Cox (J.D.)

ISTHMIA NERVOSA:

A Study of its Modes of Growth and Reproduction.

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(Reprinted from the American Journal of Microscopy).

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[*From the American Journal of Microscopy.*]

AT the time Dr. Wallich's article upon the telescopic hoops of *Biddulphia*, *Isthmia*, and some other diatoms, appeared in the *Monthly Microscopical Journal*, of February of last year, it happened that I had in hand a considerable quantity of the *Isthmia nervosa*, *in situ* upon seaweed, from the vicinity of Santa Barbara, California. I at once determined to make a somewhat full examination of this material, and to learn what facts in the life history of this diatom could be drawn from the study of the dead specimens.

Part of the material I mounted dry *in situ*, so that it could be examined as either an opaque object or a transparent one; the rest was cleaned in acids, part mounted in balsam, and part kept in distilled water for examination in that condition.

The examination fully sustained Dr. Wallich's statement, that the hoop of *Isthmia* is often found to be of two or more concentric tubes, which slide over each other during the multiplication of the diatom by division; but in regard to the mode of growth of the hoop itself, the causes of variation in the size of the frustules, and especially as to the formation and function of the conjugate (or sporangial) frustule, the facts observed seem to point to conclusions so different from his as to warrant a detailed presentation.

At the risk of repeating some things too well known, I shall endeavor to give a connected description of the appearance of the diatom at the several stages of its growth, up to the appearance of the conjugate frustule, and through such subsequent steps as I find sufficiently supported by the evidence before me.

1. *Forms of the Diatom.*—The *Isthmia nervosa* has both a rhomboid and trapezoid form. The heavy costæ, or ribs, which give the name to the species, are found on the valves only, but not on the hoop. These nervures or costæ are very similarly arranged in all the frustules. Those of one valve are nearly parallel to each other, pretty regular in direction, running from the margin next the hoop to near the summit of the dome-like valve, where they reticulate with each other. Those of the other valve start from the margin in a similar way, but branch much sooner, and run together in a coarse network, of which the

most marked lines or ribs partially surround and isolate the nose-like projection of the valve, from which the gelatinous *stipes* exudes through the fine seive-like perforations, which here take the place of the much larger areolæ found on other parts of the valve. The costæ project boldly into the interior of the shell to a depth of about one-twelfth of its shortest diameter, and their interior arrangement about the point by which the diatom is attached, is such as to indicate, or at least strongly to suggest, a separate cell, having the office of secreting the gelatinous substance of the stipes or stalk. (Fig. 1.)

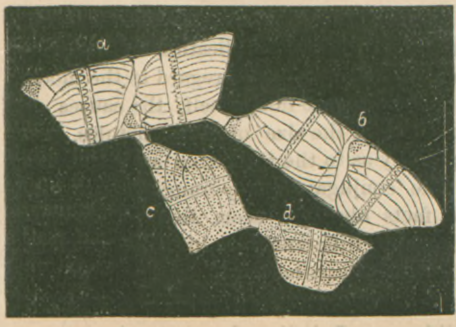

Fig. 1.

2. *Relative Numbers of the two Forms.*—The fission of the diatom is always transverse diagonally, the line of division running in such a way as always to divide a rhomboidal form into two trapezoids, and a trapezoid into one rhomboid and one trapezoid. From this it results that the number of trapezoids is largely in excess of the rhomboids, for, from the binary division of one of each kind, must result three trapezoids to one rhomboid. (Fig. 1, *a*, *b*.)

3. *Position of the Stipes and Point of Attachment.*—Assuming the point of attachment of the frond or filament to be its lower end, the valve with irregular costæ, which I will call the stipital end of the diatom, is always down. Thus, in a specimen in which division is complete, except that the two new frustules are still held within the elongated hoop of the original one, it will be seen that the stipital ends are in the same position, thus maintaining a general analogy to the positions of buds and stems upon any larger plant. (Fig. 1, *a*, *b*.)

