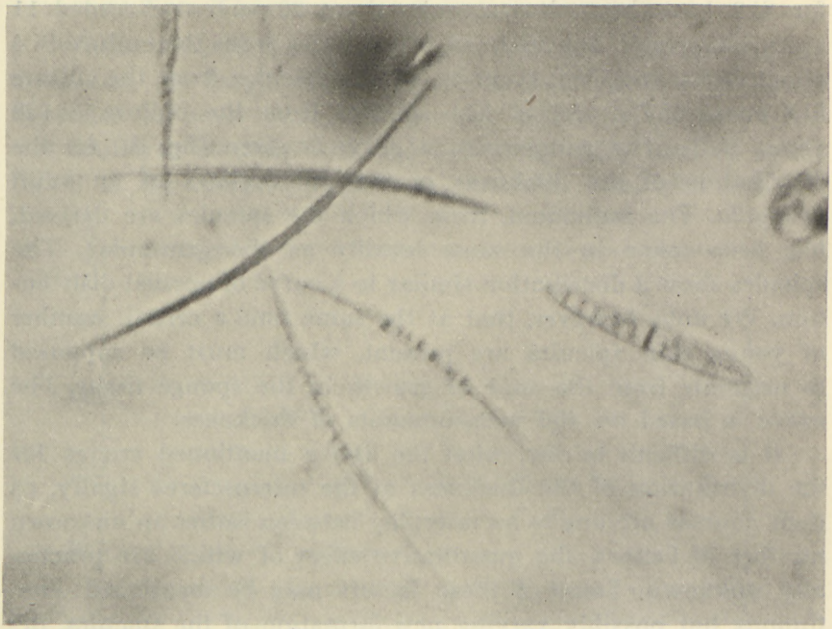

Fig. 6. Microscleres from a culture with 0.02 mM SiO_2. The spicules are slender and without middlethickenings. (The formation showing a dark cross line on the middle is a fungus germ.) (× 740).

for the thicknesses of the spicules lie higher, the higher the silica content of the culture is. It appears furthermore, as mentioned above, that the number of spicules, which attain the greatest thicknesses characteristic of each culture, are larger, the higher the silica content of the culture has been. Quite analogous conditions are also met with in the cultures with various solid silicates as substrata (see fig. 13, 14 and 15). The experiences obtained can be summarized in the following view on the action of the spicule-forming cells:

The easiest way to explain the fact that at the end of the experiment, the average maximal thicknesses were found to be the largest in the sponges from the cultures with the greatest silica content, is, to assume that the functional stage of the microscler-forming cells is limited, and that the spicule-forming faculties of each cell are exhausted, wholly or partly, within a certain space of time, in our experiments probably in the course of a few days. After the elapse of this time, further growth is

Fig. 7. Microscleres from a culture with 0.64 mM SiO_2. Three out of four spicules with more or less distinct thickenings. (× 740).

only minimal. The thickness, which can be attained by a spicule, thus depends on the quantity of silicic acid supplied to the scleroblast, while still in possession of its spicule-forming ability. Hence it follows that the rate of growth of the spicules increases with an increasing silica content of the culture medium. This view is further strengthened by the fact that with an increasing silica content, an increasing percentage accumulation of spicules takes place in the final thicknesses of spicules, characteristic of each of the silica concentrations. This may, however, also be due partly to the decreasing silica concentration of the various cultures.

Partly to estimate the absolute drop in the silica content during the experiment, but mainly to find out how the distribution of the spicules was according to volume, approximate calcutations of the volumes of the spicules from the cultures Si 2 and Si 8 were made, these being the only cultures from which a sufficient number of simultaneous measurements of the lengths and thicknesses of the microscleres were available.

The volumes of the spicules were calculated according to the formula for a spheroid (see pag. 32). The distribution according to volume is shown in the curves on fig. 11, where the numbers falling within the different size-groups are expressed in percentage of the total number of spicules. The curve "Si 2" has been drawn on the basis of 489 measurements; "Si 8" is based on 558 measurements.

From the measurements of the volumes, it is possible to

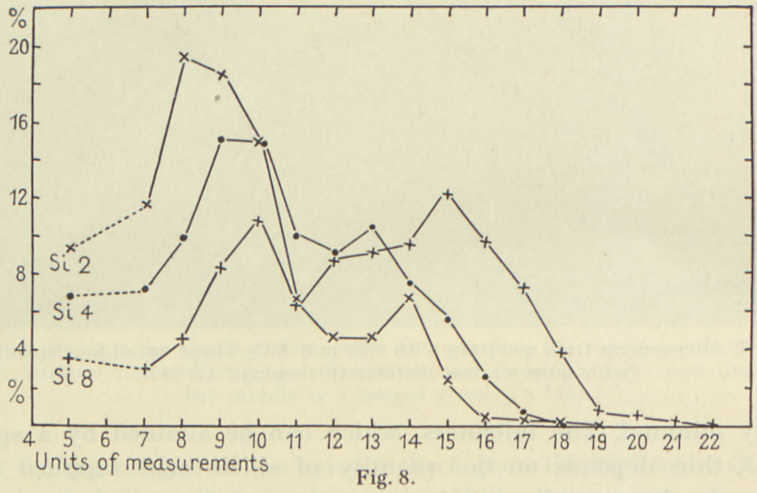

Fig. 8.

estimate the quantity of SiO_2, which has been accumulated in the spicules, although the estimation is most uncertain, especially owing to our incomplete knowledge of the total volume of the megascleres and of the actual number of spicules in the whole culture.

