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The Life History of Some Marine Plankton Diatoms

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I—THE LIFE HISTORY OF SOME MARINE PLANKTON DIATOMS

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Introduction

The diatoms are divided into two big groups, the Pennatae and the Centricae. The first group consists mainly of bottom forms which are either attached or show free movement, whereas the non-motile, suspended Centricae form the main bulk of the planktonic diatoms. The present work is concerned with the second group only.

A knowledge of the essential structure of the cell, which is very similar in both groups, is of great importance for understanding the life history of the diatoms. The cell walls consist of two parts which overlap each other like the two halves of a pill box; the valves forming the top and bottom, the connecting bands or girdle the sides of the box. In the course of cell division the two halves of the cell wall become separated and each daughter cell forms a new valve and new connecting bands to fit into the old girdle. Owing to the silicification of the cell membranes, growth is possible only along the pervalvar axis, the longitudinal axis of the "box". Consequently the diameter of one cell is slightly smaller than that of its sister cell, i.e. by twice the width of the cell wall. In a population this peculiar mechanism of cell division leads to a continuous decrease of the average cell size.

As early as 1869 MacDonald and Pfitzer (1871) established the existence of a regulatory process which counteracts the decrease of cell size by the formation of special cells which are capable of growth, the auxospores. Later work, notably by Karsten, Klebahn and more recently by Geitler (1932), produced quite a clear

picture of the life history of the Pennatae. In most forms the production of auxospores is preceded by the fusion of gametes, these representing the only haploid phase in the life cycle of the Pennatae.

Our knowledge of the centric diatoms is far less satisfactory. The life cycle is complicated by the occurrence of resting spores and microspores as well as of auxospores.

Resting spores have been frequently found in many species. They are regarded as resting stages which sink to the bottom and persist through unfavourable periods (Gran 1902). From the literature it appears that the sequence of their formation and germination has so far never been observed.

Microspores were observed by several authors (see p. 38) and regarded as gametes or as asexual reproductive cells. It must be pointed out that none of the authors has actually observed the fusion of microspores or the development of the zygote. According to Geitler (1932) this fact might perhaps find its explanation in observations recorded by Went (1924; see also his letter to Geitler (Geitler 1932)). Went studied the plankton on a journey across the Atlantic. One day the only diatoms to be found were two forms of *Chaetoceros* of which the larger was full of microspores while the smaller cells were surrounded by flagellated swarmers of similar size to those within the larger cells. Went suggested that the microspores of the larger form were microgametes which fertilized the female cells of the smaller type.

The auxospore formation in centric diatoms was formerly generally regarded as a simple growth phenomenon of the protoplast, not connected with either a sexual process or any nuclear changes. Recently, however, Persidsky (1929, 1935) found first in *Chaetoceros boreale* and *Ch. densum*, later in *Melosira varians*—the same species in which Schmidt described microspore formation—that the formation of auxospores is connected with an autogamic sexual process.

From this short review it can be seen that with regard to the life history of centric diatoms the views of different authors differ quite fundamentally. When I came to the Plymouth Laboratory last year I started culturing planktonic diatoms in the hope that a study of pure cultures might clear up some of the contradictory statements in the literature.

Apart from its purely botanical interest such a study appeared desirable from the point of view of marine biology. Plankton diatoms are of great importance as food in the sea. Their distribution and their seasonal and yearly variation in abundance, in connexion with the corresponding hydrographical conditions and chemical changes of the sea water, have been the subject of many investigations. However, owing to our incomplete knowledge of their life cycle those surveys were mainly concerned with the vegetative cells, and references to auxospores are very rare. The resting spores, evidently of some importance for the understanding of the seasonal variations in the abundance of diatoms in the sea, have been frequently found in plankton samples by various workers, but so far there does not exist any experimental evidence concerning the conditions responsible for their formation and germination.

The present work has been made possible by a grant from the Academic Assistance Council and a scholarship from the Marine Biological Association. I wish to express my sincere thanks to Dr. E. J. Allen for his kind interest and the encouragement I received from him throughout the work, and for the excellent facilities of the Plymouth Laboratory which he placed at my disposal. My thanks are also due to Miss M. V. Lebour who identified most of the diatoms I used, and to Dr. E. J. Allen, Dr. S. Kemp, and Mr. F. S. Russell who read the manuscript of this paper and made various corrections and valuable suggestions for alterations.

Culture methods

One of the reasons why the life history and physiology of the pennate diatoms is so much better known than that of the Centricae may be found in the fact that the former are more resistant and easier to culture than the latter. The classic work on culture conditions for centric diatoms was done by Miquel (1892–7), Allen and Nelson (1910) and Allen (1914). The technique used by these authors was either to pick out individual diatoms under the microscope and to place them in a sterile culture medium (Miquel's or Allen's modification of it) or to dilute a small quantity of a plankton sample containing diatoms in a certain amount of culture fluid and to subdivide this into a number of culture flasks. In one or the other culture one diatom species only would develop. Another method employed by Allen was to start a raw culture with one or two drops of plankton diluted in about 250 c.c. of sterile culture medium which was distributed over a number of Petri dishes. If these are left undisturbed for a few days colonies of different diatoms will develop at different spots on the bottom of the dish. These were picked out with pipettes and transferred into culture flasks.

I used a different technique which gave very satisfactory results. As culture medium Föyn's "Erdschreiber" (Föyn 1934) has been used. This is a modification of Schreiber's medium (Schreiber 1927), soil extract being substituted for distilled water. It consists of

$NaNO_3$	0·1 g.	Soil extract	50 c.c.
Na_2HPO_4	0·02 g.	Sea water	1000 c.c.

The diatoms were isolated from plankton samples by picking out single individuals with fine pipettes. It is essential to wash the isolated cells by passing them through three or four sterile water samples in watch-glasses. Next day the washing is repeated once or twice and the cells left for a few days in a watch-glass, well covered with a glass plate. If the diatoms reproduce satisfactorily a sample may be washed after a week or so and transferred into a Petri dish. All culture dishes, flasks, and pipettes were thoroughly cleaned and sterilized before use.

The cultures were kept at room temperature and placed near a window facing north. During the winter months a 100 W lamp hanging at a distance of about 1 m. was used to increase the light intensity during the day.

Subcultures were made every 2–3 weeks and inoculated mostly with 10–100 cells.

Bacteria-free cultures were not aimed at and no special bacterial tests made. Since the cultures were frequently examined, sometimes two or three times a day, and a large surface of the watch-glasses and Petri dishes was thus exposed to the air, the occasional appearance of mostly harmless bacteria was to be expected. On the whole I was satisfied if there was no obvious sign of bacterial contamination. A more detailed account of the culture methods has been published elsewhere (Gross 1937).

Ditylum Brightwelli (West.)

Most observations and experiments were done on this neritic species (fig. 1, Plate 1). The cells are elongated, usually solitary. In young, rapidly growing cultures they sometimes form short chains of 4–12 cells. The valves possess in the centre a strong straight hollow spine, and between the centre and the edge a collar of short, mostly curved spines. The species is described as being prism-shaped with rounded angles to nearly cylindrical. It may be noted that in cultures the progeny of prism-shaped cells gradually become cylindrical. On the other hand, the majority of cells developed from auxospores in a culture started with a single cylindrical cell were prism-shaped but also included forms with round, flat, elliptical and tetrangular valves. It seems therefore likely that the subspecies distinguished by taxonomists (see Hustedt 1930) forma *biangulata* and forma *tetragona*, with oval and tetrangular valves respectively, represent variations of the same type.

In cultures with many cells produced from auxospores there also occur forms which show a close resemblance to what is described as *Ditylum sol* Grun., the marginal ridge being more undulated, with several longitudinal lines showing up in girdle view, without the collar of small spines and with triangular valves, the sides of the triangle being slightly concave. As the distinction of this species is almost exclusively based on those characters it is not unlikely that what has been regarded as two species of *Ditylum* are again modifications of one species. This question should, however, be settled by someone with better taxonomic qualifications. I mention the above facts to show that even in pure lines there exists a considerable range of variability affecting a number of characters of systematic importance.

(*a*) *Cell division*

The cells of *Ditylum* grow in a longitudinal direction until they reach a certain maximum size which is rather uniform within a culture, but which may differ markedly from subculture to subculture (see p. 27). The division of the nucleus and protoplast then takes place and the new valves are formed inside the parental shell before the

separation of the daughter cells (fig. 1*b*, *d*, Plate 1). After separation the new valve lies at first well within the old girdle (fig. 1*c*, Plate 1) and is gradually being pushed forward out of it (fig. 1*a*, Plate 1).

The division of the protoplast takes about 30 min. Some stages of the division followed in single cells are shown in figs. 2 and 3 (Plate 1). The main bulk of protoplasm is concentrated near the centre of the cell, usually connected by two protoplasmic strands with the centre of the valves (fig. 2*a*, Plate 1). The division begins at the periphery of the cell and proceeds towards the centre. It is unlike the division of other Protozoa and Algae in that the incision is very broad from the beginning. The protoplast takes the shape of a dumbbell (fig. 2*b*, Plate 1), the prospective daughter cells being connected with each other by a protoplasmic bridge. This connexion becomes gradually very fine and eventually gets resorbed (figs. 2*c–e*, 3*a–c*, Plate 1), leaving a wide empty space between the daughter cells. The way in which the protoplasm is drawn out in the course of division, and the fact that the protoplasts of the two daughter cells together fill a space far smaller than the original parental one 20 or 30 min. before, suggest the existence of a strong bipolar tension prior to and during the division. The new central spines are formed a short time after cell division (fig. 2*f*, Plate 1, 8 min.; fig. 3*e*, Plate 1, about 40 min.) and most probably before the formation of the new valves. During their growth the spines are soft and whenever they touch another object—the opposite spine (fig. 1*d*, Plate 1) or the primary valve (fig. 7*b*, *c*, Plate 2)—they are liable to become bent. The growth of the spines takes $\frac{1}{2}$ hr. at most. The separation of the cells, however, takes place several hours later, after the formation of the small spines and the silicification of the new valves.

(*b*) *Nuclear division*

Since the first detailed investigation of the nuclear division in *Surirella* by Lauterborn a number of other pennate diatoms have been studied cytologically (see Fritsch 1935). The extranuclear origin of the spindle as described by Lauterborn has not been confirmed, and apart from the existence of centrosomes in some pennate diatoms the mitotic division does not seem to be fundamentally different from other Algae. However, Ikari (1923) has described an extranuclear spindle similar to that of Lauterborn in *Surirella* in the centric diatom *Coscinodiscus subbulliens*, and Schmidt (1927, 1928, 1929, 1931) has recorded a number of extremely peculiar cytological features in *Biddulphia sinensis* and other centric forms. His methods and interpretations were strongly criticized (Geitler 1931; v. Cholnoky 1933), and some of his statements were recently withdrawn (Schmidt 1933).

v. Cholnoky (1933) studied mitosis in *Melosira arenaria* and established a perfectly normal origin of chromosomes and spindle. In this respect I was able to confirm his results, but in some details the nuclear division in *Ditylum* differs from that of *Melosira*. A short account may therefore be of some interest.

For the cytological study of the plankton diatoms I have been using the quick and simple method of acetic carmine. This preserves and stains simultaneously and has in recent years been extensively used in plant cytology and work on *Drosophila*. It gives good results with diatoms with not strongly silicified membranes as in *Ditylum* and *Chaetoceros*. It is prepared by dissolving carmine to saturation in hot acetic acid —1 part distilled water, 1 part acetic acid—and filtering. A number of cells with as little fluid as possible are placed on a glass slide in a drop of acetic carmine and heated for a moment over a small flame. The drop is then covered with a cover-slip, a ring of hot vaseline put round the edge and the preparation is ready for use.

Dividing cells were found at any time of the day in cultures that were not over-crowded; on the average perhaps more divisions take place in the early morning.

The nucleus lies within a central protoplasmic area which is suspended in the cell and connected with the protoplasmic layer inside the membrane by means of two or more protoplasmic strands. Before or during division of *Ditylum* cells the central protoplasm with the nucleus moves towards the cell membrane. In broad cells all mitotic figures were found to lie near the side wall of the cell, the girdle, in continuity with the protoplasmic layer where the numerous small chromatophores are situated. In cells of smaller diameter this movement does not seem to take place as regularly as in broad ones. v. CHOLNOKY has found a similar movement in *Melosira* cells: the nucleus is drawn by the protoplasmic strands towards one of the valves and sometimes becomes slightly deformed.

The resting nucleus appears finely granulated and interspersed with somewhat stronger stained chromatic granules (figs. 4*a*, 5*a*, Plate 1). In the centre lies a single nucleolus, of almost homogeneous appearance, not vacuolized as in *Melosira arenaria*. In the prophase it disappears; the whole body of the nucleus is filled with numerous chromatic threads. The individual limits of the chromosomes are indistinct—a stage which formerly might have been interpreted as a continuous spirem (fig. 5*b*, Plate 1). The next figure (5*c*) shows a well-developed central spindle, while the chromosome threads have undergone little change. Centrosomes or corresponding structures appear to be absent throughout the mitosis. In the late prophase (fig. 4*b*, Plate 1) the shape of the individual chromosomes is becoming distinct. Their structure is that of long fine threads of chromomeres, the connecting substance between these being almost unstained. Some evidence for the assumption that the spindle is of intranuclear origin is produced by stages like fig. 5*d* (Plate 1), where the spindle is fully developed and the nuclear membrane is more or less intact. The chromosomes are here on the way towards the equator, their proximal parts more advanced than their distal ends. It may be noted, however, that in most cases the nuclear membrane is already absent or at least not visible at earlier stages.

In metaphase (fig. 4*c*, *d*, Plate 1) the attachment of the chromosomes to the spindle seems to be terminal. The chromosomes are stretched in almost one plane. They are very delicate and not appreciably more condensed since the prophase. Exact counting

of their number is impossible; it may be roughly estimated at 40–50. The spindle has the shape of an ellipsoid rather than that of a cylinder.

The anaphase movement must take place with great rapidity. Among many hundreds of division stages not a single middle anaphase was found. In the late anaphase (fig. 5*e*, Plate 1) the chromosomes are lying more or less parallel to the long axis of the cell. The telophase (figs. 4*e*, 5*f*, Plate 1) is perfectly normal, the chromatine structure resembling that of the prophase. A spindle remnant remains visible throughout the restoration of the resting nucleus (fig. 4*f*, Plate 1). The nucleolus appears at a very late stage (fig. 5*g*, Plate 1); this figure also shows the outline of the dividing protoplast).

On the whole the mitotic figure—chromosomes, nucleolus, spindle—are much more delicate in *Ditylum* than in *Melosira*. Their structure, however, is essentially the same. In *Melosira* the nucleolus persists through a long period of the prophase until the formation of the spindle, and it reappears after division in an earlier telophase stage. There is no "spirem" stage; in the early prophase the chromosomes appear in the shape of individual threads in smaller number than in *Ditylum* and the spindle develops very late in the prophase. At metaphase the chromosomes are much more condensed, shorter and thicker than in *Ditylum*, and form a dense and compact ring around the spindle as in the Pennatae. On the whole the mitosis of *Ditylum* does not seem to differ from that of other Algae, higher plants and animals in any essential character.

(*c*) *Secondary valves*

The formation of new valves is not entirely dependent on cell division. In dense cultures of *Ditylum* one can frequently find empty valves at the bottom of the dish. They originate from cells of the type represented in fig. 6*a* (Plate 2). Within the old shell a smaller cell with a new hypotheca has been formed. The formation of such cells with secondary valves could be followed when single cells were isolated.

On 25 November a cell which had undergone division on the same day was isolated in a watch-glass (fig. 7*a*, Plate 2). Next day both daughter cells possessed secondary valves. One of them is drawn in fig. 7*b* (Plate 2). The new spine has apparently touched the old primary valve during its growth and was consequently bent. This cell was again isolated. Next day it showed considerable growth in length, the old valve being pushed off but still hanging on the spine of the new secondary valve. (When transferred with a drop of culture fluid on a cavity slide to be drawn under the microscope the primary valve came off. It has been drawn in about the same position as it originally lay in the watch-glass.) Subsequently the cell showed normal division.

Such cases have been repeatedly observed in various lines. There is reason to assume that the formation of secondary valves is caused by a preceding contraction of the protoplast. A cell like figs. 6*a*, or 7*b* (Plate 2) must have been covered inside the whole membrane with a protoplasmic layer containing the chromatophores. Owing to the contraction of the protoplast towards the epitheca a condition is created similar to

that after the division of the protoplast and a new valve is formed. Actually a contraction of the protoplast could several times be observed when placing cells on glass slides for microscopic examination. It is a very rapid process, in these cases probably caused by mechanical irritation. This, however, does not explain the occurrence of cells with secondary valves in cultures which at least for some time had been quite undisturbed.

In crowded cultures several valves can be formed successively by a single cell. Up to eight have been observed. Fig. 6*b* (Plate 2) is a drawing of a resting spore with five valves attached to it.* The original protoplast has contracted four times and each time it has formed a new valve. Eventually a resting spore was formed.

Cells with secondary valves have also been found in nature. Meunier (1915) gives a figure of a *Ditylum Brightwelli* cell containing a secondary valve and describes it as "Phase de division avortée par résorption de l'une des deux cellules filles et le maintien d'une seule cellule jeune au sein de la matricule".