4. *Modes of Connection in the Filament of Diatoms.*—The apparently capricious way in which the several diatoms are attached to each other is easily accounted for, since a single slide of the plant *in situ* will generally give specimens which mark nearly every conceivable step in their growth and separation. Bringing these steps together, we find that the simplest form of connection is that which occurs when the stipes of No. 2 attaches itself to the top of No. 1, their growth crowds them beyond the hoop, and the two remain as shown in Fig. 1, *a*, *b*. No. 2 may attach itself to the edge of the hoop of No. 1. In this case No. 2 is gradually crowded out of the hoop by the growth of its twin brother below, until its stipes can only hold on the very edge of the hoop, and the still further growth of No. 1 makes it turn outward until it takes the position shown in Fig. 1 at *c*. If, now, No. 1 completes a second division, No. 3 may remain attached to the others as shown above (Fig. 1, *d*), or may become loosened and fall off entirely, leaving the first two in a position in which the hoop of No. 1 projects beyond the frustule, and No. 2 remains attached to its side. Besides the various combinations and permutations of these modes of attachment, the loosened frustules may attach themselves wherever they may fall, either to the alga on which the colony lives, or to other diatoms amongst which it may lodge.

4. *The Hoop.*—I have already stated my accord with Dr. Wallich in finding the hoop composed of concentric elliptical tubes, sliding over each other like the draw tubes of a telescope. Indeed this is so obvious a fact that the most casual observer could hardly be excused for overlooking it. Not only is it shown by the thicker band about the middle of the diatom, when the areolæ of the different lamellæ of the hoop are confused by lack of coincidence with each other, and where, by focussing upon

either, the areolæ of that lamella may be brought out sharply, the other becoming dim; but at the sides of the diatom, where the curve of the hoop becomes nearly perpendicular to the line of vision, the double, and sometimes triple thickness, is seen almost as clearly as if looking at the edge of a longitudinal section.

But Dr. Wallich draws the conclusion that the hoop grows by addition of silex at the free margin, "much in the same way that the epiderm of the molluscous shell is secreted by the margin of the animal's mantle." My observations tend to establish a different conclusion, viz., that the growth of the hoop is in accordance with the laws of vegetable growth, rather than animal, and harmonizing with the theory of a membrane in which the silex is deposited, which membrane may be divided and split by the process of intussusception, or a new membrane may be formed within the old, from the protoplasm or live cell contents. Let us enumerate some of the facts bearing upon the question.

Upon almost any slide of *Isthmia* will be found numerous specimens, in which will be noticed not only the arrangement of the areolæ in the form of the quincunx, made by pretty regular diagonal rows, but the face of the hoop is divided by one or more lines of clear silex, running straight around the diatom, and breaking up the dotted field into two or more parts, as shown in Fig. 2, *a*. Bringing different individuals under the eye, one will soon be found in which this clear line has divided so as to show two smooth edges a little separated, as if the two halves of the diatom had broken asunder through the middle of the hoop. This suture, however, will be found not to cut clear through the shell, but the areolæ of an inner lamella will be seen below the opening, and upon one side or the other of it careful focusing will trace successively the faint outlines of other areolæ in an exceedingly thin film of silica, which fades away to invisibility as it is traced toward the junction of the hoop with the valve.

It may also be seen that when the opening of the suture is very small, the areolæ of the inner film have evidently slid from under the exterior ones, by the same distance as the opening of the suture, thus showing that they were before concentric, and suggesting (what further observation satisfactorily proves) that the same process of doubling the membrane or film has gone on upon the other side of the suture in the other half of the diatom, but is for the nonce invisible, because the adhesion of the films is undisturbed, and their areolæ remain concentric.

If the slide is rich in broken specimens (so often the most instructive), a little search will probably be rewarded by the discovery of a cast hoop in which, beyond the suture, the thin film above described is still adherent, not as a gradual tapering or diminution of the substance of the hoop, but starting from the edge of the suture by a shoulder, or sudden diminution of the thickness, which the light marks by a distinct line, beyond which the silica grows evanescent and the areolæ gradually fainter, toward the irregular and scarcely visible edge. (Fig. 2, *b*.)

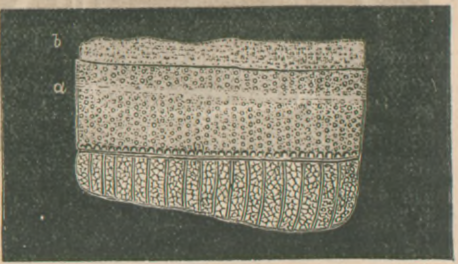

Fig. 2.

