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# ON THE SPICULE-FORMATION OF *SPONGILLA LACUSTRIS* (L.) AND *EPHYDATIA FLUVIATILIS* (L.)

## 2. THE RATE OF GROWTH OF THE SPICULES

BY

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# INTRODUCTION

In a previous paper (Barker Jørgensen 1944) it was shown that *Spongilla lacustris* is able to utilize dissolved silicic acid, only, in the formation of spicules, and that the growth of the spicules proceeds through continuous apposition of silicic acid. The present experiments were performed in order 1) to examine the rate of growth of the spicules, 2) to determine the rate of uptake of silicic acid in the spicule forming cells (the scleroblasts) and 3) to determine the dependence of the spicule size on the content of dissolved silicic acid in the environment. In the investigation gemmules of *Spongilla lacustris* and *Ephydatia fluviatilis* were used.

A few hours, only, after germination of the gemmules of *S. lacustris* and *E. fluviatilis* has commenced, the occurrence of the first scleroblasts with quite faint traces of spicules can be observed. In Figs. 1, 2, and 3 some of the first stages of development are shown. The cells where the spicules develop are tightly filled with granules. Gradually, as the spicules develop, these granules decrease in number. The spicules are laid down in the form of organic axial threads. At this first stage of spicule-formation the granules are concentrated in a central mass of protoplasm distinctly separated from the more peripheral parts of the protoplasm which have extended along the spicule (Fig. 1). Gradually, the sharp limits between the two parts of protoplasm disappear and the granules are distributed more evenly (Figs. 2 and 3). During the formation of the spicules the protoplasm is in constant movement, and the whole cell exhibitsvi vid amoeboid movements. For this reason it is difficult to indicate the thickness of the cell (the length of the cell is always very much like the length of the spicule) which, however, may be measured with rather good accuracy when the cell incidentally becomes spindle-shaped, which happens relatively often (Fig. 4). In the case of *S. lacustris*,

the thickness in the middle of the cell is for the megascleroblasts (forming skeletal spicules) about 12—14 $\mu$ and for the microscleroblasts (forming flesh spicules) about 11—13 $\mu$. There is no

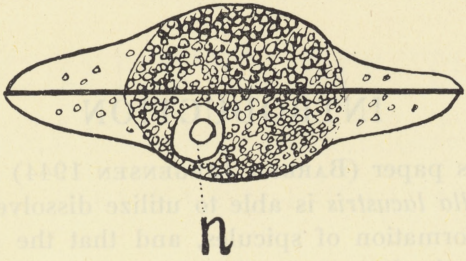


Fig. 1

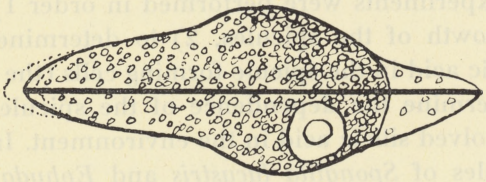

Fig. 2

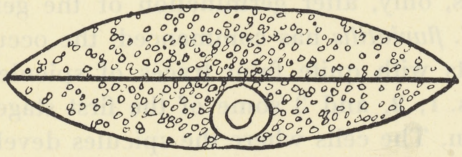

Fig. 3

Figs. 1—3 show different stages of development of the same young scleroblast (megascleroblast) from *Spongilla lacustris*. Fig. 2 shows the scleroblast at a stage five minutes later than that represented in Fig. 1, and Fig. 3 ten minutes later. In Fig. 1 the central granule-filled part of the cell is distinctly separated from the strongly moving peripheric parts which are almost free from granules. In Fig. 2 this sharp limit is disappearing and a free interchange of cell constituents takes place. In Fig. 3 the granules are rather evenly distributed over the whole cell. It was observed several times that these processes are reversible so that the stages represented in Fig. 1 as regards time may be subsequent to the stages in Fig. 2 or even in Fig. 3. The dotted lines in Fig. 2 show that the protoplasm may extend farther than the tip of the spicule and that the tip may sometimes project beyond the protoplasm. The length of the cell is about 40 $\mu$ and the maximum cell thickness in Figs. 1 and 2 is about 15 $\mu$. The thickness of spicules is about $^1/_2$ $\mu$. n = nucleus with nucleolus. Half-schematic.

detectable difference between the thickness of the cells in the young short scleroblasts and the older ones which may be up to three or four times longer. Even if due regard is paid to the

increased volume of the spicule, this means that the cell volume around the spicule proper is multiplied during spicule-formation. This strong increase in the cell body may possibly be interpreted exclusively as a consequence of the heavy vacuolization occurring in the protoplasm, which can clearly be observed in the living sponge under the microscope.

After the axial thread has been formed[1], the growth of the spicules proceeds by apposition of silicic acid.

The investigation of the growth of the spicules first aimed at continuous measurements under the microscope of the size of the growing spicules in young transparent gemmula-sponges by means of a water immersion objective. This proved, however, to

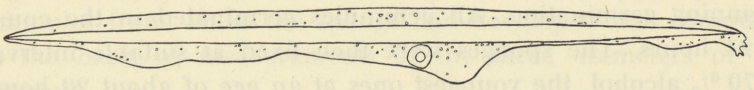

Fig. 4 shows a megascleroblast of *S. lacustris* about 160 $\mu$ long with a 3.6 $\mu$ thick spicule. The upper half of the drawing shows the spindle-shaped scleroblast; the lower half represents one of the numerous stages which occur as a consequence of the vigorous formation of pseudopodia. Half-schematic.

be unfeasible. In order to obtain a reliable picture of the growth of the spicules the very thinnest spicule stages, i. e. immediately after the formation of the axial thread, must be observed, and the measurements must be continued at suitable intervals over a period of several days. However, so long a period of observation of the same spicule was very difficult to ascertain, because the vivid cell movements in the differentiating sponge will carry the spicule forming cell out of the field of vision, and sooner or later the scleroblast will enter in opaque cell masses where the spicule cannot be observed.