Payne (1925) studied plankton from Hong Kong which contained a great number of *Ditylum sol*. Among them he found hundreds of forms of *Ditylum* "in which the frustule developed only one new frustule; the second, oldest valve being barren, and pushed off as the newcomer became fully grown". "No secondary frustule showed any indication of the development in it of a third frustule. . . ." Payne suggested that the secondary frustules may be resting spores of *Ditylum sol*.

Hustedt (1930) found similar stages of *D. Brightwelli*. He regards them as cells with incomplete division, having formed at the end of the mother cell one young daughter cell while the formation of the sister cell has been omitted.

My observations on cultures show that the interpretations of these authors were not correct. It is obvious that the formation of secondary valves does not represent an incomplete, interrupted division, nor are two daughter cells formed, one getting resorbed, as Meunier suggested. No nuclear division is connected with it, and the protoplast never shows any sign of division. Moreover, as has been stated further above (p. 4), the cells of *Ditylum* grow to a certain maximum length before cell division takes place. A comparison of the cell length in fig. 6*a* with fig. 1*b* (cells from the same subculture!) and of fig. 7*b* with fig. 7*a* (Plate 2) shows that the cells with secondary valves were far from having reached the size of cells undergoing division. No daughter cell has been formed, but the same protoplast has after its contraction produced a new valve.

From the following section it will be seen that resting spores of *Ditylum* are quite different from secondary frustules. It may well be that some of the factors responsible for resting spore formation also cause contraction of the protoplast and subsequent formation of secondary valves. This would explain why sometimes the formation of resting spores is preceded by that of secondary valves (fig. 6*b*, Plate 2).

* The resting spore was lying at the bottom of the dish with the row of valves directed upwards, so that on the camera lucida drawing the length of the valves appears shortened.

The formation of secondary valves bears some resemblance to the "moulting" of thecate dinoflagellates; a phenomenon which I have repeatedly seen in dense cultures of *Prorocentrum micans*. It is also similar to the formation of "internal valves" frequently recorded in pennate diatoms (Hustedt 1930; Geitler 1932), in which, however, both valves are newly formed, a more or less complete shell within the old parental one.

(*d*) *Resting spores*

The first culture of *Ditylum* (line A) was started on 1 October 1935 with twenty cells taken from a plankton sample. On 5 October the first subculture was made, and 16 days later many resting spores were found in it. These are more or less spherical bodies of very dense structure lying within the original cell membrane (figs. 8*a*, 9*a*, Plate 2). Some had developed a strong silicious membrane so that neither the chromatophores nor any other structural details could be seen under the microscope (fig. 6*b*, Plate 2). Each cell contained one resting spore.

On 7 November a smaller number of resting spores was found in the 15 days old subculture 2 together with some auxospores; there were also many cells with a shrunken protoplast stretching from valve to valve but, under low power, apparently disconnected from the side wall of the cell. The explanation of these peculiar cells appeared in subculture 3 which was started on 1 November. On examination after 10 days very many resting spores were seen in the morning. When, however, the culture was again examined in the afternoon to start a fresh subculture not a single resting spore could be found. All had germinated during the day into perfectly normal vegetative cells. The same striking phenomenon was observed next day: 10 a.m., culture full of resting spores; 1 p.m., few resting spores, very many germinating resting spores, i.e. cells with a long, tube-shaped protoplast; 6 p.m., two resting spores found among many thousands of vegetative cells.

As shown in Table I, this "rhythmic" process of daily formation and germination of resting spores continued until 22 November, when few spores were recorded ungerminated at 6 p.m. After 26 November all resting spores persisted, and no further germination took place in the culture. The number of cells was estimated at 120,000, or 3000 per c.c. of culture fluid, of which about 70 % had formed persistent resting spores.

Table II shows the conditions in subculture 4. Here the first resting spores appeared after 13 days. During the next 10 days a great number of resting spores were formed overnight and germinated in the course of the day (some exceptional days will be discussed later; see p. 18). After that few spores were found ungerminated in the evening, and when the culture was 33 days old they persisted in great numbers without germination.

Several parallel cultures showed very similar behaviour. In subculture 5 the first appearance of resting spores was rather late, 18 days after the subculture was made

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TABLE I—OCCURRENCE OF RESTING SPORES IN SUBCULTURE 3

Date	Age of culture in days	Room temperature in ° C. Min.	Room temperature in ° C. Max.	Illumination integral in kilolux-hours	Number of resting spores 10 a.m.	Number of resting spores 6 p.m.
Nov. 11	10	8·9	11·4	129	Very many	0
12	11	10	15	118	Very many	0
13	12	8·9	15·6	94·7	Very many	0
14	13	10·6	15·6	47·4	Few	0
16	15	10	—	107	Very many	0
18	17	8·3	15·6	106	Very many	0
20	19	10	15·6	49·6	Many	0
21	20	10·6	17·8	124	Many	0
22	21	10·6	16·7	77·7	Very many	Few
23	22	10	14·4	106	Very many	Few
24	23	8·3	13·3	—	Many	Few
25	24	6·7	14·4	71·6	Many	—
26	25	10	13·9	63·9	Many	Many
27	26	—	13·9	56·5	Very many	Very many

TABLE II—OCCURRENCE OF RESTING SPORES IN SUBCULTURE 4

Date	Age of culture in days	Room temperature in ° C. Min.	Room temperature in ° C. Max.	Illumination integral in kilolux-hours	Number of resting spores 10 a.m.	Number of resting spores 6 p.m.
Nov. 20	9	10	15·6	49·6	0	0
24	13	8·3	13·3	—	Few	0
25	14	6·7	14·4	71·6	Many	0
26	15	10	13·9	63·9	Many	0
27	16	—	13·9	56·5	Many	0
28	17	11·7	16·7	37·3	0	0
29	18	12·2	16·1	96·5	Very many	0
30	19	11·7	—	37·3	0	0
Dec. 3	22	11·7	—	33·7	Many	—
4	23	7·2	16·7	83·5	Very many	Few
6	25	9·4	16·1	49·7	Few	Few
7	26	10	15	62·2	Very many	—
9	28	10	14·4	84·1	Few	Few
14	33	7·2	15	70·4	Very many	Very many

(Table III). Four days later many resting spores were formed overnight and germinated in the daytime. When the culture was 29 days old the resting spores persisted.

Resting spores were formed in cultures of *Ditylum* irrespective of the size of the cells, by very narrow ones as well as by cells which had developed from auxospores a few

TABLE III—OCCURRENCE OF RESTING SPORES IN SUBCULTURE 5

Date	Age of culture in days	Room temperature in ° C. Min.	Room temperature in ° C. Max.	Number of resting spores 10 a.m.	Number of resting spores 6 p.m.
Dec. 14	16	7·2	15	0	0
16	18	7·2	—	2	0
18	20	10·6	—	10	0
20	22	7·2	14·4	Many	0
27	29	6·7	13·9	Very many	Very many

days before. The only difference was the larger size of the spores. Cultures of broad cells originated from auxospores also showed formation of resting spores overnight and their germination during the day.

The germination of resting spores can be followed in single cells (figs. 8–11, Plate 2). The repeated pipetting to and fro from the watch-glass where the cell was isolated to a cavity slide for drawing under the microscope slows the speed of germination down considerably. While the spores in the Petri dishes, except the persistent ones, complete germination in the evening of the same day, the isolated cells still showed some contraction of the protoplast in the evening (figs. 8*d*, 10*c*, 11*c*, Plate 2), and only regained the appearance of vegetative cells on the following day. The process is, however, not altered.

The first sign of germination is a slight elongation of the spore (fig. 8*b*, Plate 2). Fine protoplasmic filaments similar to very delicate pseudopods connect the spore with the old cell membrane. It may be noted that in most of the cases observed the first connexions sent off by the spore run towards the centre of the valve opposite the insertion of the long spine. Most of the filaments send off branches a short distance from the membrane or form a network. The elongation of the protoplast continues and the "pseudopods" become more conspicuous. Sometimes chromatophores could be watched slowly passing along a protoplasmic strand towards the membrane. Gradually the expansion of the protoplast is completed and the cell takes on its normal appearance: viz. with the inside of the thecae covered with a protoplasmic layer containing the chromatophores and in the centre the main body of protoplasm containing the nucleus, suspended on a number of protoplasmic strands.

The germination of individual spores shows some differences in detail. In narrow cells their elongation proceeds mostly in the direction of the long axis of the cells (fig. 8, Plate 2). In broader cells the protoplast stretches more irregularly throughout the inner space. Some germinating spores are suspended at or near the centre of the shell by fine or more solid "pseudopods" (fig. 11, Plate 2), others come into broader contact with one of the valves and from there send off connexions to the rest of the shell (fig. 10, Plate 2). Fig. 9 (Plate 2) shows that even in cells shortly after division where the volume of the resting spore does not greatly differ from that of the original cells germination starts by the sending off of protoplasmic filaments, and does not merely consist of a gradual expansion of the body of the spore as one might have expected.

It is of some interest to note the differences between the germination of the resting spores represented in figs. 10 and 11 (Plate 2). These were sister cells connected with each other like those on fig. 1*d* (Plate 1) and only separated in the course of isolation from the culture. In fig. 10 (Plate 2) the spore expands along the valve taking a more or less oval shape, and after a few hours (3.30 p.m.) shows inside the structure of the vegetative cell, with a central mass of protoplasm with strands extending to the periphery. The germination appears less "amoeboid" than in fig. 11 (Plate 2).

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However, the anchoring of the germinating spore to the shell by means of fine protoplasmic processes is a characteristic feature observed in all cases.

The germination described above was observed on resting spores which were isolated in watch-glasses with fresh culture medium; these would, however, have also germinated in the original cultures. In these the resting spores at the time of isolation were formed overnight and germinated in the course of the day. To induce germination of persistent resting spores which develop a silicious membrane and never show any sign of development in the culture, it is necessary to transfer them into fresh culture medium. The germination takes 2–10 days or even longer and proceeds in exactly the same manner as described above.

In old overcrowded cultures a considerable number of resting spores could be found outside the parental shells. When transferred into new culture medium they only expanded in volume, but as yet the formation of valves has not been observed.

The formation of resting spores could not be followed in single cells since isolation inhibits and usually reverses the process. But in cultures with cells slowly undergoing formation of resting spores one can easily study all stages of this process. Fig. 12 (Plate 2) gives four stages and shows that the formation of resting spores is almost exactly the reverse of germination. The protoplast becomes detached from the shell and contracts gradually into a compact, more or less spherical body. At first it remains connected with the thecae by means of a number of fine, partly branched, protoplasmic filaments which become resorbed in the course of the contraction. Occasionally some chromatophores could be observed passing along these filaments towards the central body of protoplasm. And again in most cases, the strongest connexions were those leading to the centres of the valves, the point of insertion of the long spine (fig. 12*b*, *c*, Plate 2). These also were the last to be resorbed.

Resting spores were frequently found in tow nettings. In autumn and winter after complete absence of *Ditylum* from the plankton a few resting spores were usually found together with vegetative cells and cells like those represented on fig. 13*a*, *b* (Plate 3), which might be stages of either the formation or germination of resting spores. Since their appearance coincided with that of vegetative cells of *Ditylum* after periods of absence from the plankton they probably were germinating resting spores. When isolated and placed in culture medium they developed in less than 24 hr. into normal vegetative cells.

In an attempt to analyse the factors responsible for the formation of resting spores let us begin with the observations recorded at the beginning of this section and in Tables I—III. The outstanding facts are as follows: (1) No resting spores were formed before the cultures reached the age of 9, 13, and 18 days respectively. During this period the cells reproduced actively and the Petri dish cultures reached a density of 30,000–50,000 cells in about 35 c.c. of fluid, i.e. about 1000 cells per c.c. of culture medium. (2) There followed a period of 9–15 days with formation and subsequent germination of a great number of resting spores. The reproduction of the cells not

undergoing spore formation continued and the culture became very dense. (3) Finally the resting spores developed a stronger silicious membrane and persisted. The cultures were crowded with resting spores and sometimes also with auxospores. The remaining vegetative cells became dark brown and did not divide any more.

Taking first the second phenomenon it appeared possible that the differences of the room temperature by day and night were the causes of the successive formation and germination of the resting spores. In Tables I–III the minimum and maximum temperatures are given: the differences are quite considerable. At night the temperature went down sometimes as low as 6·7° C., in the morning it was usually about 10° and in the course of the day it rose to 14–16°. I first thought that the temperature must drop below 10° to be effective. On 14 November (Table I) comparatively few resting spores had been formed; the minimum temperature was 10·6°. Again in another culture no resting spores were found on some days (28, 30 November; Table II) when the minimum temperature was 11·7°. However, very many were found on 29 November in spite of the high minimum temperature of 12·2°. Therefore probably another factor perhaps in conjunction with low temperature was affecting the cultures.

That low temperature could not be the only factor responsible for resting spore formation was clear from the beginning, because, as mentioned above, young cultures under otherwise the same conditions did not produce resting spores. Subcultures 3 and 4 were kept in the same place near the window, one Petri dish above the other. Subculture 4 did not produce a single resting spore from 11 to 23 November, while during the same period subculture 3 was crowded with them (see Tables I and II). Thus apparently the age of the culture was of importance, in that perhaps some changes were produced in the culture medium by the reproduction of the diatoms to a certain number of cells per c.c. of fluid.

To test this a number of simple experiments were made. One, ten, twenty, and 100 cells were isolated on various days into watch-glasses from cultures which produced very many resting spores overnight. The watch-glasses were placed on top of the Petri dish culture or very near it. Not a single resting spore was found either the next or the following day, while in the original culture more than half of the cells underwent spore formation.

Under these conditions—cultures with few cells in fresh culture medium—low temperature and at the same time absence of light (ice-box) did not seem to have any effect with regard to the formation of spores. On 1 November two resting spores were isolated from subculture 2. They germinated the next day. During the following days they showed a slow reproduction but no resting spores were formed. On 13 November thirty cells were transferred from that culture into a watch-glass and this placed in an ice-box (7·2° C.) for 4 days. No resting spores were formed. In other experiments a few cells were exposed to low temperature of varying degrees for periods up to a week with the same result.

On 18 November, 10.15 a.m., 100 resting spores were taken out of subculture 3, washed, and placed in a watch-glass with about 1 c.c. of fresh culture medium. They germinated in the course of the day in the same way as the resting spores which had remained in the original culture (see Table I). Division took place but no resting spores were formed during the following 4 days. On the 5th, 6th and 7th day three resting spores were seen, on the 12th day nine resting spores (and four auxospores). On the 14th day at 10 a.m. the culture contained 9–10 resting spores and was placed in an ice-box (3·3° C.). At 2.30 p.m., only $4\frac{1}{2}$ hr. later, there were considerably more resting spores of normal appearance, and almost all the rest of the diatoms formed spores of somewhat irregular shape (fig. 14*a*, Plate 3) with the contracted protoplast in the centre and few chromatophores and granules left outside the spore. The culture was left for 2 days in the ice-box and the cells counted. There were:

99	regular resting spores,
852	irregular resting spores,
9	auxospores,
2	vegetative cells,
962	

The cells were counted by cautious pipetting from the original watch-glass into a new one. They were left in it with the old culture medium, and after 2 days it was found that all irregular resting spores had taken regular, more or less spherical shape. (A similar process is described further below; see fig. 15, Plate 3.) One irregular spore, isolated into fresh "Erdschreiber", germinated in the course of 5 days (fig. 14, Plate 3). Germination of the other resting spores started only after 10 December, when they were transferred into fresh medium.

In this experiment, with almost 1000 cells in about 1 c.c. of medium, the low temperature was very effective since the resting spores were formed within $4\frac{1}{2}$ hr. instead of several days. It seems likely that the irregular shape of the spores was caused by the very sudden change of the temperature inducing a hasty spore formation. As they mostly took regular shape after the exposure to low temperature had ceased there seems to be no fundamental difference between the two types of resting spores.

It may therefore be concluded that low temperature is very effective as an additional factor to a certain degree of "overcrowding", i.e. to some changes in the medium which occur in the course of the growth and reproduction of the cells. The importance of the latter factor becomes clear from the following experiment. On 6 November a small number of cells were isolated in a watch-glass and kept at room temperature. During the following days a few auxospores and no resting spores were formed. On the 19th the great majority of diatoms had formed persistent resting spores. On

the 20th the cells were taken out with a fine pipette and counted. The culture contained:

6656 resting spores (mostly of narrow cells, some of broad cells developed from auxospores),
28 vegetative cells.

In order to find out when persistent resting spores will germinate, and whether they would do so at low temperatures, 1000 resting spores were placed in a watch-glass with fresh culture medium and left at room temperature near the window, and a further 1000 were transferred into a watch-glass with culture medium and placed in an ice-box. The number of germinated spores on the following days is given in Table IV. As soon as any vegetative cells were found—the cultures were examined daily—they were taken out so that no appreciable reproduction of the cells could have occurred. Thus the number of vegetative cells given in the table corresponds closely to the number of spores which had germinated. At room temperature germination started on the 3rd day when 126 vegetative cells were taken out and four auxospores which must have developed from vegetative cells shortly after the germination. During 12 days, counted from the start, altogether 569 resting spores had germinated. After that the culture had to be discarded owing to an infection with bacteria.