In the Si 2 culture the average volume of microscleres is 127 μ^3. If the volume of the axial thread is reckoned at 23 μ^3, the specific gravity at 1.96 and the water content at 15 % (see pag. 35) it results in the average silica content of each spicule being 0.000173 γ SiO_2. In the culture Si 2 about 50 developed gemmula sponges were found, of which the average number of spicules must be considered as having been at most 1500. The total content of the microscleres has thus been about 13 γ SiO_2. The average volume of the megascleres has roughly been calculated at about 250 μ^3, corresponding to the length of a spicule

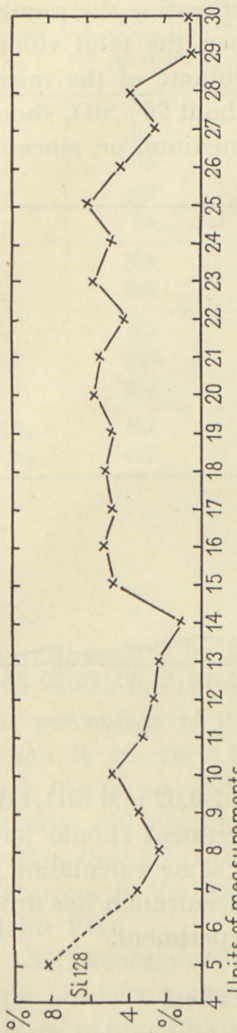
%
8
4
%
Si 128
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Units of measurements

Fig. 9.

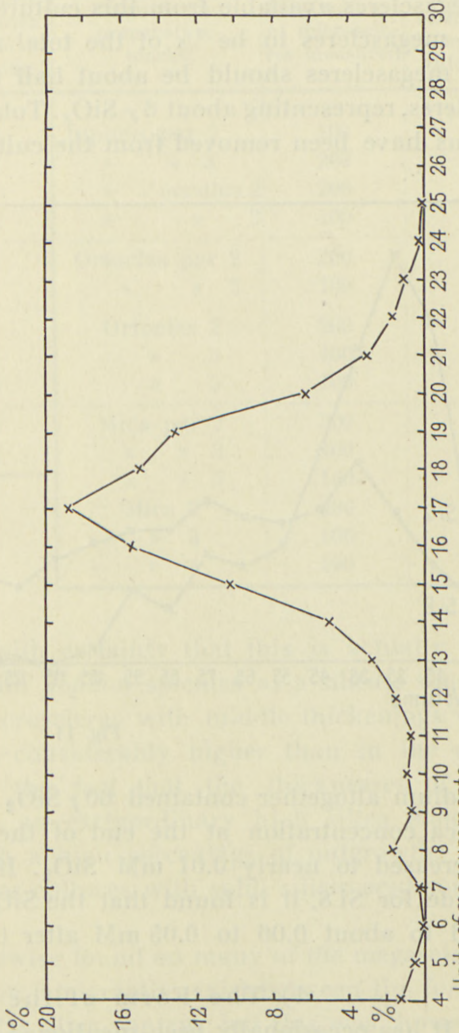
%
20
16
12
8
4
%
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Units of measurements

Fig. 10.

of about 120 μ and a thickness of about 2 μ. (This represent the average of the only 10 incidental measurements of the megascleres available from this culture.) Estimating the number of megascleres to be $^1/_5$ of the total number, the total volume of megascleres should be about half the volume of the microscleres, representing about 6 γ SiO_2. Totally about 20 γ SiO_2 should thus have been removed from the culture medium, or, since the

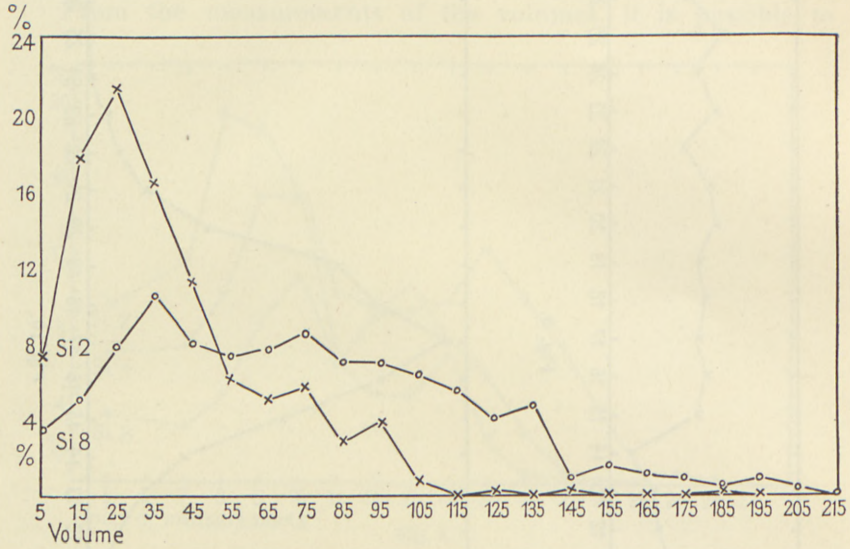

Fig. 11.

medium altogether contained 60 γ SiO_2 (50 ml 0.02 mM SiO_2) the silica concentration at the end of the experiment should have decreased to nearly 0.01 mM SiO_2. If the same calculation is made for Si 8, it is found that the SiO_2 concentration has dropped to about 0.06 to 0.05 mM after the experiment.

d) The Form of the spicules.