Instead of following the growth of the individual spicule it was, therefore, attempted to determine the average rate of growth by measurements of spicules in sponges of different ages. Two of these experiments with gemmule sponges of *S. lacustris* and *E. fluviatilis,* respectively, will be described in the following.

[1] Schröder (1936) states that the axial thread is formed by quite small granules forming a line and fusing into a thread. However, the present author never succeeded in observing such phenomena. The axial thread seems rather to be laid down in its final shape from the protoplasm and to increase in length by a continuous growth from both ends, and not by a fusion of granules with the tips of the thread, as stated by Schröder.

## I. Material and Technique.

Sponges with gemmules of *S. lacustris* were collected in Mølleaa near Copenhagen in the last few days of December 1944. All gemmules used in the experiments were thin-walled and without "granular layer" (BARKER JØRGENSEN 1946). About 50 cleaned gemmules were placed in a 14 cm Petri dish in 150 ml water from Mølleaa with additional sodium silicate to a concentration of about 0.16 mmol $SiO_2$ (measured colorimetrically according to DIENERT-WANDENBULCKE, cf. WATTENBERG 1937). The Petri dish was placed on squared paper with numbered squares, and the gemmules were placed in such a way that only one was present in each square. In this way, the age of the individual sponge could be checked. The age was reckoned from beginning germination. All gemmules germinated in the course of 24 hours. The sponges were then fixed at suitable intervals in 70 % alcohol, the youngest ones at an age of about 20 hours, the oldest when they were about 235 hours old. During the experiment the temperature varied between 13° and 19° C, the morning temperature was about 13°—14° C, in the evening the temperature reached 18°—19° C.

Sponges with gemmules of *E. fluviatilis* were collected in Hulsø in the Hareskov near Copenhagen on April 4, 1945. Some 100 gemmules, about 0.3 to 0.4 mm in diameter, were placed in a 9 cm Petri dish coated with paraffine, containing 100 ml artificial freshwater, 0.11 mmol with respect to $SiO_2$ (BARKER JØRGENSEN 1944).

The germination of the individual gemmule was not followed in this experiment, but after germination of all gemmules had occurred within a period of about 24 hours, a number of sponges, on an average ten, were fixed: in the beginning at 24 hours' intervals, later at 48 hours' intervals. The last sponges were fixed when about 240 hours old. The temperature was between 16° and 20° C.

Before the spicules in the fixed sponges could be measured the soft parts had to be removed. This was done by boiling in 70 % lactic acid. Cleaning was performed on thick slides provided with a groove, 14 mm long, 1 mm deep, 5 mm broad at the bottom, and 6 mm broad at the surface of the glass. The fixed sponges were placed in this groove in one or two drops of

lactic acid. Then, they were carefully heated above a tiny flame until the soft parts were completely dissolved. Subsequently, distilled water was added till the groove was filled. Mixture of the lactic acid concentrated by evaporation, and water and, moreover, an even distribution of the spicules in the preparation were obtained by careful sucking-up and blowing-out by means of a pipette. When the spicules had sedimented, the preparation was ready for examination without any loss of spicules during the cleaning procedure. The sizes of the spicules were measured by means of a water immersion objective and a micrometer. In a number of preparations containing spicules of *S. lacustris* the total number of spicules was counted by means of a squared eyepiece micrometer. Moreover, the volume of the gemmule or gemmules from which the sponge had developed was computed on the basis of measurements of the three diameters of the gemmule.

## II. Growth of Microscleres and Megascleres of *S. lacustris*.

In the preparations used for the measurements of the spicules on an average 10 or 15 per cent. of the total number of spicules were measured. No selection of spicules was made except for discarding defective spicules; all spicules were measured gradually as they came within the field of vision. Moreover, each preparation was moved in front of the objective several times in its whole length, so that spicules from all parts of the preparation were measured. In this way it was ascertained that the measurements represent a reliable average of the spicule sizes present in each preparation. The thickness measurements were made in the middle of the spicules. Furthermore, the length of a number of spicules was measured.

### a) Microscleres.

The results of the measurements of the microscleres will be discussed in greater detail, as they may illustrate the procedure of evaluation.

Fig. 5 shows three arbitrarily chosen examples of the distribution of the microscleres according to thickness in sponges of

different age. The thickness is plotted as the abscissa and the number of microscleres of different thickness, expressed in per cent. of the total number of microscleres measured, as ordinate. The curves which can be drawn through the plotted points show a distinct maximum falling at higher values of the abscissa with increasing age of the sponges.