TABLE IV—NUMBER OF GERMINATED RESTING SPORES ON THE DAYS FOLLOWING THEIR TRANSFERENCE INTO FRESH CULTURE MEDIUM

	1000 resting spores at room-temperature		1000 resting spores in ice-box (7·8° C.)
	No. of vegetative cells	No. of auxospores	No. of vegetative cells
Nov. 21	—	—	—
22	—	—	—
23	126	4	— (6·1° C.)
24	120	5	—
25	91	1	—
26	44	4	6 (4·4° C.)
27	25	1	—
28	37	3	—
29	45	2	2
30	19	1	—
Dec. 2	40	1	—
	547	22	8

A more unexpected result was produced by the resting spores kept in the ice-box where on the 6th day six and on the 9th day a further two vegetative cells were found. This fact shows that resting spores, provided they are placed in fresh culture medium, are capable of germination at a comparatively low temperature and in almost uninterrupted darkness (they were exposed to light for short periods during the daily examinations). Therefore the quality of the medium seems to be the most important factor for germination. This conclusion is supported by the behaviour of the other 1000 resting spores kept at room temperature where germination took place during

a period when in other cultures with an older culture medium, but not necessarily with a larger number of cells than 1000 per c.c. of fluid, very many resting spores were formed under otherwise similar conditions.

In the preceding experiment the 126 cells found on 23 November were placed in a watch-glass, and next day a further 120 cells derived from spores (Table IV) added. The watch-glass was kept on the same place as the other containing the resting spores. The 246 cells reproduced actively and after 5 days four and later on more resting spores were formed, while in the original watch-glass the germination of resting spores continued. This and other similar observations show that with about 1000 spores in 1 c.c. of fresh medium germination can go on for at least 10 days, whereas the metabolic activity of growing and dividing cells very soon produces changes which result in the formation of resting spores.

There were few exceptions to the rule that in cultures resting spore formation was preceded by a strong increase in the number of cells. In one case forty cells were transferred into a watch-glass. Next day already four resting spores were seen and two cells forming auxospores. After 11 days the culture contained:

59 vegetative cells,
10 resting spores,
6 auxospores.

Thus evidently growth and division of the cells were greatly inhibited. The explanation could be found in the presence of a great amount of bacteria which, after some time, could be seen as a "cloud" at the bottom of the glass dish. Clearly their presence was responsible for what otherwise could only be observed in overcrowded cultures: (1) inhibition of growth and reproduction, (2) formation of resting spores, (3) the persistence of auxospores without any sign of development (see p. 24).

In another case a single cell was isolated in 1 c.c. of culture fluid without washing. Only 4 days later division took place, and when drawn under the microscope many bacteria were seen attached to the diatom cells. After a further 9 days in the original medium three cells were present, all containing an irregular resting spore (fig. 15*a*, Plate 3). Immediately after being placed in fresh culture medium the three resting spores took regular shape (fig. 15*b*) and germinated in the course of several days (fig. 15*c*).

The observations described above were made in autumn and early winter. In January–February the *Ditylum* cultures gave some trouble by producing numerous cells with slightly contracted and highly refractive protoplasts. In spring and summer the formation of resting spores was delayed, less regular, and their number smaller. Subculture 7′ contained on 20 March (28 days after the inoculation) not more than a few thousands of resting spores; in later subcultures their number gradually decreased to nought. The same was observed in line C of *Ditylum*, started with a single cell on 29 January, and in another culture from one auxospore. In some overcrowded cultures

many cells were found with a protoplast somewhat contracted and irregular in shape, but the formation of resting spores was extremely rare.

These observations show that the "overcrowding factor" alone is not sufficient to induce resting spore formation, and the question arose whether low temperature applied to dense cultures would result in the production of resting spores.

On 2 May 200 cells of clone C were placed in a watch-glass with 2 c.c. of medium. On the 18th the number of cells had greatly increased, and a number of auxospores and very few resting spores had been formed. The culture was placed in the ice-box (8·3° C.) for 3 days, then diluted with culture fluid and the diatoms in a sample of 1 c.c. counted. The estimate gave: 16,400 vegetative cells—mostly of the original narrow type—and auxospores, and 1800 resting spores, i.e. about 10 %. Although this number was far greater than ever observed during spring and summer in cultures kept at room temperature it was very little compared with cultures of a similar degree of density in autumn and winter.

The next question was whether low light intensity had an effect on resting spore formation. Several cultures were placed near a west window facing a wall. No direct sunshine came through and the light intensity was further reduced by a blind. In May the light intensity was—as Mr. H. W. Harvey kindly found out for me—about 10 % of that near the north window and fell within the range of light intensity during late autumn. Dim light had no effect on dense cultures: the cells stopped division but no resting spores were found. When, however, the culture was first placed in dim light for some time and then exposed to low temperature the effect was remarkable, as the following experiment may illustrate.

On 12 May a rich, not yet overcrowded, culture of *Ditylum* (line C) contained a number of auxospores but no resting spores. At 7 p.m. it was placed in an ice-box (8·3° C.). Next morning a small number (200–300) of resting spores were found. In the course of the day no germination took place and the culture was again put into the ice-box. The following day no noticeable increase in the number of resting spores took place. The exposure to low temperature was repeated once more with the same result. On the 16th the culture was placed in a dim light near the west window. After 2 days there was about the same small number of resting spores present—no germination had taken place—and the culture was put into the ice-box (8° C.). Next day (19th) the increase in the number of resting spores was quite considerable. The culture was left in the ice-box for another 2 days, then a sample counted. The estimated numbers were:

64,000 vegetative cells and auxospores,

50,000 or almost 45 % resting spores.

This experiment resulted in a number of resting spores nearest to those observed in cultures during autumn. It gives good evidence for the assumption that low light intensity is another factor affecting the formation of resting spores.

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When these results were obtained I thought it possible that some irregularities in the number of resting spores formed in the autumn cultures on successive days (see Tables I and II) might be explained by the daily differences in the light intensity, viz. that a small number of spores would only be formed on nights following days with a high quantity of light in spite of the nightly drop of temperature. In Tables I and II data are included giving the vertical illumination integral on the roof of the laboratory in kilolux-hours.* This is a value for the quantity of light during a whole day. It includes, however, direct sunshine, whereas the cultures, placed near the north window, never received direct sunshine but only the amount reflected by a wall opposite the window. At any rate the data do not provide a direct explanation for the irregularities. For instance, on 13 November the amount of light was smaller than on the 15th (104 kilolux-hours), but the number of spores was less on the 14th than on the 16th.

The interaction of light and temperature might, however, be of a much finer and more complicated nature. The minimum of light necessary for resting spore formation probably varies with the temperature, and the temperature effective for the production of many resting spores might vary with the amount of light which the culture had previously received. For instance, on 27 November (Table II) the amount of light might have been sufficiently low to induce the formation of resting spores, but the minimum temperature at night was comparatively very high (11·7° C.), and therefore, perhaps, no resting spores were formed and recorded on the 28th. From the 28th to the 29th, although the minimum temperature was even higher, many spores were formed because the amount of light received on the 28th was very low. This kind of interpretation could be applied to most of the observations summarized in Tables I and II. It remains, however, hypothetical until more data are available, preferably of experiments under different temperatures and light intensities kept constant for long periods.

Apart from the finer mechanism of interaction there seems to be good evidence for the existence of four factors responsible for the formation of resting spores: (1) presence of bacteria—a factor which is probably of little importance in nature; (2) changes of the medium which gradually take place with the growth and reproduction of the cells, possibly consisting of the exhaustion of some nutritive substances; (3) low temperature; (4) small amount of available light. The factor (2) is ineffective alone (summer!), but seems to be the preliminary condition for the action of (3) and (4). Only in pure cultures of a certain density can low temperature and low light intensity induce the formation of resting spores. At a certain density of the culture resting spores were formed temporarily overnight and germinated in the daytime. There was apparently a kind of equilibrium between factor (2) on the one side and factors (3) and (4) on the other. The changes of the culture medium were just so far advanced that under the influence of low temperature resting spores were formed overnight. They have, however, not accumulated to such a degree as to prevent their germination in the

* I am greatly indebted to Dr. W. R. G. Atkins who kindly provided me with these data.

same medium when the temperature and light intensity increased. With increased density of the culture the effect of factors (3) and (4) result in the appearance of persistent resting spores. These will germinate when transferred into new culture medium under otherwise the same conditions which induce formation of resting spores in dense cultures.

Comparing these results with conditions prevailing in the sea one should consider the fact that the culture medium is much richer in nutrient material than sea water. The population which can be maintained by a unit of "Erdschreiber" is far greater than by the same amount of water in the sea even under optimal conditions. Therefore one would expect a lesser density of the population to be sufficient to induce resting spore formation in the sea than in cultures. For further discussion see p. 41.

(*e*) *Cell size and auxospore formation*

In *Ditylum*, as in the vast majority of diatoms, the structure of the cell entails a continuous decrease of the cell diameter in a population.* After every cell division the hypotheca of the mother cell becomes the epitheca of one daughter cell. Consequently one daughter cell remains of the same size as the mother cell and the diameter of the other becomes smaller by twice the width of the cell membrane. In some cases (cf. Fritsch 1935; Hustedt 1930) the average cell size of a population was found to decrease progressively according to the binomial law, in others the larger cells seemed to divide more rapidly with the effect that the rate of decrease in size slowed down.

Fig. 27 gives measurements of line A (circles), line C (crosses) and of the Axp.-line and of cells derived from auxospores of line A (triangles). The cells were measured with a micrometer in an eyepiece 12, with an objective 8 mm., and each point represents the average diameter in μ of 50–100 cells.

Line A, started 1 October, had in December an average diameter of 27μ. Within 5 months it decreased gradually to 13·7μ, that is to almost half of the original diameter. After that, these narrow diatoms continued to divide for some days, but no subculture was successful after the end of May. The last measurements of this line were made on 12 May (subculture 11*a*) and gave an average of 13·7μ, the narrowest cells being 10·7μ in diameter. Subculture 12 was inoculated on 7 May with twenty diatoms. On the 20th this culture was very dense; it contained, however, only broad cells which had developed from auxospores and then reproduced rapidly. Not a single narrow cell of the original size could be found. In other subcultures of 11*a* the diatoms divided for a few days and then either formed auxospores or perished.

Clone C was started on 29 January, but the culture grew very slowly during February. On 23 April the average diameter was 29·2μ, on 25 August only 11·2μ (fig. 27). Thus the diameter decreased in the course of 4 months by 18μ to about one-third of the original diameter. The rate of decrease was higher in C than in A,

* The only exceptions seem to be *Nitzschia closterium* forma *minutissima* (Allen and Nelson 1910) and *Eunotia pectinalis* var. *minor* (Geitler 1932).

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which is probably due in part to a correspondingly higher division rate in the summer as compared with the winter. However, the slope of the curve of C is steeper at the beginning, the rate of decrease greater during the first month than during the later period. Subculture 13 from 14 August was here the last in which the narrow cells still reproduced. On 25 August they had an average diameter of 11·2μ, the narrowest diatoms being 8·5μ. Subculture 14 started on 25 August with seventy narrow cells

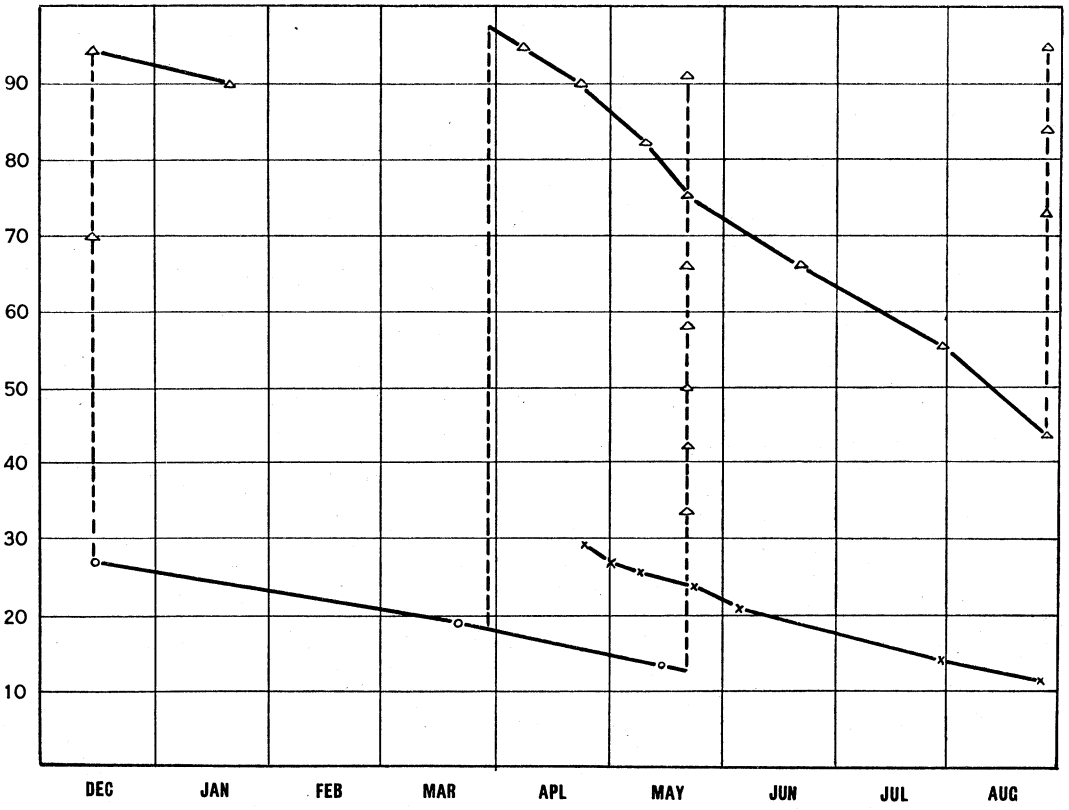

FIG. 27—The average diameter in μ of cells in successive subcultures of *Ditylum*. Line A marked with circles, line C with crosses, the Axp.-line and cells produced by auxospores marked with triangles.

showed 4 days later stages of auxospore formation and broad cells produced by them. A few cells of the original size were present with a very refractive and partly disintegrated cell content. Thus in *Ditylum* a cell diameter of 8–10μ is the minimum size at which the cells are capable of division.

In both lines formation of auxospores took place at intervals shortly after the cultures had been started until the end, when the cells reached the minimum size and died. The process of auxospore formation is entirely different from that of the resting spore formation. (*a*) All cells which undergo the formation of auxospores are very long, on the average distinctly longer than the "double cells", i.e. cells which had divided and the two daughter cells not yet separated (fig. 1*b*, Plate 1). (*b*) Auxospores are formed outside the cells by the extrusion of the whole protoplasmic content.

(*c*) The extrusion of the protoplast takes place at the junction of the two halves of the membrane (epitheca and hypotheca). Before the protoplast flows out the cell becomes bent in the middle, the two arms thus forming an obtuse, right or acute angle (figs. 16, 17, Plate 3). (*d*) The auxospores are more or less spherical bodies, much larger than resting spores, covered with the perizonium, a thin, non-silicified membrane, capable of expansion. They contain a central mass of protoplasm with the nucleus and sometimes with several protoplasmic strands extending towards the periphery. The inside of the membrane is covered with numerous chromatophores (figs. 16*b*, 17*b*, 18*a*, Plate 3).

The increased length of the cells which undergo auxospore formation becomes clear from a comparison between the average length of the "double cells", representing the maximum length during the cycle of growth and division, and the combined length of the two arms of cells in the early stage of auxospore formation (fig. 16*a*, Plate 3). To give an example: subculture 8′ (line A) showed on 20 March an average diameter of 19·2μ, and an average length of the double cells of 139·6μ. The average diameter of cells forming auxospores in the same culture was 19·3μ, the combined length of the arms 161·5μ.

This increase in length is most probably not due to actual growth, an elongation of the membrane by the formation of new intercalary bands—although such a process may occur occasionally (see p. 28)—but to the fact that the two halves of the shell separate farther apart than in cell division. As described on p. 4 cell division and formation of the new valves takes place while the parental membrane remains intact, the hypotheca being overlapped by the epitheca. In auxospore formation the growth of the protoplast continues and the thecae are gradually pushed apart.

At the moment of actual separation the bending takes place and the two halves of the shell become set at an angle one to the other. One might assume that the same tension which is present during cell division, and which is there responsible for the contraction of the daughter protoplasts and the formation of the wide gap between them (p. 5), also exists during the growth of the cell preceding auxospore formation. The tension may even become greater, since the growth continues beyond that at cell division. This increased tension would be responsible for the bending at the moment of separation of epi- and hypotheca, and the relief of the tension would be eventually produced by the detachment of the protoplast from the valves and its outflow through the split formed on one side between epi- and hypotheca in the course of bending. In an early stage of this process the outflowing protoplast is often drawn out to a point at the two poles, and as in resting spore formation remains for some time connected by a fine protoplasmic filament with the valves near the insertion of the spine (fig. 16*a*, Plate 3).