It has occasionally been mentioned in the above that microscleres with middlethickening sometimes occur to the sponges and their frequency increases with increasing silica concentrations. This appears clearly from table VII (Compare also figs. 6 and 7). It is tempting to suppose that there is a direct causal relation between the percentage of the microscleres with outgrowths and the silica content of the surrounding water. How-

Table VII.

Culture- and preparation-marks	Total number of microscleres measured	% of microscleres with middle thickening	Culture- and preparation-marks	Total number of microscleres measured	% of microscleres with middle thickening
Si 2_2	102	2.0	Jophon par 2	100	85
Si 2_3	100	2.0	» » 3	200	88
Si 2_4	299	2.3	» needles 2	200	74
Si 2_5	83	2.4	» » 3	100	90
Si 4_2	208	2.9	Ortoclas par 2	200	1
Si 4_3	408	10.0	» » 3	100	2
Si 8_3	178	14.0	Ortoclas 2	200	5
Si 8_4	205	16.1	» 3	100	6
Si 8_5	285	18.6	» 5	100	3
Si 128_2	444	56.8	Mica par 2	300	4
Si 128_3	442	65.8	» » 3	100	6
			» » 5	140	4
			Mica 2	200	4
			» 3	100	5
			» 4	100	4

ever, it cannot be said with certainty that this is actually the case, as in the culture with *Jophon* spicules as a silicate source, the percentage of the microscleres with middle thickenings was found to be very high—considerably higher than in the culture Si 128—in spite of the fact that the thicknesses of the spicules did not indicate an extraordinary high silica concentration (see fig. 15). Such a high percentage of outgrowths did not occur in the two other cultures with solid silicic acid source (table VII).

Thickenings were likewise found on many of the megascleres, and alsohere a correlation, apparently, exist between the formation of outgrowths and the silica content; but this was, however, not statistically ascertained.

Mechanism of the Formation of Spicules.

Statements dealing particularly with the mechanism of the formation of spicules are rather rare in literature. Such speculations are, however, always based primarily on the view advo-

cated by BÜTSCHLI that the spicules are congealed silica gels. SCHRÖDER (1936) is the only author who has tried to lay down a theory for the mechanism of the growth of spicules. The safe suppositions for his theory are as follows: The spicules are of intracellular origin, arising in the form of the organic axial thread, the full length of which has, however, not yet been attained. The further growth of the spicules takes place by apposition of a siliceous substance in such a way that the spicule grows in thickness primarily accompanied by a simultaneous growth in length, corresponding to the lengthening of the axial thread. As a rule, however, the final length seems to have been attained before the spicules has reached its full thickness. This is a brief outline of the observations made by many investigators since the days of CARTER about the middle of last century.

SCHRÖDER is now of opinion that he has observed in his preparations of the spicule-forming gemmula sponges of *Ephydatia mülleri* the presence of vacuoles with silicic acid gels, which would place themselves close to the developing spicule, covering it and thus involving its growth. SCHRÖDER states, moreover, that he has found vacuoles on fixed material, farther removed from the spicule and nearer to the surface of the cell, which he believes to contain silicic acid sol.[1] He therefore supposes that the sol travels from the periphery of the spicule-forming cell into the centre, becoming more and more concentrated and ultimately gelatined. Finally when the gel has completely reached the spicule it spreads and covers it and is congealed. For various reasons, which will be referred to below, I doubt the correctness of theories of this kind.

1) When observing living spicule-forming gemmula sponges, one of the most conspicuous features met with is the presence of lively and continuous currents of protoplasma, which occur in all the scleroblasts. The movements of the protoplasma are revealed by observing the movements of the numerous granulae, which characterize the spicule-forming cells.

Cells with young and quite thin spicules especially contain great quantities of such granulae, all of which show rather a

[1] SCHRÖDER do not mention the word "sol". He only speaks about "im Dunkelfeld ... grauen Vakuolen" containing silicic acid.

uniform size, about $^1/_2\ \mu$ in diameter. In older spiculiblasts there are fewer granulae, and they may almost have disappeared in cells with fully formed spicules. We have no information regarding the nature of these cell-elements. Granulae move at rates up to about $2\ \mu$ per sec., i. e. about 7 mm. per hour.

The currents of protoplasma in the spiculiblasts do not follow any fixed courses, but outside the spicule a strong mixture of all cell components takes place. It may be seen how the granulae rarely proceed more than about $5\ \mu$ at a time and often far less; then they stop, and when starting to move again, they often follow quite another course than before. Movements in both directions along the longitudinal axe of the spicule most frequently occur. Granulae situated in a layer of protoplasma near the surface of the spicule, will thus, as a rule, flow in one direction, while the layers of protoplasma in the periphery flow in the opposite direction. In the course of a short time, the current changes and goes in opposite directions. The granulae may, however, also take courses forming all kinds of angles to the longitudinal axe of the spicule, and by such courses are often led right away from the periphery of the cell to the surface of the spicule, and conversely. Thus we actually get the idea that the protoplasma elements of the spicule-forming cells are effectively mixed. It is understood without further explanation that it is difficult to imagine how vacuoles containing silicic acid should be able to migrate, independently of these confused currents of plasma, from the periphery of the cells to the surface of the spicule, while increasingly changing from the sol to the gel stage.

