Studies on a marine diatom Cosinodiscus pavillardii, Forti

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STUDIES ON A MARINE DIATOM *COSCINODISCUS PAVILLARDII*, FORTI

by

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2. ABSTRACT

A large centric diatom, tentatively identified as Coscinodiscus pavillardii Forti, was isolated from a collection of samples taken in the central area of the Sierra Leone River estuary on 23rd December, 1964. Its morphological characters are examined, and taxonomic problems involved in its identification are discussed.

Laboratory studies were made with the diatom using unialgal but not axenic cultures. The effects of light intensity, temperature, salinity, different cell sizes and vitamins on the growth rate of the diatom were examined. The saturating light intensity was 6,000 lux and there was little or no inhibition at 15,000 lux. The growth (division) rate was virtually unchanged over the salinity range of 13 - 33°/oo (one-third to about full strength sea water). The diatom survived at salinities below 13°/oo but not at 6.7°/oo and below. The smaller cells (73 - 164 µ) divided faster than the larger ones (327 - 345 µ), but their division rates were independent of the relative growth constant. Of the three vitamins - biotin, cobalamin and thiamine - only cobalamin was required for vegetative growth. The growth rate in the cobalamin medium was lower than in the complete medium (containing all the vitamins). In the absence of biotin in the complete medium the growth rate was better than in the complete medium.

Whilst in culture, spore (i.e. male gamete and auxospore) formation occurred in cells of about 20 - 60% of the mean maximum cell diameter ($464 \pm 44\ \mu$). Spore formation lowered the vegetative growth rate and occurred during the exponential phase of growth. The extent to which the growth rate was reduced was dependent on the proportion of cells involved in the activity.

The effects of light, temperature, salinity and vitamins on spore formation were also examined. The favourable conditions were - (i) light intensity of 5,500 - 8,000 lux, used either as continuous light or as an alternating twelve hour light/dark cycle; (ii) salinities of 19 - 33°/oo, (iii) temperatures of 23 - 28°C. A light intensity of 11,000 - 12,000 lux; temperatures above and below the stated range; and salinities of 16.5°/oo and below, inhibited their formation. The alternating light/dark cycle promoted better spore formation than continuous light.

Cobalamin was found to be required for spore formation although its action seemed to be linked with the promotion of vegetative growth. When biotin was omitted from the complete medium there was a noticeable increase in the number of spores produced.

Light is considered to play the most significant role in sex determination. Moreover, it appeared that spore formation

in the diatom is a result of a short-day quantitative photoperiodic response complicated by an additional ionic effect.

The methods of spore formation in the living material, as well as a probable case of isogamy are described and illustrated by photomicrographs.

2. INTRODUCTION

Previous studies on the marine phytoplankton of the Sierra Leone River estuary have been mainly systematic (Thiemann, 1934 - report on the German "Meteor" Expedition 1925 - 1927; Hendey, 1937) and ecological (Bainbridge, 1960). Watts (1957; 1958; 1960a, b; 1961) has examined various aspects of the hydrology and chemistry of the estuary. These studies give information on the basic physical conditions, species distribution, and fluctuations in the standing crop. No biological or physiological study of individual species found in the area is known to the author.

Little is also known about the growth rates of tropical diatoms. This fact prompted the desire to undertake growth studies on the principal diatoms in the estuary. A few of the diatoms isolated were successfully established as unialgal cultures in artificial synthetic media. All attempts at obtaining bacteria-free cultures of them proved unsuccessful. Among the few isolated was a large centric diatom tentatively identified as Coscinodiscus pavillardii, Forti. Cultures of this diatom were observed to produce auxospores (zygote) and motile male gametes*.

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*The phrase "motile male gamete" is used after Manton and von Stosch (1966; foot note p.124). The latter proposed the phrase as it was unambiguous and a strictly factual statement. The terms "spermium", "spermatozoid" and "microspore" are familiar botanical ones but are considered to be confusing to English and German readers and do not "do justice to the developmental uniqueness of the diatom gametes among plants."

The occurrence of such spores is always of interest to diatomologists, and records of the methods of formation have been many. Few studies have been concerned primarily with the factors which influence their formation. Therefore it was decided to investigate the method as well as how physical factors such as light intensity, photoperiod, temperature and substrate conditions influence their formation (i.e. auxospore and motile male gamete formation) in C. pavillardii. The effect of spore formation on the growth rate of the diatom has also been investigated.