The shape of the curves is the result both of the continuous production of new microscleres and of their growth. In order to

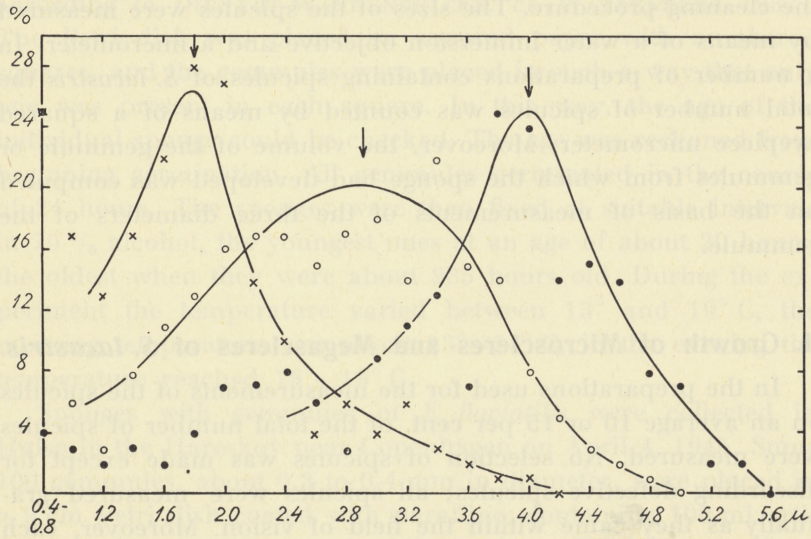


Fig. 5 shows the distribution of microscleres of *S. lacustris* according to thickness in sponges of different age. The thickness of spicules is plotted as abscissae and the corresponding number of spicules expressed in per cent. of the total number of spicules measured are plotted as ordinates. The ×-curve (200 measurements) represents the distribution in a sponge 72 hours old, the ○-curve (250 measurements) in a sponge 120 hours old, and the ●-curve (200 measurements) in a sponge 165 hours old. The arrows indicate the average values of the thickness of the spicules.

investigate the share of the continuous production of new spicules in the shape of the curve, a number of counts of the total number of microscleres in sponges of different age were made. The number was computed per 0.01 $mm^3$ of the volume of the gemmules from which the sponges had developed. In the graph of Fig. 6, these numbers are plotted as ordinates and the ages of the particular sponges, reckoned from beginning germination, as abscissae. It appears that the production of microscleres does not begin before

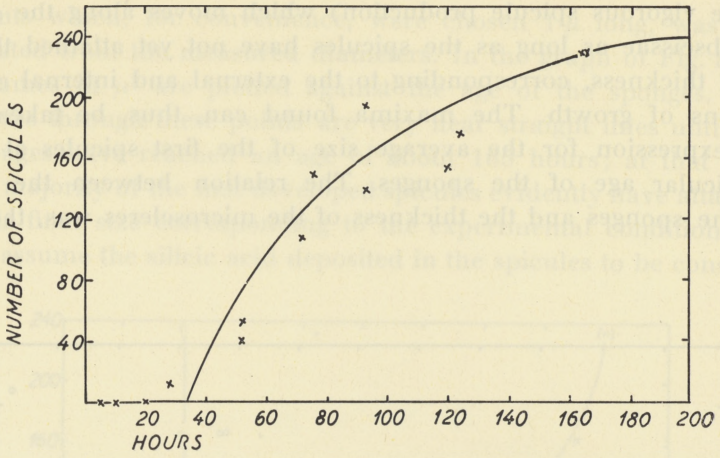


Fig. 6. *S. lacustris.* Abscissae: Age of sponge (from beginning germination). Ordinates: Number of microscleres per 0.01 mm³ gemmule volume.

the sponges are about forty hours old. From this age the production increased considerably and then decreased gradually with increasing age of the sponges. A spicule production of this type must be expected to manifest itself in curves of the present shape with a pronounced maximum (corresponding to the first

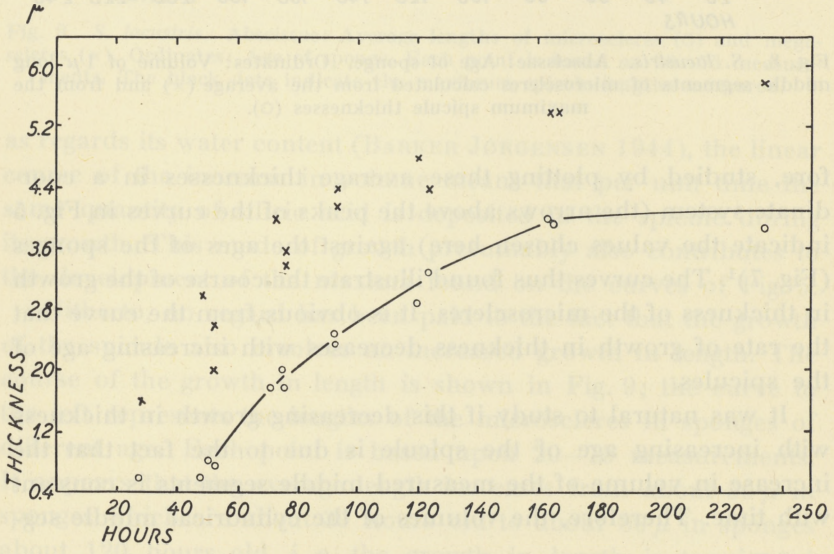


Fig. 7. *S. lacustris.* Abscissae: Age of sponge. Ordinates: Average (○) and maximum (×) thicknesses in $\mu$ of the microscleres.

more vigorous spicule production) which moves along the axis of abscissae as long as the spicules have not yet attained their final thickness, corresponding to the external and internal conditions of growth. The maxima found can, thus, be taken as an expression for the average size of the first spicules at the particular age of the sponges. The relation between the age of the sponges and the thickness of the microscleres was, there-