The above observations do not of course mean a rejection of the gel-theory in the main, but serve only to give us an idea of the difficulties in maintaining the theory in SCHRÖDER's particular form. There are, moreover, cases, which entirely contradict the gel-theories as a whole.

2) A gel must be very hydrous indeed to be able at all to spread over a surface, in this case over the developing spicule. From v. BEMMELEN's classic investigations from about the year 1900, we know the consistency of the silicic acid gel at various water contents. A gel is solid enough to be cut, when it contains

30 to 40 mol H_2O per mol SiO_2, and containing 20 mol H_2O per mol SiO_2 it is already rather stiff and quite without liquid abilities. A maximally shrunk silicic acid gel contains 1.5 to 3 mol H_2O per mol SiO_2. Such a gel is very solid. As early as at 6 mol H_2O it may be ground in a mortar to an apparently dry powder.

We may, therefore, safely conclude that the required liquid abilities of the gel are only present when it is newly formed and containing at least 25 mol H_2O per mol SiO_2 and probably more. The older gel soon looses its thixotrophy. 25 mol water per mol SiO_2 corresponds to about 88 % water or 12 % SiO_2.

In other words, it is most likely that the silicic acid gels which, according to SCHRÖDER's view, should occur in the protoplasma of the spicule-forming cells, may contain at the most about 12 % SiO_2, or rather less. In the case of *S. lacustris* the consequences of these facts will be seen from the following rough calculations.

The form of the microscleroblasts resembles somewhat that of a spheroid where the short radius equals $^1/_5$ of the long one. The volume of the cell may thus be calculated from the formula $^4/_3 \pi \cdot a \cdot b^2$, where a is the long radius and b the short one. If a length of a scleroblast is estimated to be equal to the length of a spicule, which is almost correct, and if a calculation is made for the average length of a spicule, viz. about 70 μ, a volume of the cell of about 72.000 μ^3 results. The thickness of a spicule is estimated to be 3 μ, and it has also approximately the form of a spheroid. Its volume is then about 330 μ^3. The total volume of the fully developed microscler is thus about $^1/_{20}$ of that of the spiculiblast. These figures are only recorded in order to show how the relations in regard to size may be between the cell and the spicule.

As previously mentioned a greater number of analyses have shown that the spicules of the siliceous sponges contain about 6 to 7 % water. All specific gravities determined hitherto lie between 2.096 and 2.018. (see BÜTSCHLI 1908). The specific gravities have been determined on spicules of many different sizes, both large and small, but all the spicules could easily be discerned by the naked eye. We dare not, therefore, absolutely reckon with the specific gravities and water percentages also

being applicable to the microscopically small spicules of the fresh water sponges. Consequently I have carried out some calculations of the specific gravities for megascleres and microscleres of *S. lacustris.*

The spicules were freed from the sponge tissue by boiling in 70 % lactic acid, and completely cleansed in diluted KOH (their cleanliness was controlled under the microscope) for finally to be rinsed several times in destilled water in the centrifuge. They were dried at 120° C. for half an hour and then kept about 24 hours in the atmospheric air at room temperature before the calculations of the specific gravities were undertaken[1].

The specific gravity was determined by observations on the motions of the spicules in liquid mixtures with known specific gravities. For the mixtures aethylen bromide (s. g. 2.17) and brom benzene (s. g. 1.49) were used. The calculated specific gravities of the mixtures were controlled on the Westfals scales.

Owing to the minuteness of the spicules (the megascleres were about 250 μ and the microscleres about 70 μ long), the calculations of the specific gravities had to be made in a thermostat with a constant temperature. Fluctuations of the temperature of the surroundings of a few one-hundredth degrees, involved convection currents in the liquid, which carried the spicules with them, making calculations of the specific gravities impossible. The calculations were carried out at the cyto-chemic department of the Carlsberg Laboratory. (Concerning the construction of the thermostat see H. Holter: C. R. lab. Carlsberg sér. chim. 24 nr. 18, 1943.)[2]

The liquid, containing a great number of spicules, was placed in glass tubes just inside the glass wall of the thermostat and these could be observed by the aid of a microscope standing horizontal outside the thermostat. The measurements of the motions of the spicules were made, when the system was in termical equilibrium (temperature 23.1° C.). Only the rates of motion of the vertically standing spicules were measured. It was then

[1] Being heated to 120° C. the spicules may loose some water. This amount of water is, however, soon absorbed again from the humidity of the air, when the temperature drops again (Vosmaer and Vijsman 1905).

[2] I wish to express my sincere thanks to Dr. H. Holter, the leader of the department and his assistant Mr. E. Zeuthen, M. Sc. for allowing me to make these calculations of the specific gravities in the Carlsberg Laboratory, and for their kindness and willingness to help.

found that the spicules, at a specific gravity of 1.90 of the liquid mixture, floated towards the bottom of the glass at a rate of about 8 μ per sec. (see fig. 12). At the specific gravity of 1.96 by far the greater number of the spicules also moved towards the bottom, but the speed was far slower, less than 2 μ per sec. and the rates of motion of a great number of spicules came very near to 0, while also a smaller number of spicules rose in the liquid at very small rates. At 2.00 the spicules showed a marked