I shall presently give reasons for thinking that the separation of the parts of the hoop at the suture does not take place, when the diatom is growing normally, until the inner hoop has acquired its growth in thickness, and extends from valve to valve, or at least to the next suture above or below the one at which the division is supposed to take place; but the next point to be immediately noticed is that a similar suture occurs near the edge of the hoop where it connects with the valve, and when, by the growth of the diatom, the inner or outer hoop separates at that place, the line of the division can be plainly seen running across the large horseshoe-shaped areolæ which there form the characteristic marking of the shell. We thus have lines of suture always at the two extremities of the hoop, and, frequently, one, two, or even three, crossing it at intervals. The intervening sutures are not invariably present, but are found oftenest in the largest diatoms, and especially in those whose width is greatest in proportion to their length. The suture may appear on the inner hoop at a different place from that on the outer, or when none appears on the outer hoop except at its ends, and *vice versa.* This mode of "breaking joints" will at once be seen to be useful in strengthening the structure, and preventing the slightest accident from breaking the diatom quite in two in the middle. (Fig. 3, *c.*)

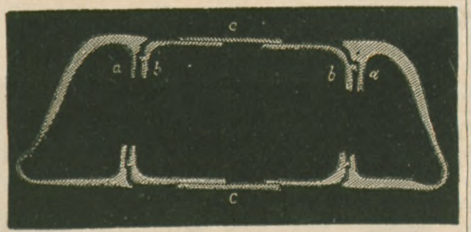

Fig. 3.

5. *The Connection of Hoop and Valve.*—Before proceeding further in the description of the manner in which the sliding of the telescopic hoops upon each other accommodates the growth of the diatom, it will be well to examine the structure of the connecting parts of hoop and valve, since these are very important items in the list of conditions which control the phenomena in question, and they do not seem to have been carefully examined or understood.

In the fully formed valve of the diatom, the edge next the hoop has a broad flange projecting inward, whose width is usually found to be about one-sixth of the shorter diameter of the valve. The costæ of the valve terminate in this flange or diaphragm, with a tapering curve reaching to its inner edge, thus supporting and strengthening it. Upon the valve, this flange makes a sharp right angle with the outer edge, or is even a little concave. The hoop has a similar diaphragm, buttressed and supported in like manner by the nervures of the large areolæ which mark the end of the hoop; the appearance of these so-called areolæ being, in fact, wholly caused by the nervures or braces which support the diaphragm, and having no real similarity to the areolæ which mark the general surface of the diatom, but are analogous to the costæ of the valves. The hoop diaphragm has one very important peculiarity, viz., that its inner edge is recurved or bent down over the edge of the valve diaphragm, with a lip of about the thickness of the latter plate, thus giving great firmness of connection, and effectually preventing lateral displacement of the parts. The hoop and its diaphragm do not meet at a sharp angle, as in the case of the valve; but the side of the hoop rounds into the diaphragm with an easy curve. The connection of the parts is shown diagrammatically, in longitudinal section, in Fig. 3, *a*, *b*.

I have occasionally found the diaphragm of both valve and hoop greatly widened, projecting into the interior of the diatom much further than common, so as to make a partition more than half closing the connection between the two parts. Its inner outline in these cases, is of the form of a broader ellipse, but it seems to have grown as a thinner film of silica, back of the ordinary diaphragm, or rather so extending from it as to leave the box-like fitting of parts, as shown in Fig. 3, so that the thinner portions are not in immediate contact with each other, as the thicker and narrower rims or flanges are. Upon these widened diaphragms the lines of the supporting nervures extend, as upon the narrower ones, but becoming fainter and wider as they go, giving a beautiful appearance of radial lines pointing to the open centre. (Fig. 4.)

It will thus be seen that the provision for a firm connection between valve and hoop indicates permanence, and the fact will be found in accordance with this. The valve and hoop never sever their connection at this place during the life of the diatom, and in the examination of a great number of individuals in which the separation has occurred, I have scarcely found half a dozen in which the recurved lip of the hoop diaphragm has not been fractured in places, thus indicating that the separation was by violence and not by natural causes. I regard it therefore as a settled point that the

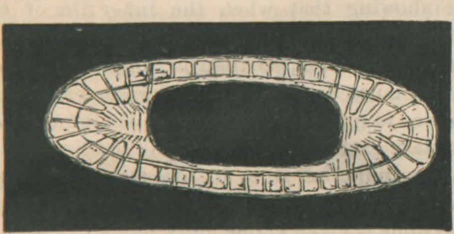

Fig. 4.

end of the hoop (including the large areolæ and the diaphragm) after it is once grown, never separates from its adjacent valve during the life of the diatom, but that all divisions by multiplication, and losses or separations of intermediate parts of the hoop, leave the connection between this and the valve undisturbed.