All these investigations have been made with a unialgal culture of the diatom, which was not strictly bacteria-free, but which remained clear for up to two weeks without any noticeable or marked turbidity.

Problems concerning the factors promoting spore formation in centric diatoms can be stated as follows:-

(a) What causes the vegetative cells to become sexual?

(b) What causes the initiation of gametogenesis in the sexual cells?

(c) What conditions influence the liberation of the motile male gametes?

(d) What causes the products of meiosis in the oogonium to enlarge?

(e) When and under what conditions does fertilization take place? Before or after the enlargement of the oogonium?

(f) What causes the auxospore (zygote) to germinate or develop?

The study has been made with these problems in mind.

1. Spore formation and Factors Inducing Sexuality

The life history of a majority of species of diatoms is characterized by a vegetative cell division which leads to a constant reduction of cell size. This is a consequence of the nature and structure of the frustule and the internal deposition of new walls. The reduction in size may in time be arrested by the formation of auxospores, which give rise to cells of the maximum size with new valves and bearing all the characteristics of the species.

Spore formation in diatoms and the conditions associated with their formation received the attention of diatomologists as early as the latter part of the nineteenth century. Auxospore formation is common to both pennate and centric diatoms. However male gamete formation is peculiar to some centric diatoms and in the past these structures have often complicated an understanding of their life history. Auxospores are large cells which develop from newly formed zygotes and, as there appear to be no confirmed reports that they are formed asexually, they are now regarded as the ultimate products of fertilization. Motile male gametes, on the other hand, are regarded as being involved in the oogamous sexual reproduction of some centric diatoms.

Motile male gametes were first described by Rabenhorst (1853) and since then there have been many records of them. In the genus

Coscinodiscus in particular male gamete formation has been reported by Murray (1896), Selk (1913), Pavillard (1914), Karsten (1928), Hofker (1928), Schmidt (1931), Gross (1937) and Subrahmanyan (1946). Views on the nature of male gametes, and their functions in the life history of the Centrales, have been conflicting (Hendey, 1964). This situation arose out of the fact that in nature motile male gametes are very seldom seen with auxospores, and it was therefore difficult to link them together. However, von Stosch (1951a, 1954, 1956) using cytological techniques has elucidated their nature and significance in the life history of the Centrales. The method of their formation involves successive mitotic divisions, which may give rise to as many as 128 rounded bodies. These are followed, after a lull, by a single meiotic division of each product, resulting in the formation of flagellated swarmers, which are immediately liberated. Geitler (1952) characterized the swarmers as spermia and the non-flagellated rounded bodies as spermatogonia. Von Stosch (1956) questioned whether the swarmers are homologous with the motile male gametes. The main objection against the immediate acceptance of the motile male gametes as spermia was that in nature motile male gametes and auxospores are never found to occur simultaneously to such an extent as would be expected if they were involved in a sexual process. Fritsch (1935) also

objected on somewhat different premises because he felt that motile male gamete and auxospore formation never seem to synchronise but occur at different phases of the life history. However, von Stosch (1956) repeatedly observed fertilization in stained slides in Biddulphia granulata and B. Rhombus. Later Ramsfjell (1959) observed in Rhizosolenia hebetata f. semispina a number of auxospores and cells containing motile male gametes. These were in two phases of formation at different depths in stations on the Norwegian Sea. The auxospores were more frequent than cells with motile male gametes and nearly all were in the same stage of development.

Auxospores were first observed by Klebahn (1896) and their formation and nature were demonstrated by the researches of Geitler (1927, 1939, 1952), Iyengar and Subrahmanyan (1944), Subrahmanyan (1945a, 1945b), and von Stosch (1951, 1954, 1955, 1956) and others. Their formation has been reviewed by Geitler (1935), Patrick (1954), Grell (1956), Lewin and Guillard (1963) and Hendey (1964).

The vegetative cells of both pennate and centric diatoms are monoecious and diploid (von Stosch, 1954), with the exception of Rhabdonema adriaticum which is dioecious (von Stosch, 1958). The gametes are produced directly following meiosis, the occurrence of which is restricted to cells of a critical size range. The

size range depends on the species but is generally 10 - 15% of the maximum valve length or diameter (Geitler, 1932; von Stosch, 1951b; von Stosch and Debres, 1964). The sexual process shows a striking difference between the pennate and centric diatoms. The pennate diatoms are characteristically isogamous and the gametes are amoeboid, whereas the centric diatoms are oogamous and the motile male gametes are flagellated.