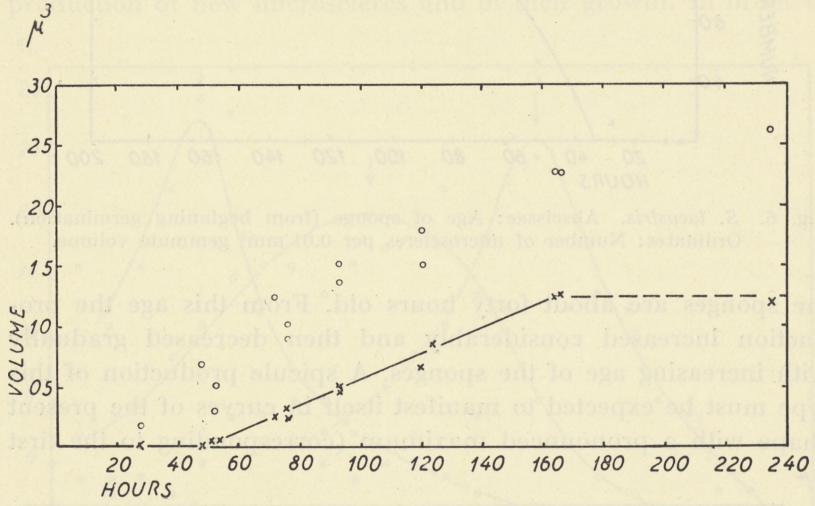


Fig. 8. *S. lacustris.* Abscissae: Age of sponge. Ordinates: Volume of 1 $\mu$ long middle segments of microscleres calculated from the average (×) and from the maximum spicule thicknesses (○).

fore, studied by plotting these average thicknesses in a coordinate system (the arrows above the peaks of the curves in Fig. 5 indicate the values chosen here) against the ages of the sponges (Fig. 7)[1]. The curves thus found illustrate the course of the growth in thickness of the microscleres. It is obvious from the curve that the rate of growth in thickness decreases with increasing age of the spicules.

It was natural to study if this decreasing growth in thickness with increasing age of the spicule is due to the fact that the increase in volume of the measured middle segments is constant with time. Therefore, the volumes of the cylindrical middle seg-

[1] The average thicknesses of the microscleres from the 48—52 hours old sponges were computed directly from the measured spicules.

ments which, for convenience, were chosen 1 $\mu$ long, was calculated from the measured diameters. In the graph of Fig. 8 the volumes in $\mu^3$ are plotted against the age of the sponges. The curves through these points are very near straight lines until the sponges have reached an age of about 165 hours; at that time the majority of the first developed spicules evidently have attained their final size corresponding to the experimental conditions. If we assume the silicic acid deposited in the spicules to be constant

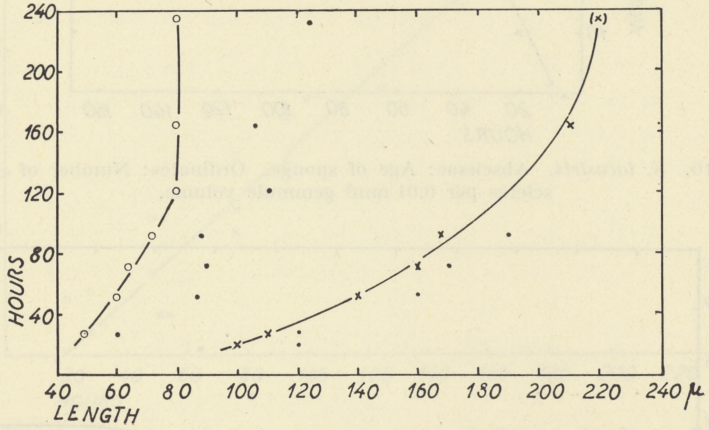


Fig. 9. *S. lacustris*. Abscissae: Average lengths of microscleres (○) and megascleres (×). Ordinates: Age of sponge. Each point is based on 20—75 measurements. The black dots indicate the maximum spicule lengths measured.

as regards its water content (Barker Jørgensen 1944), the linear course of the increase in volume means that per unit time the same quantity of silicic acid is deposited in the spicule during its growth. This mode of growth presumably also contributes to the development of the maxima found on the curves of Fig. 5.

Hitherto, no regard has been paid to the fact that the growth of the spicule also involves an increased growth in length. The course of the growth in length is shown in Fig. 9; the curve to the left represents the lengths of the microscleres in sponges of different age. Each point is based upon 20—75 measurements. It appears that the average length increases from about 50 $\mu$ in sponges which are about 30 hours old to about 80 $\mu$ in sponges about 120 hours old, i. e. the growth in length is terminated somewhat earlier than the growth in thickness.

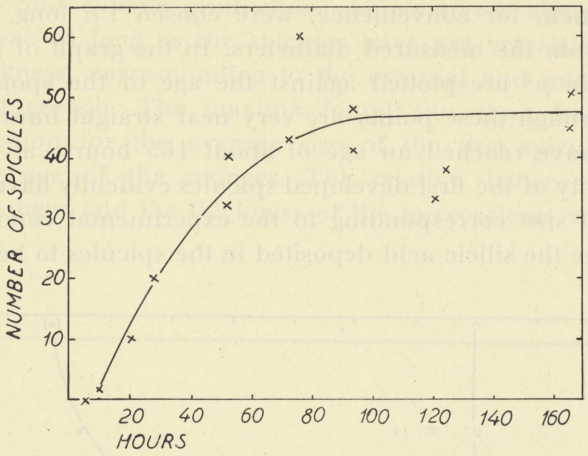


Fig. 10. *S. lacustris.* Abscissae: Age of sponge. Ordinates: Number of megascleres per 0.01 mm³ gemmule volume.