6. *The Formation of New Valves in the Growth of the Diatom.*—The diatom may properly be considered as having attained its full growth when its valves are so far separated as to leave room for the division of the cell contents into two, and the formation of two new and full-sized valves within the hoop. When this work is completed, we have no longer one diatom, but two, and their union from that time may be regarded as mechanical merely. The study of the dead specimens can tell nothing directly, about the formation of the nuclei and division of the endochrome. The growth of the siliceous film of the valve may, however, be traced by examples of almost every stage of its progress. It is found with the film so thin that it can only be discovered at all by its outline, where a portion of it is seen on edge, as it were. In these cases, no membrane could be conceived more delicate, appearing slightly crumpled by the pressure which the new valves, being back to back, seem to apply to each other by the growing force within. At this stage, no trace of costæ or areolæ can be seen. Soon, however, as a somewhat more advanced specimen is found, faint, hair-like traces of the costæ are seen. These are found soon to be more and more developed; areolæ, between them, begin to appear, and, finally, the fully formed valve, with its oval edges resting on the hoop diaphragm. The living force within each pair of valves would now seem to have gathered its energies for the next step, for we find the new valves separating from the old ones, and the pair of new diatoms severally growing as the parent did before them. In this separation the new valves leave the hoop diaphragm behind them, with its braces looking like large areolæ, and they each seem to be simply forced apart from the old valve by the growing force of the cell contents, as two pistons in one cylinder would be by steam injected between them.

7. *The Sliding of the Telescopic Hoops, and Formation of New Hoop Diaphragms.*—The growth of the diatom to a size sufficient to contain two full sized valves within the hoop, may or may not have occurred without the sliding of the tubes of the hoop upon each other. According to my observation, it depends upon the fact of the hoop of the parent diatom being broad enough at the time of its total separation from its parent, to equal the depth of two new valves.

In the stages of its history immediately following the appearance of the conjugate (or sporangial) frustule, as I shall notice presently, the hoop of the parent is narrow, the whole diatom being very broad in proportion to its length. Consequently the splitting of the hoop by intussusception, or the growth of new layers within, and the sliding of the tubes upon each other, accompany, in such cases, the growth of the cell contents, and may have to be repeated several times before room enough for the new valves is found. In other cases the parent diatom, having room enough within its girdle, the telescopic sliding does not begin till the new valves are formed, and the pair of young diatoms begin to crowd each other by their own growth.

What, then, is the process and method of this extension of minute draw tubes? I have already described the sutures which girdle the hoop at different places. The full-grown diatom seems to have its hoop also ripened into preparation for its new office. The separation of the lamellæ takes place, like the ripening of the shuck of a nut, which not only loosens it from the nut within, but prepares the seams between the segments to open at the slightest application of force. So the concentric films of the hoop, and the sutures crossing it, are prepared for separation when the growth of the cell contents demands more room. The weakest joint first yields to the strain, and the division goes on until the young plants are full grown and drop apart.

The simplest case is that of a diatom with a hoop wide enough for the formation of the new valves. Here the ripened hoop is at least double in thickness; the young diatoms press upon each other, and one thickness of the hoop separates from the other, at one end by a suture of the inside film, and at the other end by a suture of the outside film, and the two slide over each other till they slide apart. Many cases of this kind have been observed, in which the parts of the old hoop are persistent, and form the hoop of the new diatom. When the halves fall apart, the growth of the cell contents continues to force the valve out to the end of the hoop, or until the diatom is again ripened and has its growth. Then is witnessed a singular phenomenon. When the two valves are separated to the distance required for the division of the contents, and not till then, the first indications may be seen of the growth of a new hoop diaphragm, with its strong braces making the large horse-shoe areolæ. These also may be found in all stages, from the faintest trace and scarce visible outline, to the strongly marked and complete form. In one instance, not only were the faint outlines of the large areolæ seen, but through the whole interior of the hoop were traceable oval outlines partly compressed into hexagonal form, like a transverse section of a vegetable stem with closely packed oval cells. This is the only instance I have found of such indications of the beginning of the areolæ in a mere outline, but every slide I have is abundant in specimens, showing that when the inner film of the hoop is new and very thin, the areolæ are very much larger than on the exterior, often so much so as to reduce the connecting silica to quite narrow bands. Whilst the solitary example, therefore, will hardly warrant an induction from it, the statement as to the much larger size of the areolæ on new films is given as one fully proven.