If isolated in such a stage the auxospore will be completed within less than 24 hr. Mostly the protoplasm flows out simultaneously from both arms (fig. 16*a*, Plate 3) and the empty shells may remain for some time loosely attached to the auxospore. (They were, in fact, in contact with the auxospore represented in fig. 16*b* (Plate 3)

but became detached when pipetted on a cavity slide for drawing.) Sometimes, however, the protoplasm leaves one arm first while still occupying part of the other (fig. 17*a*, Plate 3). Since the empty shell drops off very easily the remaining arm with the outflowing protoplasm might suggest a formation of auxospores different from that described above and similar to that of *Melosira Borreri* (p. 35). However, in all more closely observed instances of auxospore formation in *Ditylum* it was preceded by the bending of the cell.* Thus the cell drawn in fig. 17 (Plate 3) was bent at an angle of about 120° when first noticed (11 a.m.); the outflow of the protoplasm had not yet started. At 2.30 p.m. the stage drawn in fig. 17*a* (Plate 3) was reached. The empty arm was in close contact with the young auxospore when examined first; during the transport to the microscope it had been moved away a bit. Next day, perhaps already during the night, the auxospore was completed (fig. 17*b*, Plate 3). The whole process of auxospore formation does not take more than 24 hr.

Newly formed auxospores either develop immediately into broad cells in the culture medium in which they have been formed or, in old cultures, persist as spherical bodies and develop only after being placed into fresh culture medium (see p. 24). A few stages of the development of a single, isolated auxospore are drawn on fig. 18 (Plate 3). The day after having been placed into fresh culture medium the auxospore had flattened on one side and formed a valve and a spine. (The shrinkage of the protoplast from the perizonium only occurred when transferred on a cavity slide.) Next day the opposite side was flat too and another valve and spine had been formed. The cell had, however, not yet taken its ultimate prism shape, with triangular valves (fig. 18*d*, Plate 3). This final stage was reached after one or two divisions and remained a characteristic of the whole progeny of the cell for several months. Afterwards the shape became more and more cylindrical. The perizonium was thrown off during the formation of the valves (fig. 18*c*, Plate 3). The cell produced by an auxospore is always considerably broader in diameter than the cell which produced the auxospore, and given the right conditions divides immediately after formation.

What are the conditions responsible for the formation of auxospores? It was mentioned above that both lines A and C formed auxospores shortly after the start of the cultures. This statement should be understood in the sense that the cells were capable of forming them under certain conditions. If, however, a culture is made from a big cell picked out from a plankton sample, or derived from a big auxospore, the resulting population is not capable of forming auxospores for a considerable length of time. This fact also applies for the pennate diatoms (Geitler 1932).

In fig. 27 measurements are given of a line (Axp.) derived from an auxospore at the end of March. On 7 April the average diameter was 94·2 μ. The rate of decrease in the following months was much greater, the slope of the curve being much steeper than in lines A and C. In 5 months the diameter decreased by more than half to 43·7 μ

* A similar stage of auxospore formation as on fig. 18*a* (Plate 3), with the protoplasm extruded half way out of one arm, has been found by Karsten (1899) in the plankton.

(26 August). Although the division rate was not studied in detail occasional countings left little doubt that it was equal to or rather smaller in the Axp.-line than in line C. The division rate of the latter only slowed down in July/August when the cells approached their minimum size and showed a general decrease of vitality. But up to the last subculture the cell diameter decreased and auxospores were formed. In this respect they differ from the "under normal sized" cells in cultures of pennate diatoms (GEITLER 1932) which may be cultured for some time but have lost the capability of forming auxospores.

Since the external conditions were the same for the Axp.-line as for line C the more rapid decrease of the diameter can only be explained by the assumption that in cells of big diameter the cell wall is thicker than in narrow ones, and that with the gradual decrease of diameter a proportional decrease of the thickness of the cell wall takes place with the effect that the rate of decrease of the diameter is slowed down. This is again in agreement with GEITLER's observations on pennate diatoms.

The first auxospores of the Axp.-line were found on 25 August in the dense subculture 13 with cells of an average diameter of 43·7 μ. They were few in number, and there were also two small colonies of broad cells which originated from auxospores with a diameter varying from 72·6 to 94·6 μ with an average of 83 μ (fig. 27). We may conclude that a cell diameter of about 43 μ is a preliminary condition for auxospore formation. Lines A and C showed that cells with a diameter less than 43 μ are capable of forming auxospores but need not do so until they reach a diameter of about 10 μ, when further reproduction seems to be impossible for some physiological reason and they either form auxospores or perish.

The diameter of 43 μ can be regarded as an internal factor which must be realized before auxospore formation can take place. There follows a period of 6 months—under comparatively very favourable conditions in the laboratory—during which environmental factors are responsible for the formation of auxospores. This conclusion may be drawn from the following observations.

If auxospore formation were only due to internal factors one would expect this process to take place independently of the age of the cultures. This, however, was not so. Table V gives the dates when the first auxospores or cells forming auxospores appeared in successive subcultures of lines A and C. In the first subcultures of both lines auxospore formation only occurred in old, very dense cultures, considerably later than the formation of resting spores (see Tables I–III). When transferred into fresh culture medium the cells rapidly divided, and again only after 16–30 days when the culture had become crowded did auxospore formation take place. The same could be found in later subcultures, in line A for 5 months, in line C for about 2 months. After that, when the populations have reached an average diameter of about 20 μ, the intervals between the start of the culture and the appearance of the first auxospores became continuously shorter, being finally only 2–3 days (Table V). In some of the final cultures auxospores were even found 1 day after the inoculation.

TABLE V—RELATION BETWEEN AGE OF THE CULTURE AND FORMATION OF AUXOSPORES

No. of subculture	Culture started on	First appearance of auxospores after days
	Line A	
1	Oct. 5	18
2	Oct. 21	16
3	Nov. 1	19
4	Nov. 11	22
5	Nov. 28	29
6	Dec. 16	28
7	Dec. 27	17
7′	Feb. 20	14
8′	Feb. 26	9
9	Mar. 6	5
10	Mar. 24	7
10*a*	Mar. 31	6
11a_1	May 12	3
	Line C	
2	Mar. 4	20
3	Mar. 12	19
4	Mar. 31	27
4A	April 24	8
8	June 4	7
8″	June 26	6
13	Aug. 25	2

Thus the formation of auxospores, like that of resting spores, seems to depend on certain changes of the medium occurring with the growth and reproduction of the diatoms in a culture. The degree of the changes necessary for the formation of auxospores becomes smaller with the decrease of the cell diameter of a population or, in other words, the smaller the diameter the more ready the cell to respond to those changes and to undergo transformation into an auxospore.

Another phenomenon is connected with the different intervals at which auxospores were formed in successive subcultures. In the first culture of both lines A and C the number of auxospores produced was very great; there were thousands of them when the cultures became dense. There was, however, only a negligible number of broad cells developed from them. The auxospores persisted throughout two to three weeks, or even longer, until the culture decayed and only developed into broad cells when placed into fresh culture medium. Whereas newly formed auxospores developed regularly within 2 days, older ones needed up to 5 days for the formation of broad cells. When in later subcultures the auxospores were formed in younger cultures they all developed into cells of bigger diameter and both types of cells divided actively until the cultures became very dense. In the subcultures of line A made in April/May and in those of line C made in July/August the majority of cells present after several days were those of the broad type and their preponderance increased with the age of the culture. Finally, it became difficult to find and pick out even a few of the narrow cells for

subculturing and, as stated above, those transferred either formed auxospores or soon perished.

With regard to the nature of the changes in the medium which appeared to be responsible for the formation of auxospores some experiments with pure sea water as medium may be mentioned. On 4 June sixty cells were taken out of subculture 7 of line C, washed five times in sterile sea water, then thirty cells placed into a Petri dish containing sterile sea water only (*a*), and thirty cells into another Petri dish containing the usual culture medium (*b*). Next day one cell forming an auxospore was seen in (*a*), none in (*b*). On the 6th eight auxospores and two knee-shaped cells were found in (*a*), none in (*b*). On 11 June, after a week, the cells of both cultures were counted. The sea water culture (*a*) contained:

35 vegetative cells
21 auxospores
56

The "Erdschreiber" culture contained:

378 vegetative cells
5 cells forming auxospores
0 auxospores
383

As another control the "Erdschreiber" culture 7*a* may be mentioned which was started on 5 June with fifteen cells and contained after a week:

638 vegetative cells
9 cells forming auxospores
0 auxospores
647

This experiment shows that diatoms with a diameter of about 20μ (see fig. 27) not only divide very slowly in sea water as compared with "Erdschreiber" but that auxospore formation begins sooner, within a day in sea water, after 7 days in "Erdschreiber". The degree of "overcrowding" necessary to induce auxospore formation is obviously much smaller in sea water. This may, perhaps, be explained by the fact that "Erdschreiber" contains a much greater amount of nutrient substances than sea water. One might compare sea water with uncultivated soil and "Erdschreiber" with manured garden soil.

On 23 May 100 cells of line C were placed in sterile sea water (*a*), twenty cells from the same culture in "Erdschreiber" (*b*). On 27 May not a single auxospore

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could be found in (*b*) whereas the majority of (*a*) consisted of auxospores. On 4 June the content of (*a*) was counted. There were:

15	vegetative cells
557	auxospores
3	resting spores
575	

Thus only few divisions had taken place and almost all cells had undergone auxospore formation. Culture (*b*) had to be used for another purpose and could not be counted. It was by then a rich culture with many thousands of cells, at most 5 % auxospores and some broad cells produced by auxospores.

Of the 557 auxospores in the sea-water culture (*a*) not a single one had developed or showed signs of development into broad cells. On 4 June thirty auxospores were transferred into a watch-glass with sterile sea water, thirty into a watch-glass with "Erdschreiber". No development took place in sea water; in "Erdschreiber" four auxospores had formed one valve with spine on 6 June and by the 10th twenty-four cells of big diameter had developed.

On 10 June twenty auxospores were transferred from the original sea water culture into "Erdschreiber". After 2 days one broad cell was found, and a further six were formed by the 17th. The rest had shrunk in the meantime and were not capable of development. The auxospores which had remained in the Petri dish with sea water did not show any sign of development until the beginning of July and then shrunk completely.

All these observations seem to show that the progressive changes of the culture medium which induce auxospore formation are produced by gradual exhaustion of substances which are present in "Erdschreiber" in larger quantities than in sea water, rather than by an accumulation of some metabolic products. Sea water seems to have a similar effect as the "Erdschreiber" of an overcrowded culture, where cells are induced to form auxospores which are, however, not capable, or capable only to a very limited extent, of developing into vegetative cells unless transferred into fresh culture medium.

Apart from the cell size and the changes taking place in the culture medium no other factors seemed to have any effect on either the time of appearance or the number of auxospores. They were formed under conditions described above throughout the year, whereas, as we have seen, resting spores were not formed in summer.

So far only the cell diameter has been considered. It has been shown that with the growth of a population the average diameter gradually decreased and that a regulation may take place when the diameter reached a certain size by the formation of auxospores and their development into cells of much bigger diameter. In this respect *Ditylum* shows very similar conditions to the pennate diatoms. There is, however, one

point in which they differ. Geitler (1932) found in the Pennatae that the size of auxospores and of the cells produced by them is practically constant for every species. Schreiber (1931) observed in a centric diatom, *Melosira nummuloides*, that the size of auxospores is unusually small when formed in a medium of high salt concentration. These small auxospores, however, did not develop but burst the membrane on the following day, when the protoplast was extruded and a new auxospore of normal size formed.

In *Ditylum* the size of the auxospores varies quite considerably, and the range of variation increases with the decrease of the cell diameter of a population. Fig. 27 shows that in line A the broad cells produced by auxospores in December varied from

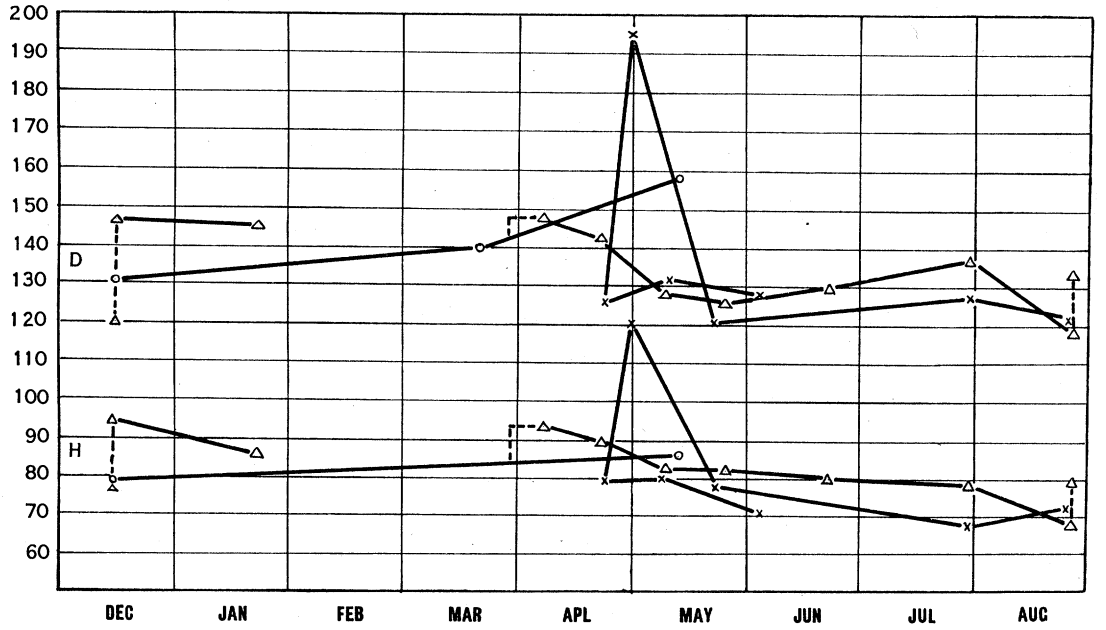

Fig. 28—The average length in μ of cells in successive subcultures of *Ditylum*. Marked as in fig. 27.

70 to 94μ in diameter. Similarly the cells developed from auxospores in the Axp.-line in August varied in diameter from 73 to 95μ with an average of 84μ. At the end of May, when the cells of line A reached the diameter of 13μ, the cells developed from auxospores varied from 33 to 90μ. Thus cells were produced by auxospores which in view of their diameter would be expected to form auxospores as soon as overcrowding of the culture occurs. This was actually so. On 5 June in subculture 12—the same from which a sample had been measured on 20 May—a number of cells with a diameter from 30 to 40μ were found to have formed or be forming auxospores; cells which had themselves developed from auxospores only 2–3 weeks ago. The broad cells produced by auxospores in line C have not been measured but the general behaviour was very similar.

In pennate diatoms the pervalvar axis—corresponding to the long axis of *Ditylum*—remains practically constant (Geitler 1932). In *Ditylum* there exists on the other hand

a considerable variation which shows no correlation with the decrease of the diameter and its increase after auxospore formation. Since the individual growth of the cells takes place in a longitudinal direction not all cells are suitable for measurement and comparison. Two stages appear obviously to be the best, viz. the "doubles", cells which have undergone division, the daughter cells not yet being separated (fig. 1 *b*, *d*, Plate 1), and the "half-cells", daughter cells shortly after their separation with the new valve still lying within the epitheca (fig. 1 *c*, Plate 1). The latter measurement is evidently the more reliable one, since the length of the "double cells" may vary according to the degree of separation of the daughter cells. The difference between the length of "double cells" and "half-cells"—the distance between the two curves on fig. 28—represents the range of individual cell growth. The average length in these two stages of line A, C, and Axp. are plotted on fig. 28. The dates of the measurements are the same as for the diameters on fig. 27.

In line A the "double cells" (= D) had an average length of 131 μ, the "half-cells" (= H) of 79 μ in December. In March the D cells were 139·6 μ; of the H cells only three were measured, they were 65, 75, and 80 μ respectively long. In May the D cells showed a marked increase to 157·8 μ, the H cells to 85·4 μ.*

That this increase in length is not necessarily connected with the decrease of the diameter becomes clear from the measurements of cells of other lines. In clone C (fig. 28) the average length of D cells was on 23 April 125·8 μ, of H cells 79 μ. On the 24th two subcultures were made: subculture 5 with forty cells in a Petri dish, subculture 4A with fifty cells in a watch-glass. On 1 May the number of cells in 4A was greatly increased, but the cells were unusually long. 100 cells were measured; the average length of D cells was 194·8 μ, that of the H cells 120·5 μ. A sample of 100 cells of subculture 5 was measured on 8 May. It showed only a slight increase in length as compared with that in April: the D cells were 131·2 μ long, the H cells 80 μ. On 2 May twenty-five of those extremely long cells of subculture 4A were washed and placed in a Petri dish. On the 22nd a sample of 100 cells from this culture gave an average cell length of 120 μ for the D cells, of 77·1 μ for the H cells. The length of the cells had become "normal" again.

Unfortunately it was impossible to discover the cause of the enormous growth in length of the *Ditylum* in subculture 4A. It might have been an infection of the watch-glass culture with some bacteria which had no effect on the division rate but had an effect on cell growth.

In later subcultures of line C the length of the cells did not differ much (fig. 28). The values obtained in August compared with those of April show on the whole a slight decrease in length.

* In all lines the D cells appeared to be considerably smaller than the double length of H cells. This is due to the fact that only those D cells could stand the pipetting and transferring on glass slides (for measurement) which had the original hypotheca well overlapped by the epitheca. D cells with the daughter cells being far advanced in separation broke apart during that procedure and arrived as two H cells on the glass slide.