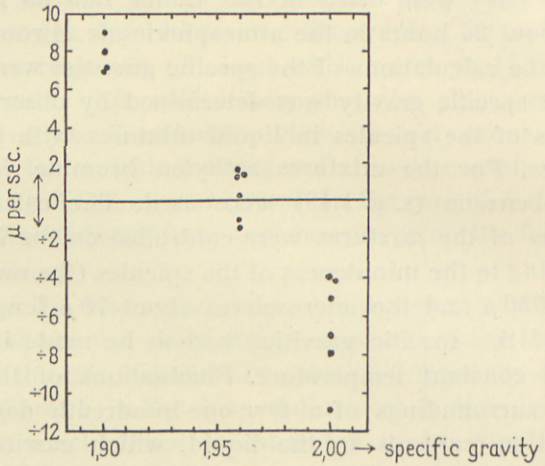

Fig. 12.

tendency to rise at rates up to 11—12 μ per sec. Quite a few spicules, however, likewise moved towards the bottom at this specific gravity of the surrounding liquid. Since the latter, on all sides and at a distance of less than 100 μ, were surrounded by spicules that were lighter than the liquid, the downward motion cannot be due to termical currents but must indicate that these spicules were actually heavier than the surrounding liquid.

It may be seen that the specific gravity is not quite the same in the case of all the spicules, but by far the majority showed specific gravities very near to 1.96. There was no assignable difference between the megascleres and microscleres. The specific gravity of the small spicules of *S. lacustris* is, therefore, somewhat lower than that of the larger ones examined earlier, which is probably due to a somewhat greater water content in

the latter. It is difficult to say how great the water content actually is in the spicules of *Spongilla*, as there is no agreement between the statements in literature of the specific gravity and of the water content of the silica spicules; thus a determination of the water content of the *Spongilla* spicules by extrapolation cannot be made.

The substance of the siliceous spicules is very similar to the opals. The chemical compositions, specific gravities, refractive index, etc. both of the substance of the spicules and that of the opal, particularly hyalith, are very closely related. As the specific gravities of the opals nearly always lie between 1.9 and 2.2, I tried to compare the *Spongilla* spicules with some of the lighter opals. Neither in the case of the opals is there complete correlation between the specific gravity and the water percentage. For instance, it may be mentioned that the water contents of two opals with low specific gravities, viz. kachelong (s. g. 1.884) and michalith (s. g. 1.886) are 7.74 % and 1.35 % respectively. Two siliceous sinters with specific gravities of 2.046 and 2.031 contained 12.86 % and 3.06 % water respectively (see Bütschli 1908). As a rule, the specific gravity for transparent opals, with a SiO_2 content of about 90 % and a water content of about 7.5 %, lies between 1.9 to 2.03. As to non-transparent opals with about 87 % SiO_2 and 9 % water the specific gravities are determined at 1.94—1.97. (The statements are recorded from Gmelin-Kraut, Handb. anorg. Chemie III, 1. 1912.)

Since the water percentage, even at specific gravities under 1.9, did not rise higher than to about 17, we may surely conclude that neither do the water percentages of the *Spongilla* spicules exceed values of this size. If the water content of the spicules is considered to be about 15 %, this is certainly a high estimate; the water percentage surely comes nearer 10.

In the following calculations the SiO_2 percentage is reckoned at 85 and the water percentage at 15.

The volume of a normal microsclere is, as mentioned, 330 μ^3. If a correction is made for the organic axial thread, which, at a high estimate, is 0.8 μ thick with a volume of about 23 μ^3, the spicule is found to contain about 0.00051 γ SiO_2. This quantity of silica distributed on a 12 % gel gives a gel weight of 0.0042 γ. If the specific gravity of pure SiO_2 is reckoned at 2.3, the spe-

cific gravity of this gel becomes about 1.16. The volume will thus be 3600 μ^3,

If the volume of the hypothetic silica gel, before it is deposited on the spicule, is supposed to have a volume equal to one half of the fully developed spicule, i. e. about 165 μ^3,[1] this will mean that about 22 of such gels are required to form a single spicule.

Our knowledge of the rate of the spicule-formation is rather uncertain, but 2 days must be considered to be ample time for the formation of a spicule 3 μ thick and 70 μ long. In many cases only half the time may be required. But supposing that the duration of formation equals 50 hours, this must mean that a gel containing 12 % SiO_2 must be ready for deposition, every second hour, at least, and probably far more frequently. Since in the course of comparatively few minutes many hundreds of developing spicules could be observed in a culture with young gemmula sponges, it might be expected that, if the gel theory was correct, the formation of gels and the deposition upon the spicules would be a phenomenon frequently met with in such cultures. As a matter of fact, however, the deposition of the gel has never been directly observed, in spite of the fact that numerous trained observers have closely studied and watched the spicule-formation in many live fresh-water sponges and other siliceous sponges. SCHRÖDER, however, believes he has witnessed the formation of a siliceous globe on a spicule in one single case. He saw, how an amoebocyt, moving along a megascler which was so far developed that it was no more enclosed by the scleroblast, stopped for a few seconds, then it suddenly contracted, whereby a siliceous globe appeared on the spicule. The globe did not spread on the spicule. The amoebocyt passed on. Being the only support in favour of the gel-theory this observation must be considered to be insufficient evidence. One cannot completely free oneself of the suspicion that the thickening of the spicule may have been present beforehand.