Here, however, I must notice a fact which stumbled me. In the cases which I have noticed in paragraph 4, where the thick hoop has been found with a thinner part attached, and this last diminishing to a knife edge, the areolæ on the thin part have not been larger, but smaller if anything, as if split from the bottom of a depression corresponding to the outer areolae, (Fig. 2, *b*). After careful examination of numerous instances, including those in which the loosening at the suture is only partial, the best solution I can suggest is the following. These cases would seem to exist only when the separation has been forced, and premature, *i. e.* before the splitting of the hoop by intussusception is fully ripened. For in most cases of apparently natural growth, where the separation of the hoop takes place at one of these sutures, the doubling of the lamellæ on the loosened side extends all the way from the suture to the diaphragm, or to the next suture, and the sliding of the parts follows the usual appearance, the areolæ of the two films being similar in size. The weight of evidence seems to be that the doubling or tripling of the lamellæ occurs in both of the two methods indicated, viz., by the splitting of the exterior lamella, and by the rapid growth of a new one within, the latter case being the one in which the areolæ appear of very considerably greater size.

The telescoping of the hoops exhibits all the combinations which could be expected from the arrangement of sutures which have been described. The thick band commonly visible in the growing diatom may be caused by the simple lapping of two tubes, but it may also be caused by a real band, the division taking place at its two edges simultaneously, and the divided inner hoop sliding both ways out of the band, which is loosened, and falls when the separation is complete, giving one of the most common forms in which a perfect cast hoop is found, both margins being smooth and unbroken, (Fig. 3, *a*).

Before leaving this part of the subject, I will mention the fact that with high powers the areolæ on both valve and hoop can be seen to have a thin film of silica over or under them, the margin of each areola having an indented appearance, which I interpret to be little thickened projections from the thick margin upon the surface of this thin film, analogous to the braces or projections of the costæ upon the diaphragm of valve or hoop above described.

8. *The Conjugate or so-called Sporangial Frustule.*—For manifest reasons, it would appear to be improbable that much evidence on the subject of conjugation should be found in the dead remains of diatoms. But in this respect, as in many others, the indestructible character of the material has been the means of preserving testimony which presents itself unexpectedly, and is of decisive weight on one or two important questions.

I regard it proven by my observations that the so-called sporangial frustule in Isthmia propagates by division in the same manner as all the other frustules. It seems almost equally clear that from this large frustule its descendants decrease regularly in width, but gain in length, until the proportions are exactly reversed, the width of the conjugate frustule being equal to the length of that from which it sprung, at the moment of its full growth, and the consequent division of its cell contents, while its length, when leaving its shell, is only equal to the width of its attenuated parent.

The facts illustrating and supporting these positions are as follows: In carefully going over my slides of the dry diatoms on algæ *in situ*, I happened upon a specimen in which the end of a very wide but short frustule was still inserted in a small hoop and valve. The line of division between the valves of the large diatom was at right angles (or nearly so) to that of the small. Here was evidently a case of a chick with part of its shell still on its tail. Had the same been found in a cleaned specimen, it might have been accounted for by supposing the two accidentally to have fallen into such juxtaposition; but they were here as they had grown, and were the terminus of a filament which was plainly before my eyes. (Fig. 5, *b*). A hunt stimulated by

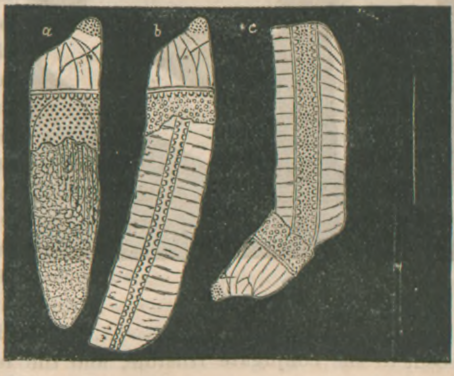

Fig. 5.