There is also no definite correlation between the length of narrow cells and that of broad cells derived from auxospores. Among the cells developed from auxospores of line A there were from the beginning some longer and some shorter than the cells by which the auxospores had been formed (fig. 28, December). The Axp.-line showed in April an average length of cells of 147μ (D cells) and 93·5μ (H cells) respectively, slightly higher values than those of line A at the same time (fig. 28); in August it was distinctly smaller than in April.

All these facts show that there is no correlation between changes in the length and the progressive decrease of the breadth of *Ditylum*. Whereas the decrease of the diameter is determined by the structure and division process peculiar to the diatoms there apparently exist environmental factors affecting the extent of the individual growth in length of cells. From the slopes of the curves of fig. 28 it may be justifiable to assume that under more constant culture conditions—i.e. controlled temperature and light, and more frequent subculturing—the variability of the length of the cells would be greatly reduced and the length would remain practically constant in successive subcultures.

In spite of the variation of the length of cells in the cultures there is no doubt that on the whole the cell volume decreased with the decrease in diameter.

No special study was made of the ratio of length to breadth of cells developed from different auxospores, but it was found to be extremely variable even within the same culture.

It was mentioned in the introduction that until quite recently the formation of auxospores in centric diatoms had been regarded as a growth phenomenon not preceded by any nuclear changes or sexual phenomena. Both reduction division and the sexual phase were rather vaguely connected with the formation of microscopes. Only Persidsky (1929, 1935) described the occurrence of reduction division and autogamic sexual fusion of two out of four nuclei previous to auxospore formation in two *Chaetoceros* species and in *Melosira varians*. Particularly for the latter species the description and drawings of some important stages of the reduction division and nuclear fusion are rather convincing. v. Cholnoky (1933) found young auxospores of *Melosira arenaria* containing one big and two small nuclei, which he interpreted as being the result of a reduction division and the formation of four nuclei, of which two fused while the remaining two degenerated.

Cytological observations on *Ditylum* give a similar support to Persidsky's views. From the outset it was found that in every culture in which formation of auxospores and their development had taken place the broad cells contained a much bigger nucleus than the original narrow ones (fig. 19*a*, *b*, Plate 3). This alone would be difficult to explain on the assumption of growth only. Nuclei which were about twice as big as those of the sister cells were also found in bent cells before the actual formation of auxospores. Cells whose protoplast had been partly extruded in the course of auxospore formation frequently contained two nuclei (fig. 19*c*, Plate 3), mostly without

nucleoli, or with one nucleus again much larger than any nucleus of vegetative cells. One cell, shortly before the formation of the auxospores proper, contained one big nucleus with rather indistinct boundaries (shortly after fusion?) and two small degenerating nuclei (fig. 19*d*, Plate 3). No nucleolus could be seen in the big nucleus. The most instructive stages so far found were several young auxospores containing one big nucleus lying near the periphery of the auxospore within a protoplasmic area and two small nuclei similarly situated on the opposite side of the cell (fig. 19*e*, Plate 3).

As yet no stages preceding the appearance of two or three nuclei as described above have been found. But even these incomplete results, similar to those of v. CHOLNOKY, taken in conjunction with the more complete results of PERSIDSKY, leave little doubt that the auxospore formation in *Ditylum* is, or may be, preceded by a reduction division, the first meiotic division resulting in the formation of two nuclei, the second in four, two of which fuse while the others, taking no part in this very reduced sexual process, degenerate.

No microspores were found in *Ditylum*, and although that would not be sufficient proof for their non-existence the conclusion may be safely drawn that they do not take part in the formation of auxospores. The cultures of *Ditylum* were examined at frequent intervals for about a year under the binocular microscope, and many samples, both living and preserved, were studied under high power. No structures or bodies, whether flagellate-like or not, were observed apart from vegetative cells, resting spores, auxospores and occasionally bacteria. Thus the possibility of a fertilization of cells by microgametes before the formation of auxospores, suggested by WENT and GEITLER (see p. 2), certainly does not apply to *Ditylum*.

CHAETOCEROS DIDYMUS EHR.

Ten chains of this species were isolated from a plankton sample of 18 September 1935. On 21 November the culture was continued as a clone. This diatom forms very long chains which remain suspended until the culture becomes very dense. Then the majority of chains are found lying at the bottom of the glass dish. It may be noted that the long chains are slightly bent, not straight as usually described (GRAN 1908). The reason is probably that in tow-nettings long chains are broken into several pieces which then appear straight. Long chains of broad cells are also always slightly twisted, showing the flat surface in front, the narrow side view in the middle and again the broad surface at the end of the chain.

Two varieties are distinguished (MEUNIER 1913; GRAN 1908; LEBOUR 1930; HUSTEDT 1930): v. *genuina* with rather strong bristles crossing one another near the base, and v. *anglica* with fine bristles crossing far outside the chain. Both types occur in clone cultures at various periods (figs. 20–22, Plate 4), all representing specimens from one clone). It seems that chains of narrow cells which are capable of auxospore formation mostly belong to the type of v. *genuina* (fig. 22, Plate 4), whereas in broad chains

the bristles may cross either near the base (fig. 21, Plate 4) or farther off (fig. 20*b*, Plate 4).

Resting spores have been described by MEUNIER (1913). In the cultures they were mostly found in spring, lying singly within normal, not specially formed mother cells (fig. 21, Plate 4). Apart from the fact that resting spores of *Chaetoceros didymus* were also found in dense cultures only, the environmental conditions for their formation must be somewhat different from those in *Ditylum*. They were never found in such great numbers as in *Ditylum*—this applies also to all other diatoms which I cultured—and were not regularly formed in successive subcultures throughout a long period.

The first auxospores were noticed on 29 October in a 25 days old culture of the line mentioned above. These auxospores did not develop in the original culture. The first chains of broad cells were found in a later subculture on 11 November. They appeared in all subsequent cultures, always at a considerable interval—8–28 days—after the start of the culture.

The auxospores are similar to those described in other species of *Chaetoceros* (cf. FRITSCH 1935). The mother cell remains straight and the auxospore is formed laterally, the protoplast being extruded through a split between the epitheca and hypotheca of the cell (fig. 22, Plate 4). The auxospores develop immediately after their formation or, in very dense cultures, persist until they perish. On 18 March a chain containing an auxospore was isolated, on the 19th the auxospore had given rise to a broad cell which had divided in the meantime (fig. 22*b*, Plate 4). Behind the cells the empty perizonium was attached to the mother cell and in a neighbouring cell auxospore formation had started. On the 20th there were three broad cells, but the young auxospore was not yet completed. A small colony of bacteria was seen attached to it which evidently caused the delay. The other cells of the same chain continued to divide. On the 19th three cells were in front of the auxospore, next day six. The cells behind had divided into four, three of which broke off when the chain was transferred on a cavity slide. The broad cells developed from the auxospore produced a chain at right angles to the long axis of the parental chain and broke off on the 22nd.

As can be seen from fig. 22 (Plate 4), the empty mother cells of the auxospores are distinctly longer than the vegetative cells. On 23 March the average length of twenty-five "double cells" was 36·8μ, that of twenty-five auxospore producing cells from the same culture 46·4μ. If cells which undergo auxospore formation do not grow longer than the vegetative cells we must assume that in the latter the epitheca overlaps the hypotheca along a distance of 5μ.

Measurements of the cell diameter are given in fig. 29. In March the average diameter of the original line (from September) was 7·5μ. It had decreased to 3·8μ by June, after which no further subculture was successful. The cells which did not form auxospores were no longer capable of division and died. Broad cells developed from auxospores in March had a diameter of 17·3μ. Those of a culture started with six broad chains on 20 January showed a diameter of 22·1μ in April (fig. 29, Axp.).

There followed a rapid decrease to 7·6μ in September, that is a decrease by about two-thirds of the original diameter during 5 months. As in *Ditylum* the rate of decrease is much greater in broad cells than in narrow ones.

The Axp.-line of broad cells did not produce auxospores until the middle of August. The first auxospores were produced by cells with a diameter of about 9μ. This diameter, with a certain range of variation, must therefore be regarded as the maximum size of cells capable of forming auxospores. A population of *Ch. didymus* with a diameter

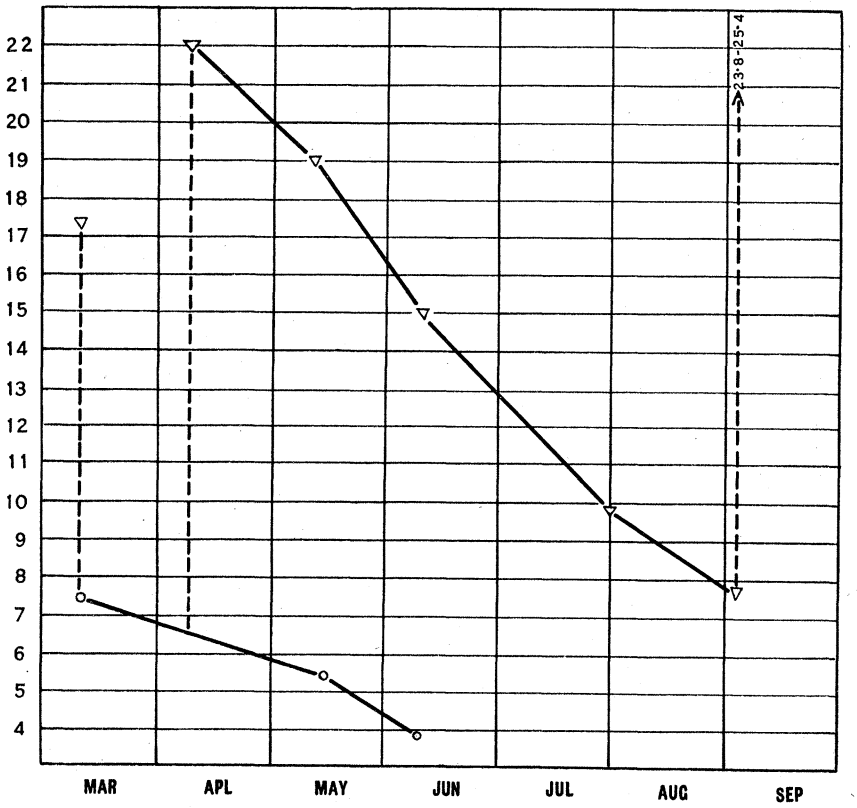

FIG. 29—The average diameter in μ of cells in successive subcultures of *Chaetoceros didymus*. The original narrow line marked with circles, the Axp.-line and cells developed from auxospores marked with triangles.

of 30μ—the size of the original cells of the Axp.-line which we may estimate from the slope of the curve—must therefore reproduce for a period of 7 months under very favourable conditions before the cells obtain an average diameter of 9μ. Then a period of 3 months follows when, as in *Ditylum*, environmental factors may induce some cells to form auxospores. That the main factor again changes in the medium produced by the rich growth of a culture may be concluded from the fact that whenever a new subculture was made a period of reproduction by division followed varying from 12–28 days in relatively broad cells to 1–2 days in narrow cells approaching a size at which division stopped and the cells died.

By transferring samples at frequent intervals (4–7 days) in fresh culture fluid it was possible to eliminate the formation of auxospores in two lines for a period of 6 and 8 weeks respectively. The smaller the diameter of the cells the more difficult it becomes to eliminate auxospore formation.

With regard to the size of auxospores and the diameter of cells produced by them a certain variability can be seen in the values given in fig. 29 for the broad cells in March and those formed in April. It must, however, be pointed out that as in *Ditylum* the variability greatly increases with the decrease in diameter of the cells producing the auxospores. In the subculture from June containing cells of the original line with an average diameter of only 3·8 μ the majority of cells produced by auxospores were of a diameter varying from about 7 to 12 μ, a size at which the cells become capable of forming auxospores again. Under low power it becomes difficult to distinguish these cells from those of the original line and to pick out the narrow chains for subcultures without contamination by cells developed from auxospores.

When in the Axp.-line the cells reached the diameter of about 9 μ the first auxospores formed in overcrowded cultures in August and September did not develop in the original medium. It was only after having been transferred into fresh culture medium (1 September) that the auxospores produced broad cells and chains after 5–7 days.

The length of the cells shows no regular increase or decrease in successive subcultures but fluctuates in both narrow and broad cells between 32 and 41 μ ("double cells").

Chaetoceros pseudocrinitus Ostenf.

The culture was started with one chain isolated from a plankton sample on 31 January 1936. In March the average diameter of the cells was 15·1 μ; it decreased to 7·2 μ in August. The first auxospores were formed in October. Occasionally other cytological phenomena were observed which shall be briefly described.

In very overcrowded cultures, or in dense cultures contaminated with bacteria, where reproduction had practically ceased and many chains showed signs of disintegration, cells were found containing mostly two to four (fig. 23, Plate 4), sometimes six ellipsoid-shaped bodies instead of the normal protoplast. When there were three such bodies (fig. 23*a*, *b*, Plate 4), one was much bigger than the rest, apparently lagging behind with division. Most of them contained a nucleus near the centre and distinct chromatophores at the surface; some were more or less empty and obviously dead (fig. 23*c*, Plate 4, lower cells).

The interest of these structures lies in their resemblance to what has been described by various authors as reproductive bodies or, later, microspores (see p. 38). It may therefore be pointed out that there is no evidence whatever that these microspore-like bodies found in the cultures of *Ch. pseudocrinitus* acted as reproductive cells. They were never liberated or at any rate never found outside the parental cells, and flagellate-like cells were never found in the cultures where their formation had taken place.

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Chains with cells containing such bodies were isolated into watch-glasses with fresh culture medium. There the neighbouring cells often recovered and started dividing again, but no trace of microspores in the shape of motile or non-motile gametes or asexual swarmers could be found.

Under similar conditions the formation of such bodies also occurred in *Ch. didymus* cultures but less frequently.

As they only occurred in very overcrowded cultures, or in such which were strongly contaminated with bacteria, their formation might perhaps be explained by the assumption that under certain unfavourable conditions cell division is not followed by the separation of the thecae and the formation of new valves. The nucleus retains the capability for further division which is followed by the division of the protoplasm and the formation of "daughter cells" inside the old membrane. It would thus be a degenerative process similar to the so-called plasmogamies in Thecamoebae. In these protozoa Belar (1921) found that in every dense agar-agar culture abnormal cells appeared after some time: viz. big cells with two or more nuclei the division of which has not been followed by cell division, or several cells which, derived from a single cell by division, were unable to separate and remained fused at the base of their pseudopods.

Skeletonema costatum (Greville)

This diatom divides at a rate of one to two divisions per day. It forms long, mostly straight chains, but in every culture a considerable number of curved and spiral-shaped chains can be found. The spaces between the cells are usually rather short (fig. 24*a*, *b*, Plate 4). This mode of growth is in agreement with Karsten's observations (1898) that the spaces are short in still water, and long in cultures which had been shaken.

The culture was started with six chains from a tow-netting on 23 September 1935 and continued as a clone on 19 November. The first broad chains developed from auxospores were found on 27 March (fig. 24*a*, *b*, Plate 4). It seems probable, however, that auxospores were formed some time before and were overlooked, since also in later subcultures they showed far less readiness for development than *Ditylum* and *Chaetoceros* and remained in great numbers at the bottom of the Petri dishes. Even when transferred into fresh culture medium only about 50% will develop after 5–8 days (compared with 1 and 2 days for *Chaetoceros didymus* and *Ditylum* respectively). Also the number of auxospores found in crowded cultures was small compared with *Ditylum*. They were formed by cells bent to $\pm$ right angles (fig. 24*c*, Plate 4) and were never found to develop in connexion with the parental chains as in *Chaetoceros*.

In April the original line showed a cell diameter of 3μ; broad cells that of 11·3μ (fig. 30). The diameter of the narrow cells decreased to 2μ in June, and it became difficult to distinguish them from chains of an average diameter of only 5·4μ developed from auxospores. A line (Axp.) started from a single broad chain in March possessed

a diameter of 11·4μ in April. The next measurement in May gave only the slightly smaller value of 11·3μ. Either the sample of sixty chains measured was too small or the division rate was lower than usual. In September the diameter had decreased to 8·5μ.

Resting spores (fig. 24*d*, Plate 4) were observed only once in May when a small number was formed after the exposure of a dense culture to dim light for 4 days and subsequently to low temperature (8·3° C.) for 3 days. Low temperature alone seemed to have no effect.

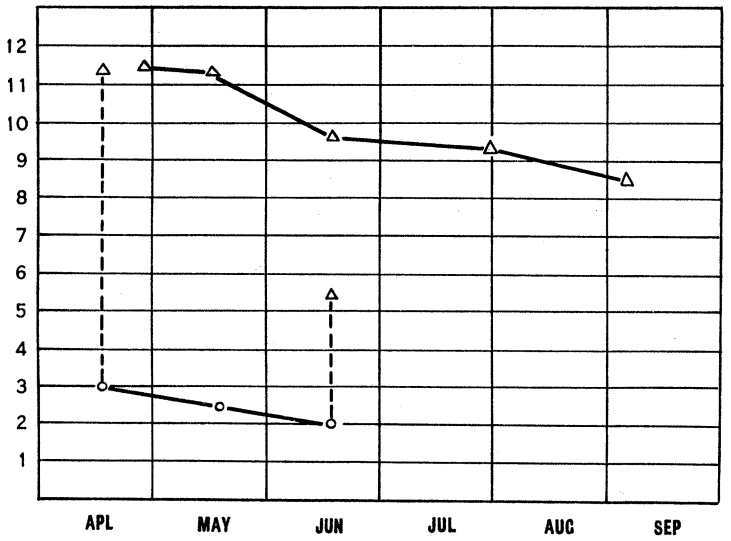

FIG. 30—The average diameter in μ of cells in successive subcultures of *Skeletonema costatum*. The original line marked with circles, the Axp.-line and cells produced by auxospores marked with triangles.