At any rate, the latter is the only record of the growth of a spicule taking place discontinuously. Otherwise the growth of spicules is stated as being invisible to the eye, which also corresponds with my own experience obtained after lengthy micro-

[1] Such a gel will, if of globular shape, have a diametre of about 7 μ and this corresponds rather closely to the size of the "silica" vacuoles in SCHRÖDER's fig. 6.

scopic observations of young gemmula sponges with lively formation and growth of spicules.

3) Finally still another fact should be mentioned which seems to contradict the gel-theory. If the formation of gels of silicic acid were to take place before the silica was deposited upon the spicule itself, it would be expected that these gels could be demonstrated by staining with basic vital dyes, as for instance methylen blue, which especially is strongly adsorbed to silicic acid gels *in vitro*. One might, therefore, expect that bluish vacuoles of the spicule-forming cells would fairly often be demonstrated in the young gemmula sponges in cultures with e. g. methylen blue. This was, however, not found to be the case, as will appear from the following.

The gemmulae were placed in petri vessels with staining-solutions in tap water with a power of about 1/100.000—50.000, 50 to 100 in each vessel[1]. The vital dyes used were methylen blue, neutral red, Bismarck brown, brilliant cresyl blue and methylen green.

Furthermore a silicic acid gel of about 10 % was made in the usual manner i. e. by neutralizing a 10 % sodium silicate solution with HCl. After the neutralization the gelatination took place in the course of a few minutes. The gel thus formed was broken up and washed for several days in tap water, which was often changed. Very small fragments of the washed silicic acid gel were placed in petri vessels with the same vital staining-solutions as used in the cultures with gemmulae. In the course of a few minutes the gel fragments had been intensively coloured in the methylen blue culture, and in the course of some hours a strong absorption of methylen green and brilliant cresyl blue had also taken place. Not until 24 hours later did the staining with neulral red and Bismarck brown also occur.

The staining gels were observed under the microscope with the water immersion objective, magnified in such a way as used

[1] The gemmulae were procured on March 3rd 1943; they were kept for some hours at room temperature before they were placed in the refrigerator, where they were left until the 9th April. At this juncture they were all frozen up. In spite of this their germination was almost complete in the experiments (more than 90 %) and spreading and differentiation had a normal course. Germination took place at room temperature on the 12th and 13th April 1943. They had been thawed on the 9th April, immediately before they were placed in the experimental cultures.

for the gemmula sponges. Also when thus magnified the staining was intensive and homogeneous even of the smallest particles of the silicic acid gel.

During and after the germination and spreading the gemmula sponges took up abundant dye in all cultures, excluding that with methylen green. (After being made up the methylen green solution soon became completely colourless). The dyes were mainly accumulated in some of the intracellular green algae but also in some of the granulae not further identified and of which the most were about $^1/_2\,\mu$ in diametre. There was no indication that the cell components of the scleroblasts were more frequently coloured than the other cell-types, on the contrary, they contained fewer coloured elements than did the dominating cell-type viz. the amoebocyte or cell-type II (BRØNDSTED 1936), since the scleroblasts do not contain algae cells, as is the case with the latter. The remaining coloured granulae also occurred rather less numerously in the scleroblasts than in the other cells of the sponge. Most frequently the spicule-forming cells contained no coloured constituents at all, and but rarely more than one coloured concretion was found at a time, which, apart from the colour, could not be distinguished from the other granulae, which are so numerous in the young scleroblasts.

Is there, however, not a possibility that these few formations in the spicule-cells which actually take up basic vital dyes may be silicic acid gels? In any case, being far too small, they do not correspond to SCHRÖDER's siliceous vacuoles, and besides it is difficult to prove or disprove such an assumption. A quite simple quantitative estimation, however, shows that this supposition is improbable, and at any rate it may be seen that if they are silicic acid gels, these few coloured granulae are quite insufficient in number to be solely responsible for the formation of the spicules.

The diametre of the granules is about $^1/_2\,\mu$ i. e. their volume is about $0.07\,\mu^3$. Supposing that they contain about 12 % SiO_2, as mentioned above (pag. 32), about 50.000 such granulae would be required to form an average-sized microscler ($70\,\mu$ long and $3\,\mu$ thick, corresponding to a gel volume of about $3600\,\mu^3$ and with 12 % SiO_2). In other words, reckoning, as before mentioned,

with a period of formation of 50 hours, this must mean that the deposition upon the spicule of 20 such coloured granulae per minute might be observed. This is, however, not at all the case. On the contrary the microscopic observations show that the coloured granulae, as would be expected, behave in the same way as the numerous non-coloured granules. They are conducted round in the cell by the protoplasma currents, now along the spicule, now in directions more or less at an acute angle to the longitudinal axe of the spicule.

Judging from the facts stated above, it must be considered improbable that the silicic acid would occur in the form of a gel in the protoplasma before it is deposited on the axial thread or on the part of the spicule already formed. The most probable theory is that the silicic acid, whether it is absorbed in an ionised, molecular or more or less polymerised form, is deposited directly on the spicule in whatever form or manner this process may take place. Investigations dealing with this question are under preparation, and I hope later to get an opportunity to return to this problem.[1]

Is *Spongilla* able to dissolve and utilize solid silicic acid?

Although it must be regarded as most likely that the silica required by the sponges is mainly supplied through the content of dissolved silicic acid in the surrounding water, one cannot beforehand exclude the possibility that the sponges may be able to dissolve solid silicates and thus procure silica from an underlayer of encrusted particles containing it, as presupposed by SCHULZE (1923). In order to decide this question the following experiments were made.