this discovery, resulted in the finding of over a dozen more instances, all attached to the small parent frustule in a similar manner, but varying in their own length as they had progressed in growth, by the widening of the hoop. These being arranged in the order of their growth toward maturity and division, made a regular series, ending with two, one of which showed the faint outline of the nascent pair of new valves within the hoop, (Fig. 6, *a*), and the last was a completed example of division into two new frustules (Fig. 6, *b*). Later, among the cleaned but unmounted material was found an aborted frustule, in which conjugation had taken place, but the perfect growth of the valves had been stopped apparently by the breaking of the shell of the parent diatom too soon. As in the other cases, the end of the conjugate frustule was inserted into the parent shell, of which nearly half remained, and from which it projected straight out. Its coat was, of course, silicified, having withstood the boiling in acids, but its areolæ were confused and its shape imperfect, and no demarcation of valves distinguishable, (Fig. 5, *a*).

The relation of other filaments to these, among the specimens *in situ*, was such as to force the mind very powerfully toward the conclusion that the strings of large diatoms were those nearer in the degrees of

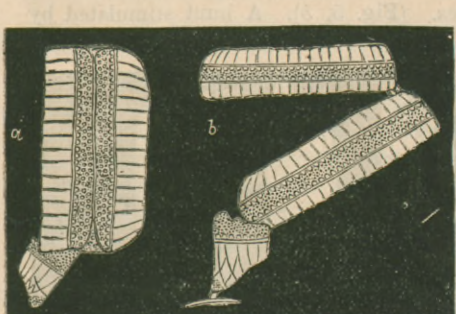

Fig. 6.

descent to the conjugate frustule, and the strings of smaller ones were those further removed from the ancestor, but nearer to the advent of a new conjugation. In the presence of these facts it must require direct and positive evidence to support the theory that the conjugate frustule is a sporangium, giving rise to a numerous brood of new diatoms at once.

Whoever will take pains to compare the phenomena of the growth and division of Isthmia with the results of modern botanical investigations into the origin of the cell wall, its growth and division by intussusception, and the division of the cell contents, so as to form two or more "daughter cells," will, I am sure, be impressed with the evidence he will find that the general laws of vegetable physiology, as seen in larger plants, are as implicitly obeyed in these minute structures as anywhere in the vegetable kingdom.

We have only to bear in mind the fact, discovered early by Prof. Bailey, that cellular membrane may be demonstrated to coexist with the silica in the diatom walls, and the following general statement of Sach's in regard to the morphology of the cell, will be found to harmonize very perfectly with the phenomena I have attempted to describe: "The systems of striation and stratification of a cell wall, intersect one another, like the cleavage planes of a crystal splitting in three directions. But since the striations and laminæ consist of lamellæ of a measurable thickness, composed of alternately denser and less dense substance, the cell wall appears to be composed of parallelolipedal pieces, distinguished by their contents of water. If we for a moment disregard the stratification, and assume that we have two intersecting systems of striation, then where two denser striæ intersect, the densest or least watery places are always to be found; where two less dense ones intersect, the least dense or most watery; and where places of greater and less density intersect, areolæ of intermediate density are formed." ("Text-Book of Botany," Eng. Ed., p. 30.) The whole context of the passage quoted, the succeeding section on intussusception, and the treatment of the origin of the partition walls in tissue cells, which increase by bi-partition, in the same work (p. 72), describe the steps of growth in this diatom as perfectly as general statements can do. The specific peculiarity of the diatom is that the deposition of the silica in the membrane of the cell wall, so stiffens the parts as to make the separation of the lamellæ of the cell wall appear more like mineral cleavage, and has brought about the adaptation of the parts and of the natural forces to their peculiar work, so that the singular feature of the sliding of the hoops upon each other in mechanical fashion, has taken the place of the sort of accommodation which the softer parts of the common vegetable structure make for the growth of the new cells.

Many of the appearances of the diatom, at different stages of its growth, indicate a degree of plasticity and elasticity of the walls. especially in the case of the new forming valves, which very evidently yield to each other, crumpling the membrane more or less, before the silication has become strong, and presenting quite a different appearance from that of the strong and mature shell, which is finally exposed to the action of the outer water.