MELOSIRA BORRERI GREVILLE

This is a coastal bottom form with detached strands occurring occasionally in the plankton. In culture the end-cells of the filaments attach themselves to the bottom of the glass dish. Auxospores have been described by MIQUEL (1892) and KARSTEN (1899). Usually they appear as end-cells of narrow filaments, one theca of the mother cell and the rest of the chain becoming disconnected in the course of their formation. Occasionally, however, they remain connected with the neighbouring cells at least until the auxospore has formed a new epitheca (fig. 25, Plate 4).

The culture was started with one narrow chain on 1 April. On the 18th a few auxospores were found and two short chains of broad cells. The latter were isolated and cultured separately (Axp.-line). On 13 May the narrow cells had a diameter of 10·9μ, and the cells of the Axp.-line one of 18·6μ (fig. 31). Later, the narrow cells produced an ever-increasing number of auxospores, and in June broad chains had completely overgrown the original line. The Axp.-line produced no auxospores until 5 July. In August many auxospores and broad chains were formed with an average

diameter of 18·6μ, that of the original Axp.-line having decreased in 3 months by 8·5 to 10·1μ.

Schreiber (1931) has shown that *M. nummuloides* can stand salinities varying from 0·5 to 6 %. If cells of a diameter of 12μ, capable of auxospore formation, were transferred into a medium of higher concentration, no auxospores were formed. When placed into lower concentration they were formed readily, the relative not the absolute salt concentration being the decisive factor.

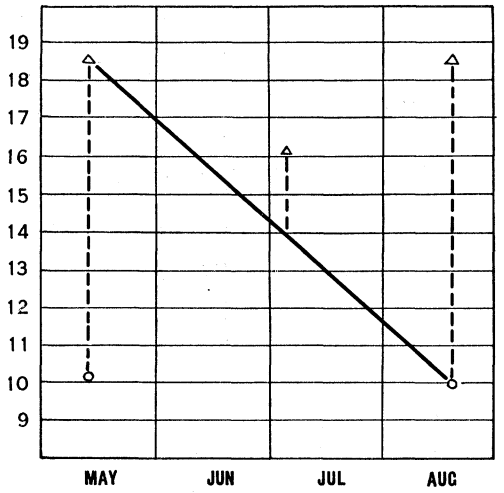

Fig. 31—Showing the differences in the size of the diameter of narrow cells (circles) and cells developed from auxospores (triangles) in *Melosira Borreri*.

Notes on other Species

In December 1935 the plankton contained a small number of very broad *Coscinodiscus* cells. One cell was picked out on 18 December; the cells of the resulting culture had an average diameter of 188·2μ with a maximum of 195μ on 22 January. It decreased to 139·7μ by the end of July 1936. No auxospores have been yet formed (November 1936). The species was difficult to identify. According to Miss Lebour, the type to which it showed the greatest resemblance was *C. obscurus*. This pelagic species, however, has so far only been found in Languedoc and in the North Atlantic (Hustedt 1930, p. 419).

In January two cells in a sample of the first culture showed a curious phenomenon. The girdle, or perhaps the connecting band of one theca only, had burst, the two ends being detached from the cell (fig. 26, Plate 4). In subculture 1 six cells out of a sample of forty-three showed the same phenomenon. In some only a short piece of the connecting band was attached to the cell, the rest having apparently been thrown off. In February they numbered twenty-six in a sample of 400 cells; in April only two were found among 100 cells, and in subsequent cultures they did not occur any more.

At first I was tempted to regard this process as a regulation of the cell diameter independent of auxospores.* The rigid girdle being broken the cell could expand and form a new and broader girdle and valve. But, as mentioned before, on the whole the diameter of the cells of successive cultures showed a steady decrease. Thus, should that phenomenon in fact represent a secondary growth, it might have slowed down the rate of decrease of the size in the population but did not prevent it altogether.

C. radiatus Ehr. has been cultured since 23 September 1935, when two cells were isolated from a plankton sample. In February the average diameter of the cells was 110μ, in August 69μ. No auxospores have been formed yet (November 1936).

Two lines of *C. Granii* Gough have been kept in pure culture, one from 31 October 1935, the other from 11 March 1936. The diameter of the cells showed a steady decrease, but in neither of the lines has auxospore formation been yet observed.

General Considerations

The observations on cultures described above showed that in all the species investigated, even in a thin-walled form like *Ditylum Brightwelli*, the average cell diameter of a population is bound to decrease progressively. The life cycle of centric diatoms appears to consist of successive periods of reproduction by cell division and of formation of auxospores. The diameter of cells developed from auxospores is twice to ten times larger than that of the mother cells. The process of auxospore formation only occurs when the cells have diminished to a certain size. When the cells have reached this size they become capable of producing auxospores but need not do so. They continue to divide, and for a considerable length of time environmental factors decide whether and to what extent auxospores shall be formed. The cell diameter continues to decrease until it reaches a minimum size and the cells either form auxospores or die.

In this respect the centric diatoms differ from the Pennatae (see p. 23). Another difference is found in the fact that in centric diatoms the auxospores formed by cells of different diameter show a great variability in size, whereas this is rather uniform in pennate diatoms.

Cytological studies in *Ditylum* have produced some evidence in support of Persidsky's views, that a reduction division and an autogamic sexual process precedes the formation of auxospores in centric diatoms. The observation of young auxospores of *Ditylum* containing one big and two small nuclei especially strengthens the probability of the occurrence of these processes in *Ditylum*. The possible interpretation of the stages of reduction division described by Persidsky as preceding the formation of macrogametes which need to be fertilized by microgametes or microspores (Geitler 1932), was eliminated by observations on cultures of *Ditylum*, *Chaetoceros*, *Skeletonema* and *Melosira*.

* The "dogma" of the auxospores being the only means for size regulation has been repeatedly attacked (Gemeinhardt 1927; see also Geitler 1927). So far, however, the observations concerning the occurrence of a secondary growth have not been convincing.

In all these species auxospore formation took place in great numbers, and although frequently examined no swarmers or microspores were ever found.

But, when reduction division and sexuality are connected with auxospore formation, what place in the life cycle of centric diatoms is taken by the microspores? The formation of cells resembling stages in the formation of microspores as described by various authors from plankton catches was only found in *Chaetoceros didymus* and *Ch. pseudocrinitus*. There is, however, reason to assume that these bodies, although containing a nucleus and chromatophores at first, do not take part in the reproductive cycle of the diatoms but represent stages of a peculiar degenerative process (see p. 33). The following observations may be recorded in connexion with the problem of microspores. On 20 December 1935 a raw culture of various diatoms, among them *Coscinodiscus Granii*, was made by inoculating a Petri dish with a small sample (about 1 c.c.) of plankton from a very fine-meshed tow-netting. On 3 January the culture was examined and several shells of *C. Granii* were found containing numerous motile flagellates, 10–12μ long, with a distinct brown chromatophore at the base of the cell. Three such *C. Granii* cells were washed and isolated in a watch-glass. On 5 January a number of flagellates were found swimming outside the shells. During the following days most of them had left the shells, and the number of flagellates in the watch-glass seemed to be much larger than that contained previously by the three shells. However, bacteria had developed in great numbers and on 14 January the free-swimming flagellates had disappeared. In the diatom shells a few were still present but had lost their motility.

No doubt the flagellates had nothing to do with the life cycle of *C. Granii*. Evidently the diatoms in the raw culture had reproduced very rapidly during the first week or so, and so did the flagellates which were introduced into the culture with the plankton sample. It is a common experience of all workers who try to obtain pure cultures of marine diatoms that nanno-planktonic flagellates present in a diatom culture inhibit the growth of the diatoms. It seems very probable that the *Coscinodiscus* cells mentioned above had died in the crowded raw culture, that the cell content had disintegrated, and that one or few flagellates present in the raw culture found their way into the shell where they stayed and divided until all organic substances were exhausted. Then they gradually left the shell and lived in the surrounding medium until the strong contamination with bacteria made an end of them.

These observations offer an example of how various flagellates might be mistaken for microspores or microgametes. The possibility that some of the microspores described were in fact parasites has been often discussed (Geitler 1931; Fritsch 1935). Where the formation of microspores was preceded by nuclear divisions of the diatom cells (Gran 1902, 1904; Karsten 1904, 1907; Bergon 1907; Schiller 1909; Hofker 1928) the simple assumption of parasitic infection is clearly unsatisfactory.

My own observations point to the possibility of interpreting the great diversity exhibited in the observations of various authors with regard to the formation, the

ultimate shape and the supposed fate of the microspores as being due to two phenomena: (*a*) an abnormal division process similar to that found in *Chaetoceros didymus* and *Ch. pseudocrinitus* (p. 33), resulting in the formation of a number of small naked cells within the parental shell; (*b*) the occasional presence and reproduction in dead diatom cells of flagellates not necessarily of parasitic nature. This would lead to the view that what have been described as microspores are either abnormal products of the diatom cell or alien flagellates, neither participating in the life cycle of the diatoms.

This rather radical view, tentatively put forward, may be based on the following considerations. From the observations on cultures one can see that microspores do not take part in auxospore formation. There is very little evidence that they represent gametes, and there is accumulating evidence for the assumption that an autogamic sexual process is connected with auxospore formation. If, however, they were asexual spores directly developing into normal vegetative cells it would be very unlikely that such extremely competent observers as Gran, Karsten, Bergon and others had missed the various stages of their development.

The number of microspores recorded in cells of different species varies from eight in *Melosira varians* (Schmidt 1923) to 128 in *Rhizosolenia styliformis* (Gran 1902). Another difference in their formation in different species is of interest. In diatoms where up to 128 microspores were recorded (*Rhizosolenia styliformis*, *Corethron Valdiviae*) the nucleus underwent first a multiple division and only in a late stage the nuclei appeared surrounded by a protoplasmic film. In other forms the microspores were formed as in *Chaetoceros pseudocrinitus* by successive divisions of the nucleus and the protoplasm.

Schiller (1909) found two types of microspores, spherical ones, 2–3·3 μ in diameter, and oval ones of 5 μ length, which he regarded as macro- and microgametes respectively. Both types had no flagella and were non-motile. Bergon (1907), who has studied the microspores of *Biddulphia mobiliensis* most carefully throughout five years, described the formation and liberation of microspores possessing two flagella. He was unable to establish when the flagella were formed; he assumes that their development takes place between the stage of 16 and 32 cells. When the microspores had formed flagella further reproduction took place within the diatom shell. After they got out of it they swam about for a few days, then became motionless and died. They never copulated and therefore Bergon did not regard them as gametes but as asexual swarmers. It may be pointed out that the microspores exhibited strong differences in different years with regard to their shape and movement. One year they were spherical and showed rotating movement, another year they had an irregular oval shape or were pointed at one pole and their movement was more oscillating.

In view of these differences one is tempted to suppose that Bergon put two types of *Biddulphia* cells into one developmental series: cells which for some reason had abnormally formed 16–32 cells—corresponding to those found in *Chaetoceros didymus* and *Ch. pseudocrinitus*—and cells containing flagellates—corresponding to the *Coscinodiscus Granii* cells in my raw culture mentioned above.

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With regard to the conditions for auxospore formation the centric diatoms dealt with in this paper show a similar behaviour to that of the Pennatae studied by Geitler (1932). In both groups only cells of a certain range of diameter are capable of auxospore formation.

In the Pennatae it seemed that once the right size has been obtained the sexual reproduction would take place under optimal culture conditions without any changes of environmental factors. Thus the diatoms appeared to be the only exception to the rule, first conceived clearly by Klebs, that in Protozoa and Algae sexual reproduction is induced by definite changes in environmental conditions. However, Geitler found that if *Navicula seminulum* is cultured on alkaline Knop-agar with addition of 1% NaCl normal growth, division and decrease in size will take place but the sexual reproduction —fusion of gametes and auxospore formation—is completely suppressed, in spite of the fact that they were of a size which would permit auxospore formation. When transferred on Knop-agar without the additional NaCl they readily formed gametes and auxospores. These and experiments with low light intensity with similar results show that apart from the "internal factor", a certain cell size, certain environmental conditions must be realized before sexual reproduction can take place, but these conditions do not seem to differ from the optimal conditions for growth and asexual reproduction. The better the conditions for growth and division the more auxospores were formed.

For the centric diatoms it could be shown that the optimal conditions for growth and division on the one side and for auxospore formation on the other are different. During a period of several months after the cells had obtained the right size auxospores were formed by *Ditylum*, *Chaetoceros didymus*, *Skeletonema* and *Melosira* only in old cultures, where the medium had undergone certain changes and the rate of division had decreased. The degree of these changes necessary for inducing the formation of auxospores became smaller with a further decrease of the cell diameter, but only when the diatoms had reached a size which sets a limit to further asexual reproduction by division were auxospores formed in the old medium, as well as immediately after having been placed in fresh culture medium, thus resembling the behaviour of the Pennatae. It was shown that cells of the same culture of *Ditylum* formed auxospores much sooner in pure sea water than in sea water enriched with nitrate, phosphate and soil extract ("Erdschreiber"). In the centric diatoms the causation of auxospore formation by external, environmental factors is thus much clearer than in the Pennatae.

Resting spores only occur in centric diatoms. Hensen (1887) and Schütt (1888) found that the appearance of resting spores in plankton diatoms is an indication that they will soon disappear from the plankton. The resting spores sink slowly towards the bottom; in greater depths they were found to be more numerous than near the surface. Gran (1902) pointed out their biological importance and opposed the views of Cleve who tried to explain the seasonal variation of planktonic diatom populations

exclusively on the basis of currents, without considering the possibility of their reappearance from the sea bottom.

A discussion of the literature on seasonal variations in the abundance of planktonic diatoms and their causes deduced by various authors from analyses of the hydrographic and chemical conditions would fall beyond the scope of this paper. The observations and experiments on cultures described above have a bearing on those plankton problems only so far as resting spores are concerned.

It was found that apart from the presence of great quantities of bacteria three factors are responsible for the formation of resting spores in *Ditylum*. (1) "Overcrowding" of the culture, i.e. changes in the medium caused by the growth and reproduction of many diatoms. Some evidence was produced for the assumption that the growth of a culture to about 1000 cells per c.c. of culture fluid is a preliminary condition for resting spore formation. (2) Low temperature. (3) Low light intensity. The "overcrowding" factor has no effect at high temperature and strong light intensity—no resting spores were formed in the cultures during summer. Low temperature and dim light have no effect in young cultures with less than about 1000 cells per c.c. of culture medium.

At this degree of overcrowding during autumn and winter, the cultures often produced thousands of resting spores overnight when the temperature dropped and the resting spores germinated during the following day when the temperature rose again. The changes of the culture medium were sufficient to induce resting spore formation at low temperature and low light intensity but not advanced enough to prevent germination immediately after the disappearance of these two factors. After a few days the higher degree of overcrowding resulted in the production of persistent resting spores which germinated only when transferred into fresh culture medium. For germination fresh culture medium, i.e. the absence of the "overcrowding" factor, is of almost exclusive importance. It takes place in fresh culture medium under otherwise the same conditions which induce the formation of resting spores in old cultures.

How can these observations be applied to the conditions in the sea? The growing diatom population during the spring and autumn outburst can certainly be compared with a growing culture, and there is enough evidence for the fact that the overcrowding of the surface layers of the sea results in the exhaustion or, at any rate, a considerable decrease in the amount of nitrates and phosphates. The same may be true for other substances which have not yet been studied quantitatively. It may well be that the exhaustion of some substances during the outbursts is partly responsible for the formation of resting spores just as in the cultures. It has been pointed out before (p. 19) that the degree of "overcrowding" in the sea, i.e. the density of the plankton population, need not be as high as in the cultures to be equally effective.

It appears, however, difficult to compare the two other factors in their effect upon the plankton and the culture populations respectively. "In the Channel... *Ditylum*

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Brightwelli is a perennial species, but has, however, a distinct flowering period in the winter half-year and a pronounced depression in the summer" (Ostenfeld 1913). It occurs in Plymouth waters mainly during autumn and winter. It may take part in the spring outburst of diatoms in March, and shortly afterwards disappear from the plankton (Harvey, Cooper, Lebour and Russell 1935). If the disappearance were due to resting spore formation this process would appear to take place under increasing light intensity and temperature as compared with the conditions in winter—contrary to the experience with cultures. However, the water temperature in March is well below 10° C. and thus sufficiently low for resting spore formation. With regard to the light intensity there is another possible interpretation of the conditions for the production of resting spores in nature which would bring them in accordance with the observations on cultures.