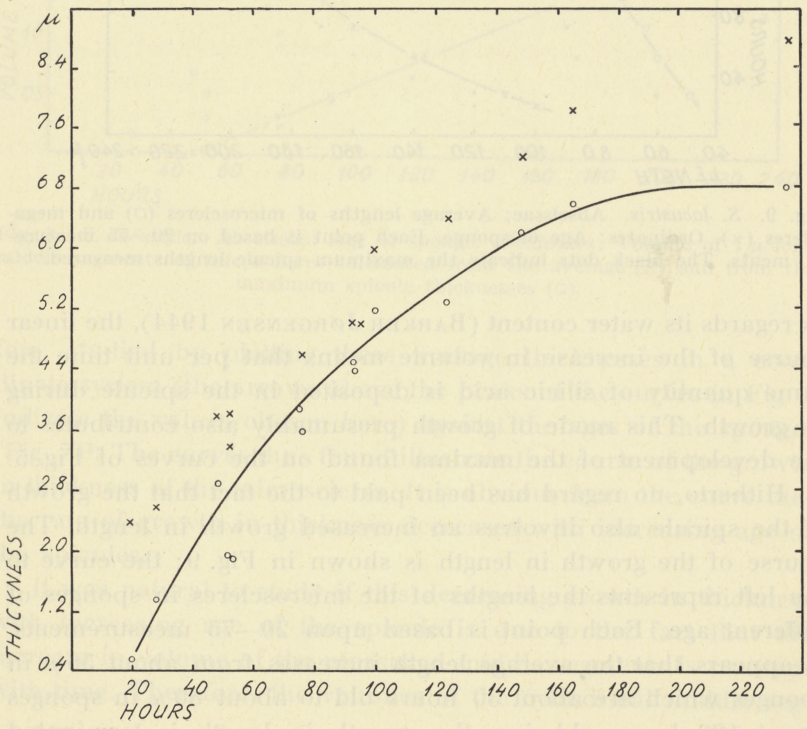


Fig. 11. *S. lacustris.* Abscissae: Age of sponge. Ordinates: Maximum (×) and average (○) thicknesses in μ of megascleres.

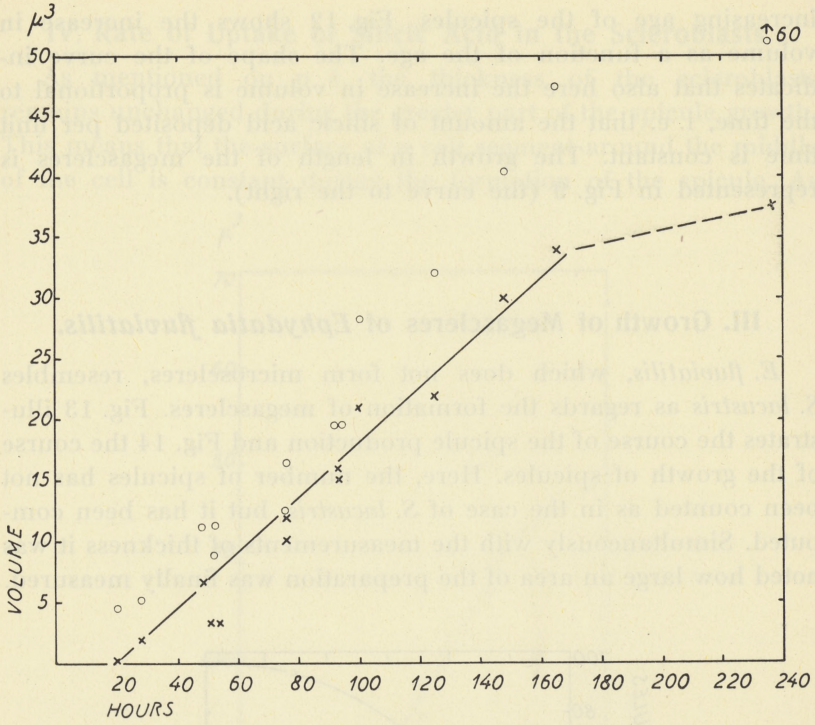


Fig. 12. *S. lacustris.* Abscissae: Age of sponge. Ordinates: Maximum (○) and average (×) volume of 1 μ long megasclere segments.

### b) Megascleres.

The course of the megasclere production is depicted in Fig. 10. The shape of the curve is almost the same as in the case of microsclere production, however, the production of megascleres sets in earlier, i. e. already when the sponges are 20 hours old[1]. As it was to be expected, the distribution curves for megascleres as a function of the age of the sponges are very similar to the corresponding curves for microscleres. The average and maximum rate of growth in thickness could, therefore, be determined in the same way as for the microscleres. This is shown in Fig. 11 which also reveals that the growth in thickness decreases with

[1] The reason why the curve of the megasclere production flattens more rapidly than that of the microscleres must undoubtedly be that part of the megascleres in the older sponges already were built-in in the skeleton which adhered to the support, so that some spicules were lost when the sponges were loosened.

increasing age of the spicules. Fig. 12 shows the increase in volume as a function of the age. The shape of the curve indicates that also here the increase in volume is proportional to the time, i. e. that the amount of silicic acid deposited per unit time is constant. The growth in length of the megascleres is represented in Fig. 9 (the curve to the right).

## III. Growth of Megascleres of *Ephydatia fluviatilis.*

*E. fluviatilis,* which does not form microscleres, resembles *S. lacustris* as regards the formation of megascleres. Fig. 13 illustrates the course of the spicule production and Fig. 14 the course of the growth of spicules. Here, the number of spicules has not been counted as in the case of *S. lacustris,* but it has been computed. Simultaneously with the measurements of thickness it was noted how large an area of the preparation was finally measured.