In three paraffined petri vessels, each containing about 50 ml artificial fresh water without silica (As to composition see pag. 9) spicules of *Jophon piceus*, pulverized ortoclas and pulverized

[1] In order to explain the lamellar structure occurring in the larger siliceous spicules, but lacking in the homogeneous microscleres, P. SCHULZE (1925) assumes that each lamel is laid down in the form of a silicic acid sol, derived from the scleroblast or scleroblasts and covering that part of the spicule already formed. Not until the sol has passed into the gel stage and has congealed, is silicic acid sol again deposited upon the spicule, thus forming a new lamel. As will appear from the above statements, all the observations on the formation of spicules in *Spongilla lacustris*, however, contradict such a mechanism of formation of its spicule-types.

dark mica were placed, covering the bottom with a thin layer. Finally a control vessel without substratum was arranged. In each of the vessels 20 gemmulae were placed which germinated in the course of a few days. Three or four days later a rather strong development of spicules could be noted in all the cultures with substratum, especially in the culture with pulverized mica, whereas no spicule-formation could be observed in the control

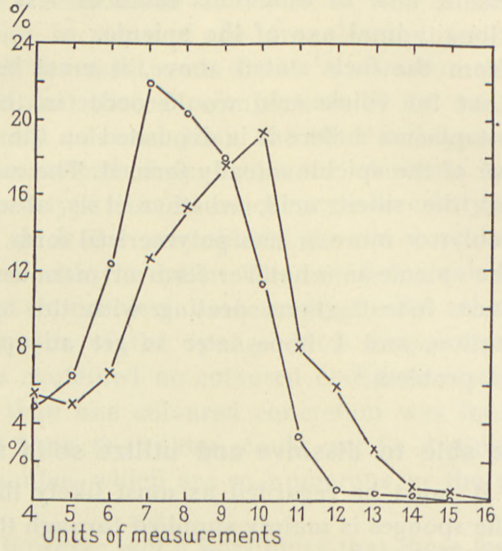

Fig. 13. Distribution of spicules from sponges which have grown on paraffin —×—×— and directly on ortoclas —o—o—.

culture. In order to decide to what degree the formation of spicules is due to the silicic acid dissolved in the water, the young sponges were removed from the petri vessel, and about 4 weeks after the various substrata had been put into the culture vessels without silica, these were again filled with gemmulae, which were placed, partly directly on the substratum and, partly, on a great paraffined cover-glass placed over the mineral constituents at the bottom. In each vessel about 100 gemmulae were placed, one half of which was put directly on the paraffined cover-glass, and one half on the ortoclas, mica or *Jophon* spicules respectively. In the culture vessel without silica about 60 gemmulae were placed. The gemmulae were newly gathered. The experiment started on the 17. 4. 43. at normal room temperature.

18. 4. 43. the germination had started. 23. 4. 43. the experiment was suspended and the young sponges originating partly from a single gemmulae, partly from several fused together, were fixed in 70 % alcohol. The counting of numbers and measuring of thickness of the spicules was made as recorded on pag. 9.

The thicknesses of the spicules from the ortoclas and mica cultures cannot directly be compared with those of the microscleres from sponges which have grown in the culture with *Jophon*

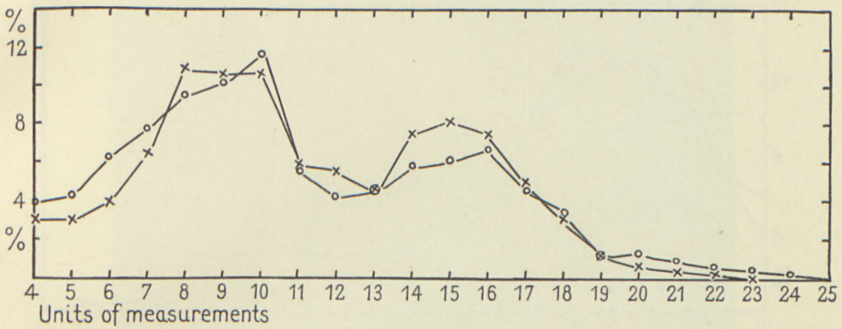

Fig. 14. Distribution of spicules from sponges which have grown on paraffin —o—o— and directly on mica —×—×—.

spicules, as the latter have almost all of them outgrowths on the middle (about 80 % of all the microscleres). The thicknesses of the latter were therefore measured just below the point where the middle outgrowths made the spicules extremely thick. The existence of this formation of outgrowths is the more striking because the 20 gemmulae in the first experiment in the same culture did not show any middlethickenings. Almost all the microscleres from the cultures containing ortoclas and mica had no middlethickenings (table VII).