Some of the cultured species, *Chaetoceros didymus*, *Ch. pseudocrinitus*, *Coscinodiscus excentricus*, *Skeletonema*, *Streptotheca* and *Thalassiosira*, are freely suspended in the cultures. As soon as these become crowded they sink to the bottom. *Ditylum* is not suspended to the same extent as the other species just named. The cells are lying mostly at and near the bottom but in young cultures slight shaking, e.g. by placing a Petri dish culture under the microscope, is sufficient to upset their position and to lift many cells towards the surface, whereas in old cultures their position at the bottom of the glass dish gets less easily disturbed.*

If in the sea, as in cultures, the diatoms tend to sink when overcrowding occurs, and if with overcrowding in the upper water layer their capacity for suspension were reduced, the cells would gradually sink down and reach water levels with lower light intensity, and perhaps with lower temperature, than at the surface. Here the actual formation of resting spores would take place. The same factors which in a culture induce *Ditylum* cells to undergo resting spore formation would in nature act in a somewhat different way. The temperature being sufficiently low for this process to take place, the overcrowding factor is responsible for the sinking of the cells and their passing through water layers with light conditions which favour the formation of resting spores. As we have seen, their germination in cultures can take place in almost complete darkness and at comparatively low temperature, provided the cells are placed in fresh culture medium. The same conditions may prevail in the sea. The resting spores would remain suspended in a deep-water layer or lie at the bottom until the sea water becomes enriched with those substances which probably became exhausted by overcrowding.

The validity of certain conclusions drawn above may be tested on some interesting results of plankton investigations by Lohmann (1908). This author found for *Chaetoceros*

* We have very little knowledge of the mechanism which enables diatoms to remain suspended. Karsten (1907) draws attention to the possibility that there may exist in some diatoms, particularly in those without long bristles or spines which increase the resistance towards sinking, as in *Coscinodiscus*, a similar storage of CO_2 in the vacuole fluid for the regulation of suspension as was found in *Radiolaria* by Brandt. There is evidence for the same mechanism being responsible for the floating of *Noctiluca* (Gross 1934).

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that during the spring and autumn outburst, as soon as these diatoms reached their maximum number, resting spores occurred in the plankton.

On the whole the vegetative growth and reproduction during the spring outburst takes place almost exclusively in the surface layer of 0–5 m. Towards the end of this period, when the number of vegetative cells decreased in the uppermost layers, many vegetative cells and resting spores occurred at 5 m. and finally many vegetative cells and enormous numbers of resting spores—up to 14,000,000/100 l.—at 15 m. depth.

The facts that in the sea *Chaetoceros* cells start sinking when overcrowding of the plankton sets in and that the main bulk of resting spores is formed at 5–15 m. depth, i.e. in a layer of lower light intensity and possibly lower temperature, seem to support the conclusions drawn from the experiments on cultures.

A perfect parallel to Lohmann's observations was recorded by Gran (1915). This author found in the North Sea on a certain day in May that the diatoms were distributed vertically throughout the whole of the water mass. The maximum production had passed and a great number of resting spores of *Chaetoceros debile* and *diadema* were found throughout the water column, but in particularly great quantities in the deep water. There were of the first-named species at 0 m. 1580 vegetative cells and 12,400 resting spores per litre, at 68 m. depths 6500 and 195,100 respectively.

The conditions for resting spore formation are probably different, at least in degree, in different species of diatoms, as are the optimal conditions for reproduction in the sea. The occurrence of a number of resting spores at 0 m., as found by Lohmann in *Chaetoceros*, if not due to currents which had brought them up from deeper water layers, might not perhaps be found in *Ditylum* which, as Lohmann states, was only found in September, October and November, at first and for the longest time in a depth of 15 m.

The connexions drawn above between observations on cultures and those on plankton are only of a preliminary nature. But it seems certain that it would be profitable in future plankton studies concerned with the abundance of diatoms in different seasons to pay more attention to resting spores and auxospores. Their occurrence and distribution might turn out to be an indicator of some definite changes in the chemical and hydrographical conditions of the corresponding waters. In work on cultures concerned with the chemical and physical conditions for the growth of diatoms it would appear equally profitable not to concentrate on the division rate only but to analyse the conditions for the formation and germination of resting spores respectively, and the formation and development of auxospores.

Summary

A number of centric plankton diatoms have been kept in pure cultures in Föyn's "Erdschreiber" as culture medium. The cultures were started with one or few cells isolated from plankton samples and washed repeatedly in sterile medium.

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Most observations and experiments were made on *Ditylum Brightwelli*. In this species the cell division is remarkable because of a wide space formed between the daughter cells before their separation. The formation of new valves is not entirely dependent on cell division (see secondary valves, p. 7).

The mitotic division has been found to be very similar to that of other Algae. Neither centrosomes nor an extranuclear origin of the spindle could be observed.

Resting spores occurred regularly and in great numbers in *Ditylum* cultures during autumn, winter and early spring, not, however, in summer. They are spherical bodies formed inside the parental shell by contraction of the protoplast. Their membrane becomes silicified.

The interaction of three factors was found to be responsible for the formation of resting spores. (1) Certain changes of the culture medium, probably due to the exhaustion of some substances, gradually taking place with the growth of the culture. Except in a few cases of heavy contamination with bacteria no resting spores were formed unless the culture reached a density of about 1000 cells per c.c. of culture medium. (2) Low temperature. Most resting spores were formed over night when the room temperature dropped to 10° C. and below. When rich cultures which have not yet produced resting spores at room temperature are placed in an ice-box, almost 100 % of cells will form resting spores within a few hours. (3) Low light intensity. In summer low temperature has very little effect on overcrowded cultures with regard to the formation of resting spores, unless the cultures are placed in dim light for some time before the exposure to cold. Low light intensity alone does not induce resting spore formation.

Before the density of the cultures reaches such a degree that persistent resting spores are formed, their formation in autumn and winter takes place for some time overnight, followed by germination in the course of the next day. Persistent resting spores germinate only if transferred into fresh culture medium, and then under otherwise the same conditions which induce their formation in old cultures. Their germination may take 3–12 and more days.

At the beginning of the germination the resting spores expand slightly and fine protoplasmic filaments are sent off towards the original cell membrane. These connexions become stronger, and by gradual elongation and expansion of the protoplast the cell gains normal appearance. The formation of resting spores is almost exactly the reverse process.

A continuous decrease of the average cell diameter could be observed in cultures of *Ditylum Brightwelli*, *Chaetoceros didymus*, *Skeletonema costatum*, *Melosira Borreri*, *Chaetoceros pseudocrinitus*, *Coscinodiscus Granii*, *C. radiatus* and *C. obscurus*. In the first four species the formation of auxospores was studied, a process by which a broad cell diameter is restored. When the diameter of the diatoms reaches a certain minimum size no further growth or division is possible and they perish unless they form auxospores which develop into big cells. The maximum and minimum diameters respectively were

found to be 100 and 10μ in *Ditylum*, 26 and 4μ in *Chaetoceros didymus*, 12 and 2μ in *Skeletonema*, 19 and 10μ in *Melosira*. The average length of the cells may vary considerably in successive subcultures, but no progressive decrease or increase takes place corresponding to the steady decrease of the diameter.

Auxospores are the only stages in the life history of diatoms which are capable of growth along all axes. It is by means of these special cells that the cell diameter of a diatom population is restored. They are spherical bodies formed by the outflow of the protoplast of cells grown to a length greater than that at which otherwise cell division would occur. Auxospore formation only takes place in diatoms of a certain diameter, not larger than 45μ in *Ditylum*, 8μ in *Chaetoceros didymus*. Once this preliminary condition is realized, auxospore formation may be induced by environmental factors; the main factor being again progressive changes of the culture medium due to the growth of the culture population. Comparatively broad cells only form auxospores in old, very dense cultures, and only very few if any of the auxospores will develop into broad cells unless transferred into fresh culture medium. The smaller the cell diameter the more ready become the cells to form auxospores. The degree of overcrowding necessary to induce auxospore formation becomes much smaller in narrow cells and the auxospores develop into broad cells which reproduce side by side with the cells of the original size.

Cells from the same culture will form auxospores much sooner in pure sea water than in "Erdschreiber". They will, however, not develop into broad cells unless transferred from sea water into "Erdschreiber".

Broad cells develop from newly formed auxospores in 2 days, from older ones in 3–7 days.

Cytological observations on *Ditylum Brightwelli*, particularly the fact that young auxospores were found containing one big and two small nuclei, give support to Persidsky's view that reduction division and an autogamic sexual process precede auxospore formation. No microspores take part in this process.

Intracellular bodies resembling microspores as described by several authors were only found in *Chaetoceros didymus* and *Ch. pseudocrinitus*. They are interpreted not as reproductive cells but as products of an abnormal cell division.

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Description of Plates

Plate 1

Fig. 1—*Ditylum Brightwelli*. *a*, growing cell; *b*, cell after division; *c*, one daughter cell after separation. *a*, *b*, *c* from the same culture. *d*, a broad cell developed from an auxospore after division. Acetic-carmine. × 520.*

Fig. 2—Some division stages of a *Ditylum* cell. The corresponding time given on each figure. × 250.

Fig. 3—Some division stages of a narrow *Ditylum* cell. × 250.

Fig. 4—Some stages of the nuclear division of broad cells (Axp.-line). *a*, resting nucleus; *b*, late prophase, optical section; *c*, *d*, metaphase, *d*, side-view, optical section; *e*, telophase; *f*, interphase. Acetic-carmine. × 1400.

Fig. 5—Some stages of the nuclear division of narrow cells (line A and C). *a*, resting nucleus; *b*, early, *c*, late prophase; *d*, metakinesis; *e*, late anaphase; *f*, telophase; *g*, daughter nuclei. Acetic-carmine. × 1400.

* This and all the subsequent figures were drawn with the aid of a camera lucida.

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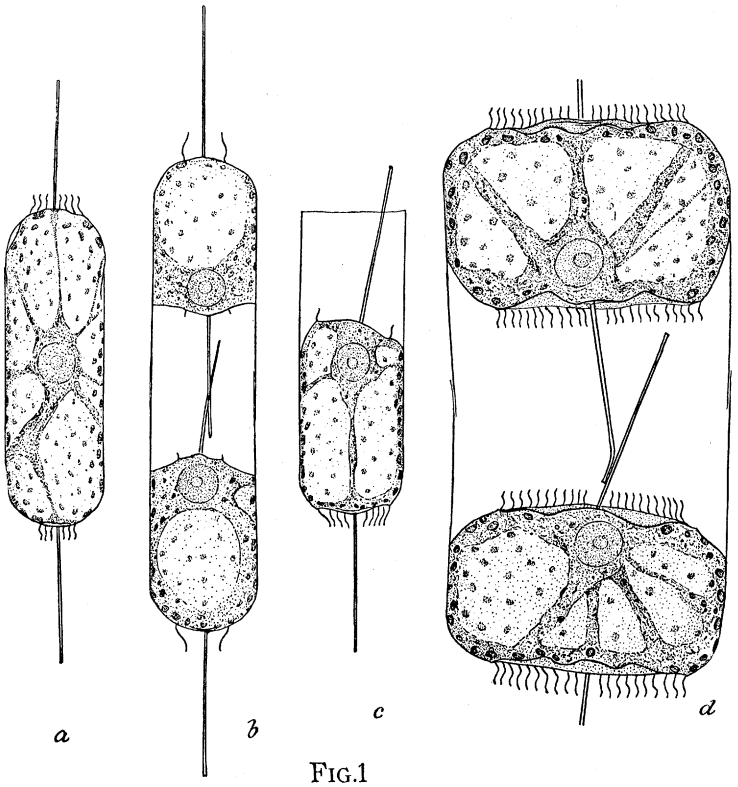


FIG. 1

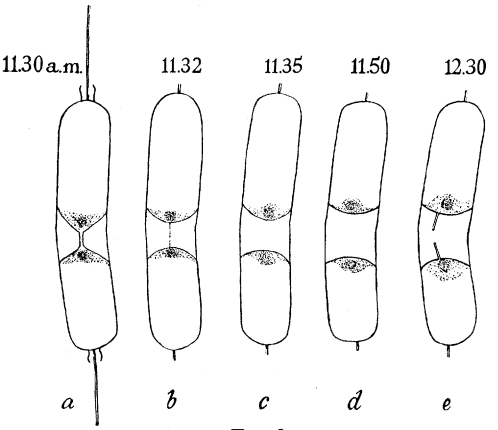
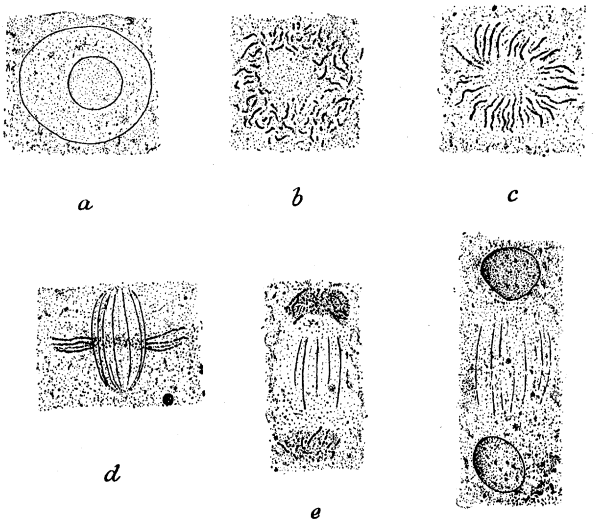


FIG. 3



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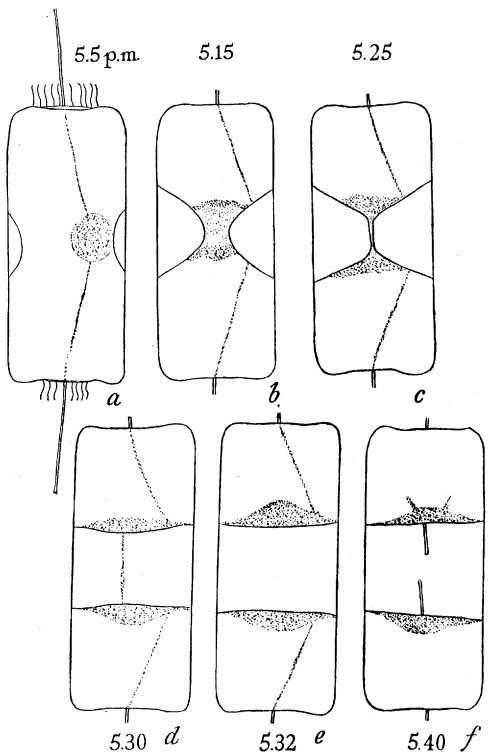

FIG. 2

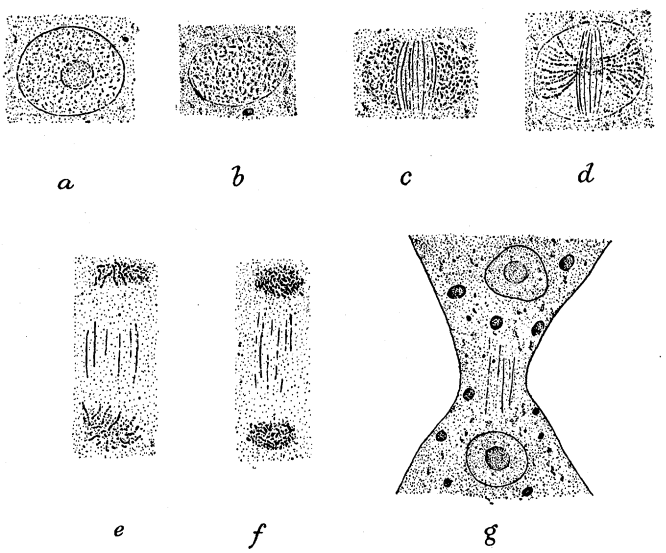

FIG. 5

Plate 2

Fig. 6—Secondary valves. *a*, from the same culture as fig. 1 *a–c*; *b*, resting spore with five secondary valves. *a* × 560; *b* × 180.

Fig. 7—*a*, cell after division; *b*, one daughter cell of *a* with a secondary valve; *c*, the same after having grown and pushed off the primary valve. Below the figures the dates at which they were drawn. × 350.

Fig. 8—*a*, resting spore; *b–d*, stages of germination of *a*; *e*, the same cell after division. × 350.

Fig. 9—*a*, resting spores formed in a cell short time after division; *b*, first stage of germination. × 350.

Fig. 10—Some stages of germination of a resting spore formed by a broad cell. × 250.

Fig. 11—Germination stages of a sister cell of that on fig. 10. × 250.

Fig. 12—Four stages of the formation of resting spores (cells from the same culture). × 250.