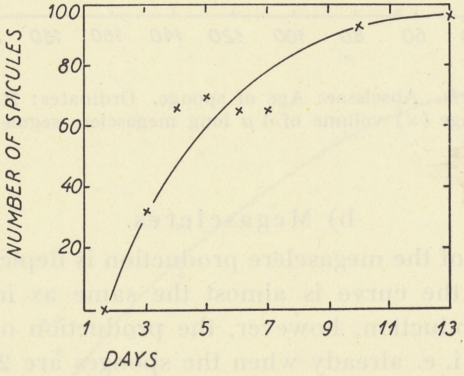


Fig. 13. *Ephydatia fluviatilis.* Abscissae: Age of sponge. Ordinates: Number of spicules per gemmule sponge formed by one gemmule.

When the area examined and the number of gemmule sponges on the preparation were taken into account, the number of megascleres per gemmule could be calculated. During the measurements $^1/_5$—$^1/_2$ of the whole preparation was examined. The course of production as well as the course of growth are analogous to those of *S. lacustris.*

## IV. Rate of Uptake of Silicic Acid in the Scleroblasts.

As mentioned on p. 4, the thickness of the scleroblasts remains unchanged during the greater part of the spicule growth. This means that the surface of a cell segment around the middle of the cell is constant during the formation of the spicule. As

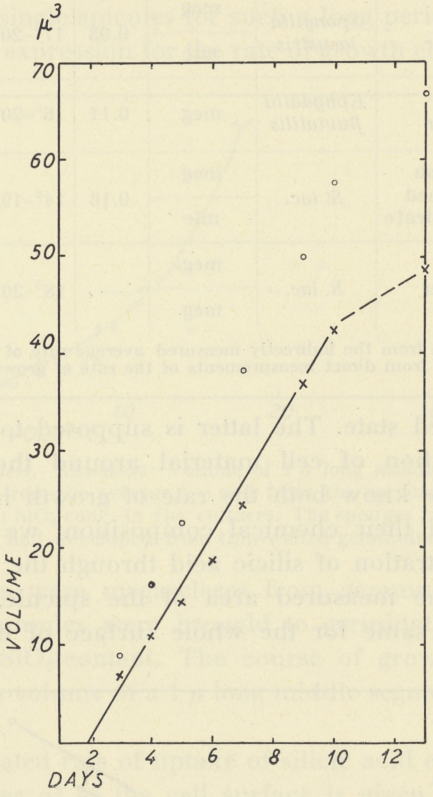


Fig. 14. *E. fluviatilis.* Abscissae: Age of sponge. Ordinates: Maximum (○) and average (×) volume of 1 μ long megasclere segments.

the increase in volume of the corresponding spicule segment is also approximately constant, the quantity of silicic acid passing this part of the cell surface must be almost constant. It can be assumed that most of the silicic acid penetrating a surface segment is precipitated on the corresponding segment of the spicule proper. This assumption is supported by the agreement found between the shape of the normal spicule and the scleroblast in

Table I.

| Time | Culture medium | Species | Spicules meg = megasclere mic = microsclere | $SiO_2$ conc. in mmol. | Temp. | Uptake in mols $SiO_2$ per min. per $\mu^2$ of the scleroblast surface | |
|---|---|---|---|---|---|---|---|
| March 1945 | Artificial fresh water | *Spongilla lacustris* | meg | 0.03 | 17°–20° C. | $0.9 \cdot 10^{-18}$ | 1 |
| | | | mic | | | $0.8 \cdot 10^{-18}$ | 2 |
| April 1945 | Artificial fresh water | *Ephydatia fluviatilis* | meg | 0.11 | 16°–20° C. | $1.6 \cdot 10^{-18}$ | 3 |
| January 1945 | Natural fresh water enriched with sodium silicate | *S. lac.* | meg | 0.16 | 14°–19° C. | $2.4 \cdot 10^{-18}$ | 4 |
| | | | mic | | | $1.5 \cdot 10^{-18}$ | 5 |
| August 1943 | Pond water | *S. lac.* | meg | — | 18°–20° C. | $5.4 \cdot 10^{-18}$ | 6 |
| | | | meg | | | $6.1 \cdot 10^{-18}$ | 7 |

1–5 are computed from the indirectly measured average rate of growth of spicules. 6 and 7 are computed from direct measurements of the rate of growth.

its spindle-shaped state. The latter is supposed to represent the average distribution of cell material around the spicule (see Fig. 4). Since we know both the rate of growth in thickness of the spicules and their chemical composition, we can calculate the rate of penetration of silicic acid through the surface of the scleroblast in the measured area of the spicule. This rate is presumably the same for the whole surface of the scleroblast.

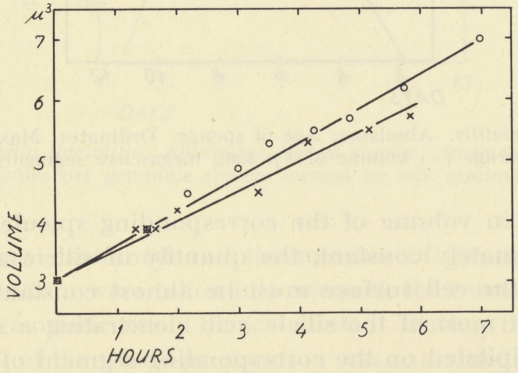


Fig. 15. *S. lacustris*. Rate of growth of two megascleres calculated on the basis of direct measurements of thickness. Abscissae: Time in hours. Ordinates: Volume of 1 $\mu$ long middle segments of the spicules.