The results of the measurements are given in the curves in figs. 13, 14 and 15 and in tables III and IV. It clearly appears that neither as regards the thickness of the spicules, nor their number (the microscleres alone have been considered) is there any difference between the sponges, which have grown on paraffined cover-glasses and those, which have directly been in contact with the substance containing solid silica. A microscopic observation showed that in the latter case the contact between

the sponge tissue and the substratum has actually existed. The gemmula sponges had completely embodied large quantities of quite small ortoclas and mica particles, and they had stretched themselves between the *Jophon* spicules which seemed to be completely incorporated in the sponge tissue (fig. 16). In spite of this, there is nothing to indicate that these sponges might have had more silica at their disposal for the formation of spicules than those which have only been in contact with

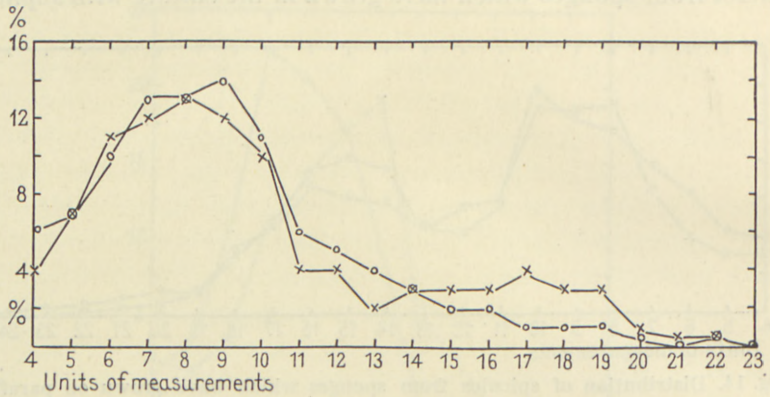

Fig. 15 Distribution of spicules from sponges which have grown on paraffin —o—o— and directly on spicules of Jophon —x—x—.

the paraffined underlayer. However, there is a marked difference in the size of spicules of the various cultures. On an average the spicules are stronger in the cultures with mica and *Jophon* spicules as substratum than in the case of the ortoclas.

In the control culture only extremely few thin spicules were developed, as mentioned before. We may, therefore, conclude that of the silicic acid used for the formation of spicules in the experiments with ortoclas, mica and *Jophon* spicules as substratum, by far the greater part is, at any rate, derived from silica dissolved in the water without the aid of the sponge. The greater silica acid concentration, which one would expect to be present in the immediate vicinity with the sponges growing directly on the substance containing silica, has apparently been too small to involve an increased growth of the spicules, which could make itself felt against the individual variations in the microscler production of the gemmula sponges.

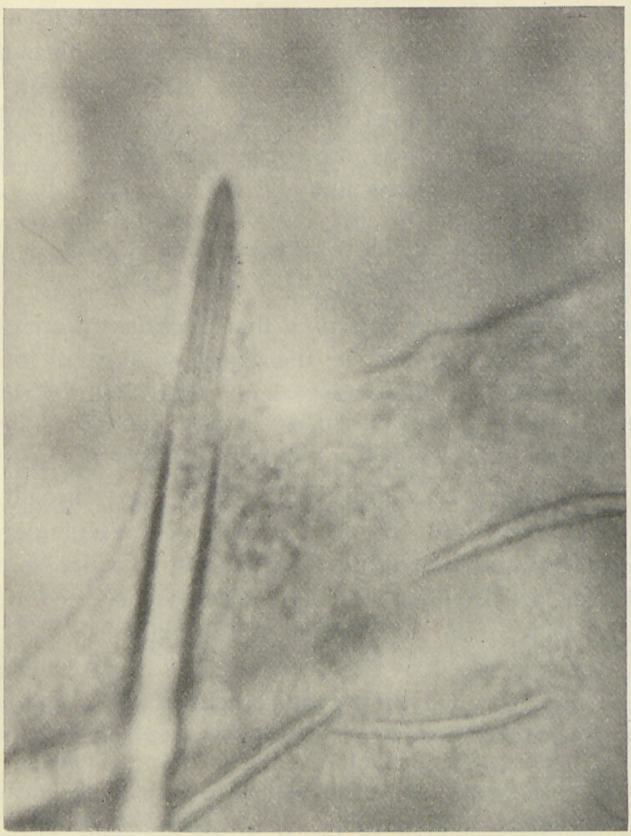

Fig. 16. *Halichondria* spicule completely embodied in the spongilla tissue (× ca. 400).

Summary.

The formation of spicules, mainly the formation of microscleres of the gemmula sponges in *Spongilla lacustris* has been examined with special regard to its dependence on the silicic acid content of the surrounding medium. The gemmula sponges were kept in artificial salt solutions, partly without silica and partly containing known silica contents varying from between 0.02 to 1.28 mM SiO_2.

It was found that the number of spicules formed per unity of volume of gemmula during the experiment was independent on the silica concentration, when this was over a certain minimum value. Under the conditions of experiment in question

the limit for full spicule production came close to 0.02 mM SiO_2 in the case of production of microscleres. The formation of megascleres is less sensitive to a low silica content. At the highest silica concentrations used, i. e. 1.28 mM SiO_2, the production of megascleres was, however, retarded, while the production of microscleres was not effected.

The length—unlike the thickness—of the microscleres was not affected by variations in the silicic acid content in the surroundings. The maximum thickness of the spicules and the percentage of microsleres attaining this thickness increased with the increasing silica content in the liquid of the culture.

The percentage of the microscleres with middlethickenings rises with increasing silica content of the water.

The theory put forward by SCHRÖDER that the spicule-formation takes place by deposition on the axial thread of preformed silicic acid gels, or on the part of the spicule already formed, has proved untenable. The growth of spicules must be considered to be brought about by the protoplasma continuously depositing silica upon the surface of the spicules.

The view expressed by SCHULZE that the fresh-water sponges should be able to meet their requirement of silica by dissolving silicic acid from solid substances containing silica, does not seem to hold good.

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