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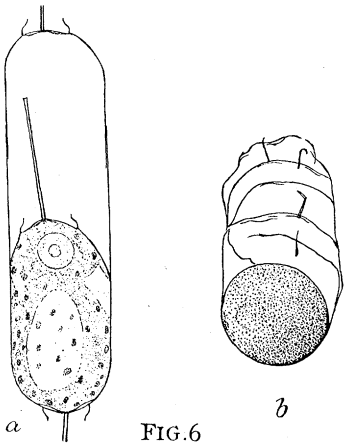
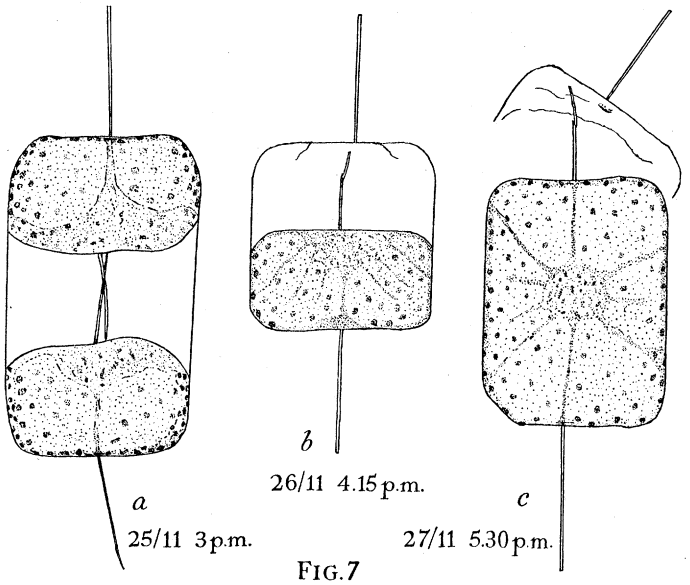


FIG. 6



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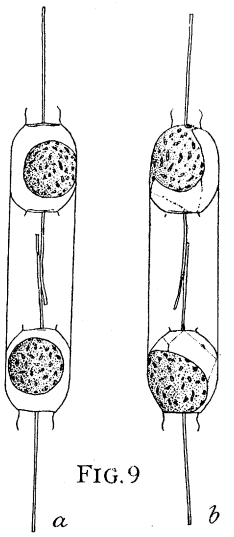
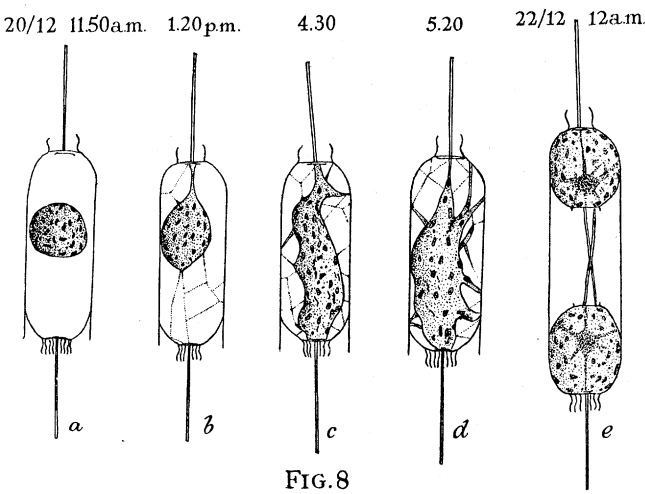


FIG. 9



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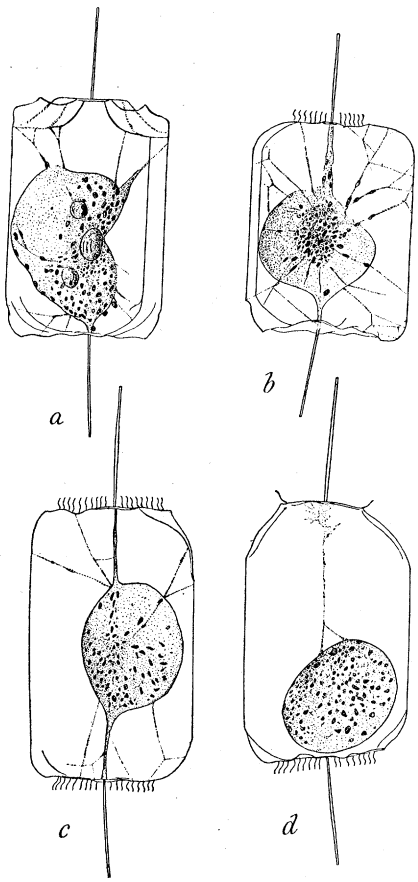

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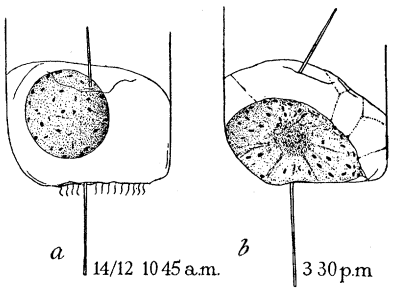

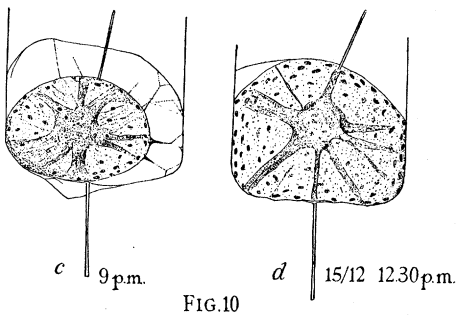

FIG. 10

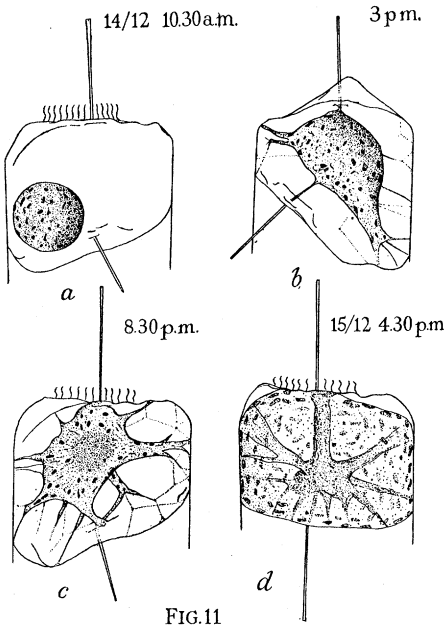

FIG. 11

Plate 3

Fig. 13—*a*, *b*, two cells isolated from the plankton, probably germinating resting spores; a^1, b^1, the same cells next day. × 250.

Fig. 14—*a*, an irregular resting spore formed after exposure to low temperature (4 December 1935); *b*, the same after germination (9th). × 250.

Fig. 15—*a*, irregular resting spore formed under the influence of bacteria; *b*, the same immediately after transference into fresh culture medium; *c*, the same four days later, germination almost completed. × 250.

Fig. 16—*a*, cell undergoing auxospore formation; *b*, completed auxospore. × 350.

Fig. 17—Auxospore formation. *a* and *b* as in fig. 16. × 170.

Fig. 18—Stages of development of one auxospore into a broad cell. × 250.

Fig. 19—*a*, outline of the nucleus of a narrow cell, *b*, of a broad cell from the same culture, developed from an auxospore; *c*, *d*, stages of auxospore formation, *c*, containing two nuclei, *d*, one big and two small degenerating nuclei; *e*, young auxospore with one big and two small nuclei. Acetic-carmine. *a*, *b* × 980; *c*–*e* × 650.

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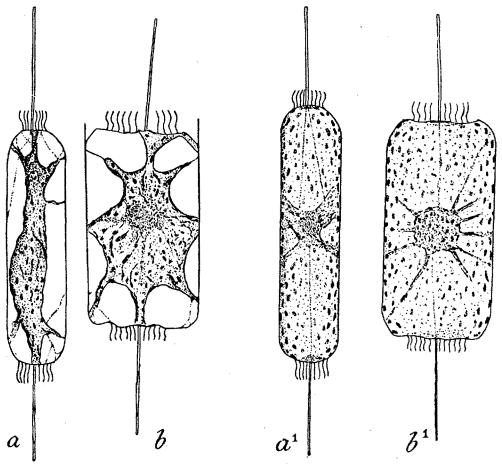

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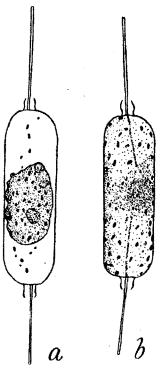

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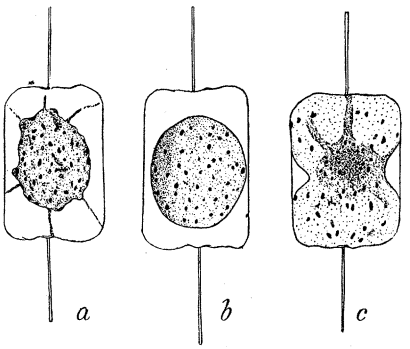

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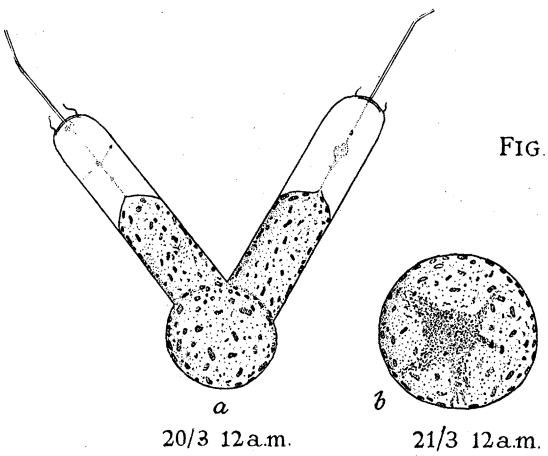

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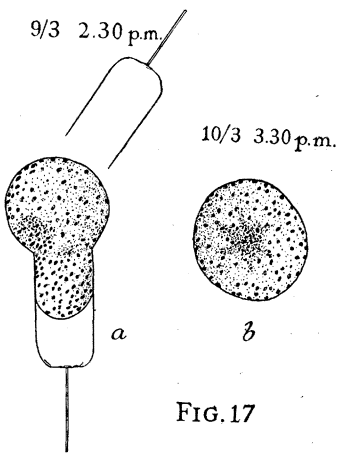

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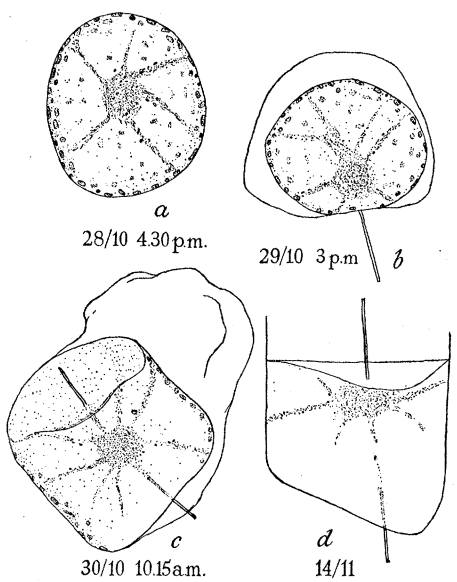

FIG. 18

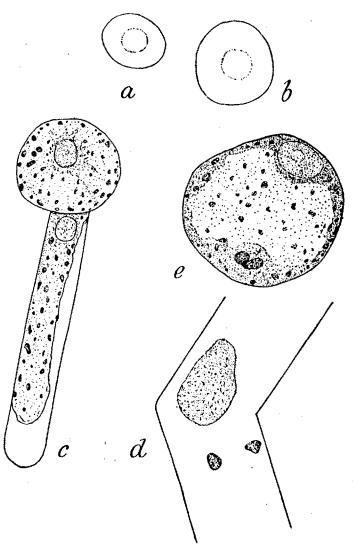

FIG. 19

Plate 4

Fig. 20—*Chaetoceros didymus*. *a*, part of a chain of narrow cells, *b*, of broad cells developed from an auxospore in the same culture. × 250.

Fig. 21—Resting spores of *Chaetoceros didymus*. × 250.

Fig. 22—*a*, chain containing a cell which had formed an auxospore; *b*, chain with two broad cells developed from an auxospore, one cell (below) undergoing auxospore formation; *c*, the same next day. × 250.

Fig. 23—Microspore-like bodies in cells of *Chaetoceros pseudocrinitus*. *a–c*, from living cells, × 920. *d*, acetic-carmine, × 1400.

Fig. 24—*Skeletonema costatum*. *a*, part of a chain of narrow cells, *b*, of broad cells developed from an auxospore in the same culture; *c*, auxospore; *d*, resting spore. × 560.

Fig. 25—*Melosira Borreri*. Auxospore with newly formed epitheca. × 930.

Fig. 26—Outline of a *Coscinodiscus obscurus* cell with burst connecting band. × 250.

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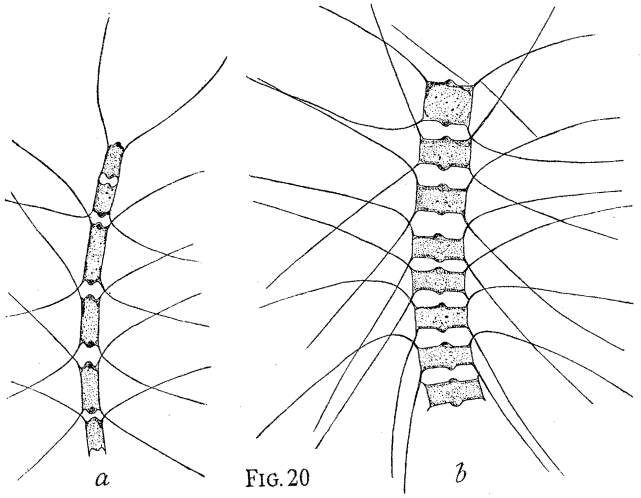
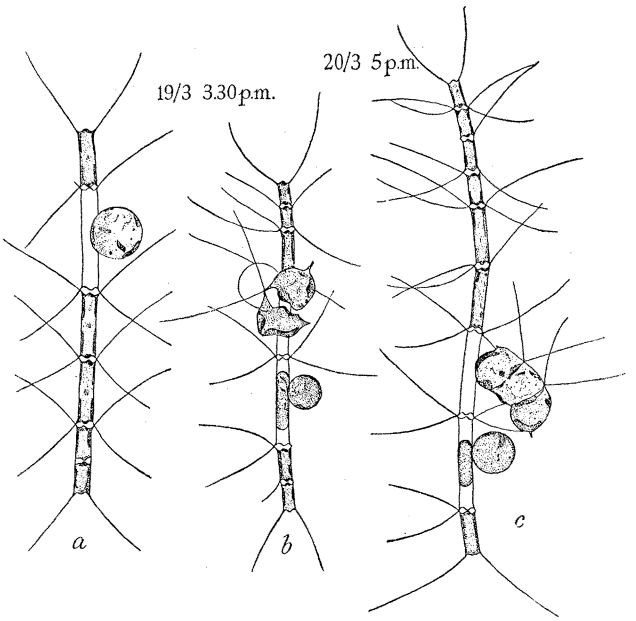


FIG. 20



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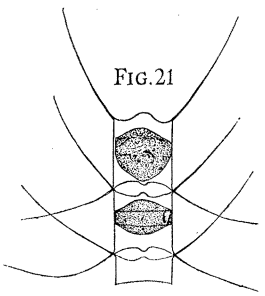

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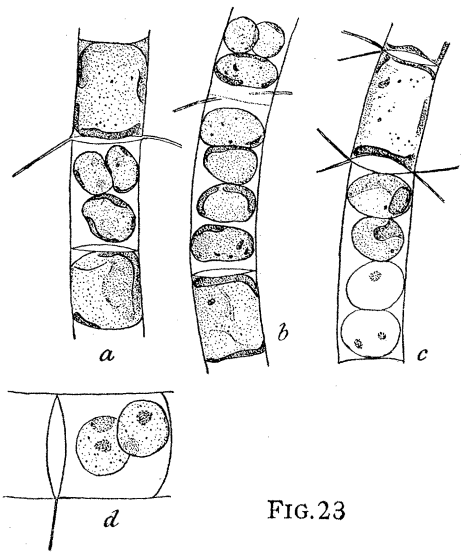

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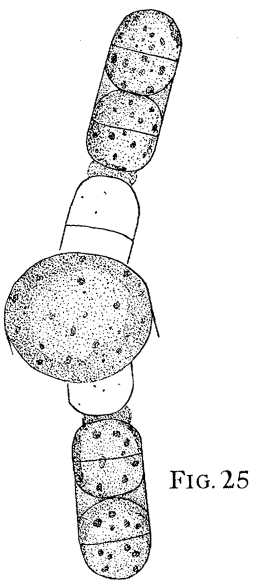

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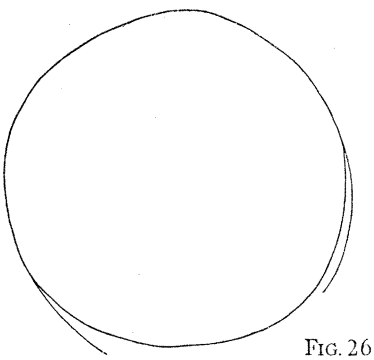

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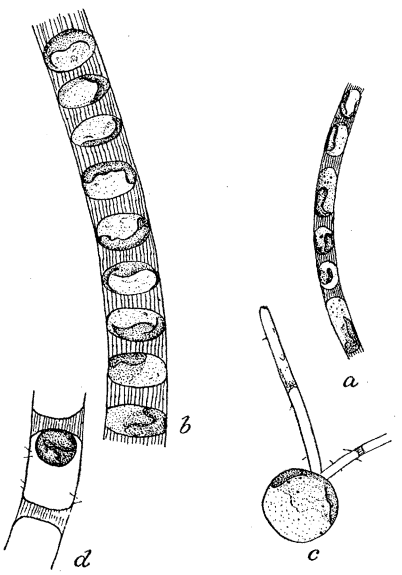

FIG. 24