The calculation of the rate of uptake of silicic acid was made on the basis partly of the above mentioned material partly of experiments of a similar kind, viz. the rates of growth of megascleres and microscleres of *S. lacustris*. In this last mentioned experiment the $SiO_2$-concentration in the culture medium was 0.03 mmol. Besides, it has been possible in two cases to follow the growth of single spicules for such a long period (6 to 7 hours) that a reliable expression for the rate of growth could be obtained.

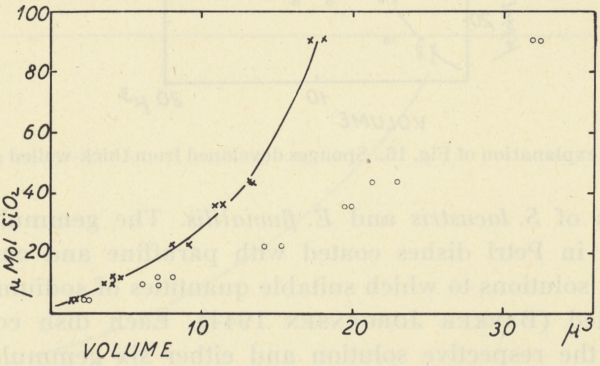


Fig. 16. *S. lacustris*. Abscissae: Volume of 1 $\mu$ long middle segments of microscleres calculated from the average (×) and from the maximum (○) spicule thicknesses. Ordinates: $SiO_2$-conc. in the cultures. The sponges from which the microscleres originated had developed from thin-walled gemmules (see foot note p. 19).

These spicules were megascleres from gemmule sponges of *S. lacustris*[1]. Gemmules were brought to germinate in pond water of unknown $SiO_2$-content. The course of growth calculated as the increase in volume of a 1 $\mu$ long middle segment is represented in Fig. 15.

The calculated rate of uptake of silicic acid expressed in mols per minute per $\mu^2$ of the cell surface is given in Table I. The specific gravity of the spicules is taken to be 1.96 and the $SiO_2$-content to be 85 per cent. (Barker Jørgensen 1944).

## V. Spicule Formation at Different Silicic Acid Concentrations.

The experiments on the spicule formation at different concentrations of $SiO_2$ in the culture medium were performed with

[1] The gemmules were from a sponge taken in Fantasidam (a pond near Hillerød) on August 18, 1943. In this case, the formation of gemmules thus had set in at an unusually early time of the year.

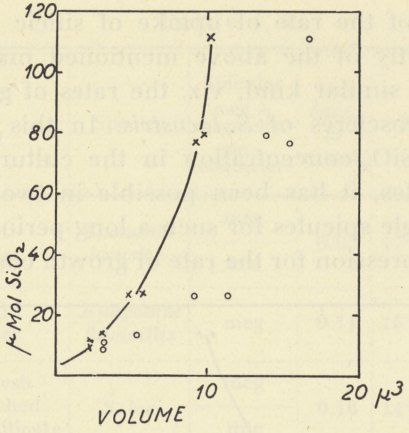


Fig. 17. See explanation of Fig. 16. Sponges developed from thick-walled gemmules.

gemmules of *S. lacustris* and *E. fluviatilis*. The gemmules were cultivated in Petri dishes coated with paraffine and containing dilute salt solutions to which suitable quantities of sodium silicate were added (Barker Jørgensen 1944). Each dish contained 75 ml of the respective solution and either six gemmules of *S. lacustris* or five gemmules of *S. lacustris* plus four gemmules of *E. fluviatilis* which, as is well known, are much smaller than *S. lacustris*. Thereby it was ascertained that during the formation

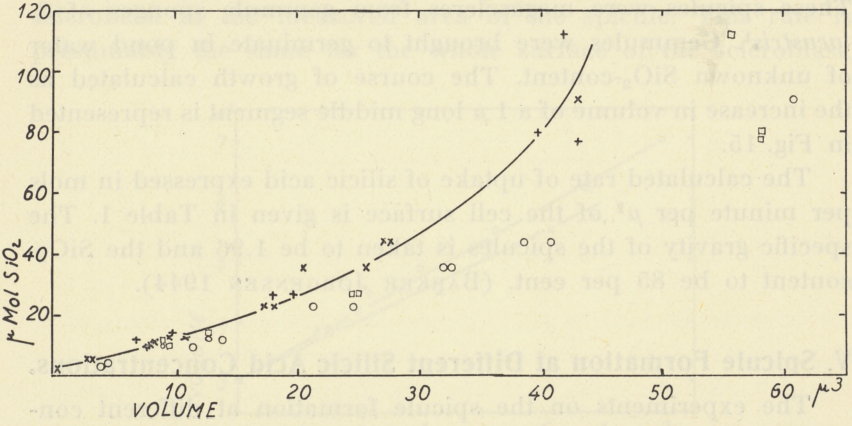


Fig. 18. *S. lacustris*. Abscissae: Volume of 1 μ long middle segments of megascleres calculated on the basis of the measured thicknesses, both originating from sponges developed from thin-walled gemmules (×=average thicknesses and ○=maximum thicknesses) and from sponges developed from thick-walled gemmules (+=average thicknesses and □=maximum thicknesses).

of spicules the gemmule sponges did not take up so much silicic acid from the culture medium that its $SiO_2$ concentration was changed perceptibly in the course of the experiment. The sizes of the spicules were calculated as above on the basis of the average thicknesses obtained, viz.: as volumes of 1 $\mu$ long middle segments. The ratio between the calculated volumes and the $SiO_2$-concentrations in the cultures are shown in Figs. 16, 17, 18, and 19 for microscleres and megascleres of *S. lacustris* and for