I have spoken of the gelatinous stipes as exuded from the sieve-like perforations of the lower end of the shell. This is, of course, an induction from the appearance of different examples of this stipes, and from the manner in which it is attached to other shells, or to the algæ on which the plant is found. It would be difficult to describe all these appearances, but a summary of them in one's own mind gives great strength to the conviction that the mode of growth is as stated. It is quite easy to understand how a similar exudation may give rise to the longer stipes of Gomphonema, Achnanthes, Licmophora, etc. It is only necessary to imagine the exudation more rapid, and rapidly solidifying on exposure to the surrounding water, to see how a long column may thus be built up. I, however, fully recognize the fact that scientific methods forbid that this should be regarded as anything more than a suggestion to be verified or disproved by actual observation of the life history of these plants, an investigation quite within reach, and which we may hope will stimulate the zeal of the members of the vigorous microscopical society of the Pacific coast, where these forms abound.

It is a little singular that the observed facts which have been detailed in the foregoing paper, tend strongly to support the earlier view of the character and office of the conjugate or sporangial frustule, as against the later one. As early as 1848, Mr. Thwaites, in the paper in the "Annals of Natural History," in which he made known his discovery of conjugation in certain species of diatoms, spoke of the occurrence of this phenomenon as "a mixture of Endochromes; after which process fissiparous division proceeds as before," and this view prevailed until arguments from analogy, but, so far as I know, without definite support from actual observation, led to the opinion that the conjugate frustule was in fact a sporangium. Dr. Carpenter has expressed this opinion in the last edition (1875) of his work on the "Microscope and its Revelations," saying (p. 317): "It has been already shown that the sporangial frustule, even when it precisely resembles its parent in form and marking, greatly exceeds it in size; and this excess seems to render it improbable that it should reproduce the race by ordinary self-division. Appearances have been observed which make it probable that the contents of each sporangial frustule break up into a brood of gonidia, and that it is from these that the new generation originates."

In like manner, Dr. Wallich, in the article to which reference has already been made, says, "The sporangial frustule, instead of being, as heretofore assumed, the primary or parent frustule of a new and vigorous generation, constitutes in reality the expiring phase in the life cycle of a generation that is passing away,"—that it is a "sporangial cell," "doomed, on the liberation of its living contents, to death and immediate decay."

The observations I have detailed appear to me a demonstration of the fact that the conjugate frustule *is* the "parent of a new and vigorous generation," and that the earlier belief is the better one. But whilst thus enforcing the danger of making negative assertions in matters of science, I will not fall into the same error by denying that there are among the diatomaceæ any sporangial cells producing broods of gonidia. The cases I have observed in *Isthmia nervosa* show that this diatom is propagated from the so-called sporangial frustule by ordinary self-division. I have looked with some care for evidence of distinct observation by others of the origin of any diatom from gonidia and have not lighted upon it. In regard to the *Isthmieæ*, at least, Dr. Carpenter says the phenomena of conjugation "have not been clearly made out in this group." ("The Microscope and its Revelations," p. 314.)

Upon the topic of the mode of division of the hoop and the sliding of the laminæ upon each other, the same caution should be observed lest too large or too hasty conclusions are drawn from the facts. In regard to *Isthmia*, the cases observed seem conclusively to prove that the hoop proper is a permanent part of the diatom, except when the separation at the sutures is such as to leave a portion of the hoop free at both edges, in which event the separation of the young frustules drops this segment of the hoop. In other cases it remains attached to one or the other valve, and forms the outer coat of the wall of the new growing diatom. If we suppose that in any species no sutures occur in the hoop, except near the connection between hoop and valve, no cast hoops would be found, except when accident had broken the frustule, for the two lamellæ would adhere, one to the old and the other to the new valve, and this would satisfactorily answer the queries propounded by Dr. Carpenter in another passage, where he says, "In some other cases all trace of the hoop is lost, so that it may be questioned whether it has ever been properly silicified, and whether it does not become fused (as it were) into the gelatinous envelope."—*Id.*, p. 314.

The field is a most fascinating and an open one, and let us hope that careful observations will be pushed until the life history of all the diatomaceæ shall be thoroughly known, a work which the venerable authority just quoted well says, "may advantageously occupy the attention of many a microscopist who is at present devoting himself to the mere detection of differences, and to the multiplication of reputed species." It is also to be hoped that the valuable work already done in this direction by such able naturalists as Prof. H. L. Smith, and perhaps others, may be soon brought within the reach of all who are interested in these studies, so that the task of investigation may be lightened by avoiding unnecessary repetition of steps already taken, or by the encouragement of finding verification for observations which might otherwise be involved in doubt.