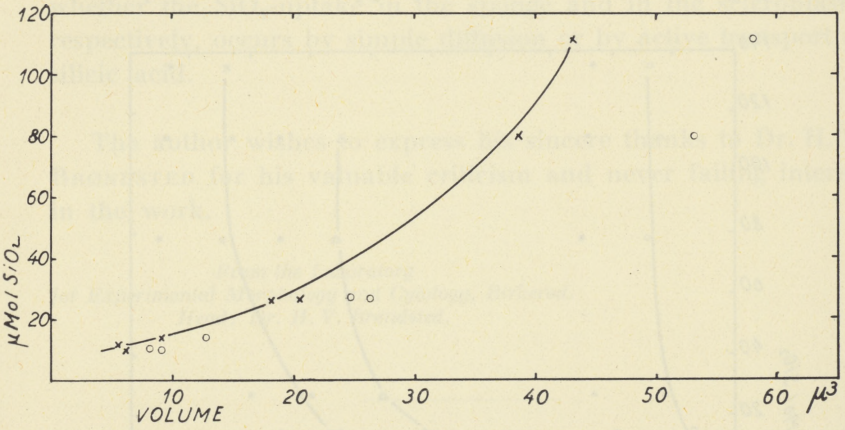


Fig. 19. *E. fluviatilis*. Abscissae: Spicule volume. Ordinates: $SiO_2$-conc. in the cultures.

megascleres of *E. fluviatilis*. Also the length of the spicules depends on the silicic acid concentrations, as it appears from Fig. 20[1].

The curves representing the relation between spicule segment volume and silicic acid concentration in the culture medium are supposed also to represent the relation between the rate of uptake of silicic acid per segment surface and the $SiO_2$-concentration in the culture medium. However, this implies that the period of growth of the spicules, i. e. the period of functioning of the sclero-blasts, is independent of the silicic acid concentration. On this assumption, the rate at which silicic acid penetrates the surface

[1] In the experiments on the gemmule sponges of *S. lacustris*, gemmules of both the thick-walled and the thin-walled type were used. A comparison of Figs. 16 and 17 reveals that the thicknesses of microscleres corresponding to a given $SiO_2$-concentration are considerably larger in sponges developed from thin-walled gemmules than in sponges from thick-walled gemmules. A similar difference does not exist regarding the thicknesses of the megascleres (Barker Jørgensen 1946).

of the scleroblast seems to depend on the $SiO_2$-concentration in the culture medium when the concentration is below a certain value, in the present experiments somewhat above 0.1 mmol. At very low concentration in the culture medium the dependence is almost linear.

## Summary and Conclusions.

The growth in thickness of the spicules of *Spongilla lacustris* and *Ephydatia fluviatilis* occurs by apposition on the spicules of

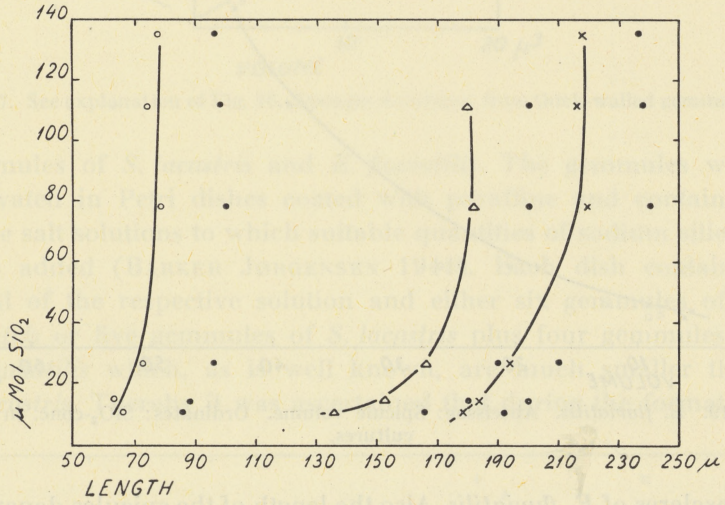


Fig. 20. Abscissae: Length of microscleres (○) and megascleres (×) of *S. lacustris* and megascleres (△) of *E. fluviatilis*. Ordinates: $SiO_2$-conc. in the cultures. Each point is based on 25—50 measurements. The black dots indicate the maximum lengths.

an approximately constant quantity of silicic acid per unit time in a constant environment.

The rate of uptake of silicic acid is calculated on the basis of measurements of the rate of growth in thickness and of the size of the scleroblast surface around the measured part of the spicule. On the supposition that the quantity of silicic acid deposited on the measured spicule segment corresponds to the quantity of silicic acid which penetrates the surface of the scleroblast around the segment, the rate of uptake is found to increase from about $0.8 \times 10^{-18}$ to about $2.4 \times 10^{-18}$ mol $SiO_2$ per minute

per $\mu^2$ of the scleroblast surface when the $SiO_2$-concentration in the outer medium rises from 0.03 to 0.16 mmol.

The spicule size attained depends on the $SiO_2$-content of the medium for concentrations below a certain value; in the present experiments c 0.1 mmol. At very low $SiO_2$-concentrations we find an almost linear dependence between the $SiO_2$-concentrations and the total quantity of $SiO_2$ deposited per unit of spicule length at the end of growth.

In the present state of our knowledge it cannot be decided whether the $SiO_2$-uptake in the sponge and in the scleroblasts, respectively, occurs by simple diffusion or by active transport of silicic acid.

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