In the centric diatoms the oogonia are usually slightly extended cells with an elongated or inflated nucleus, (von Stosch, 1954). Normally one egg nucleus is produced, without cytokinesis, in each oogonium, since one nucleus degenerates at the end of each of two meiotic divisions, e.g. Melosira varians, Cyclotella tenuistriata and Coscinodiscus spp. However, in certain cases, two egg cells (e.g. Biddulphia mobiliensis) and one functioning egg and polar body (e.g. B. rhombus) are formed per oogonium. The egg cell normally remains enclosed in the oogonium, or attached to it, until fertilization, but in a few genera (e.g. Lithodesmium and Streptotheca), the unfertilized eggs are liberated (von Stosch, 1954).

The method of formation of a spermatogonium (male sex organ) in centric diatoms is very variable (von Stosch, 1954). A vegetative cell may give rise to a spermatogonium directly (e.g. Cyclotella tenuistriata). Alternatively, it may give rise, by mitosis, to a

small number of spermatogonia (e.g. 4 - 8 in Melosira varians), or to a larger number of spermatogonia (e.g. 16 in Lithodesmium undulatum; 32 - 128 in Coscinodiscus spp.) Usually reduction divisions in each spermatogonium result in four uniflagellate motile gametes per spermatogonium. In species with a smaller number of spermatogonia all of the intracellular material of the spermatogonium including the plastids, is distributed among the newly formed motile gametes, whereas in those with a larger number of spermatogonia, much of the cytoplasm and the chromotophores remain behind in a residual body, leaving the motile gametes free of plastids (von Stosch, 1954 ; 1958).

In some centric species (e.g. Cyclotella meneghiniana (Iyengar and Subramanyan, 1944) and Melosira nummuloides (Erben, 1959)) no separate gametic cells have yet been found and it has been suggested that auxospore formation in them is a result of intracellular autogamy.

In both pennate and centric diatoms the zygote develops directly after nuclear fusion into an auxospore which is generally about three times the length or diameter of the mother cell. Two mitoses then take place resulting in the formation of the vegetative shell which has the maximum dimensions of the species (Geitler, 1953).

Several factors, internal as well as external, are believed to influence the formation of auxospores. Chief of the internal

factors is the development of a specific physiological state in cells of a definite size range. Bruckmayer-Berkenbusch (1955) stated that the necessary internal condition which favours auxospore formation in cells of Melosira nummuloides within their critical size range is attained through a "shift" in the nucleus/cytoplasm ratio in favour of the nucleus. Thus the relative nuclear size constantly increases with decrease in cell size and this effect becomes more marked with progressive decrease of cell dimensions. Only when this "shift" is established can external factors effect a promoting or inhibiting influence. She concluded that initiation of gametogenesis and auxospore formation in the diatom is a consequence of abnormally strong formation of plasma protein, diminution of which correspondingly gives an inhibition of the developmental process.

Erben (1959), using the same diatom, tested the conclusions of Bruckmayer-Berkenbusch on auxospore formation by determining the influence of nitrogen deficiency or sufficiency in a synthetic medium, in which rapid vegetative growth of the diatom occurred. He argued that one should expect a great inhibition of spore formation in N-deficient cells undergoing vegetative growth because their protein content soon drops to a minimum as new syntheses of protein become impossible. He found that auxospore formation was possible as long as there was vegetative growth. He also found that their occurrence, and the conditions favouring it, were

independent of modifications in the nitrogen content of the medium which were required for normal vegetative growth.

The observations of Bruckmayer-Berkenbusch have been described as "astonishing" by von Stosch and Debres (1964). Recent studies by Holmes (1967) on the cells of the diatoms Coscinodiscus concinnus and C. asteromphalus, which were producing auxospores and motile male gametes, revealed no morphological differences between them and vegetative cells. Very few studies have been directed at the internal condition of cells in "the critical size range which promotes the development of sexuality". (In this thesis this will be referred to as the "induction size range"). The problem, therefore, remains unresolved.

The external factors favouring auxospore formation are believed to be light, temperature, and substrate conditions. Von Stosch (1954) found that sex determination in Lithodesmium sp. was influenced by light. A clone of cells in the induction size range produced almost exclusively female cells in continuous light, whereas in weak alternating light (day and night) only female cells were produced; and in strong alternating light, equal numbers of male and female cells. Unfortunately he did not give details of the photoperiod employed. Bruckmayer-Berkenbusch (1955) working with Melosira nummuloides found that low temperature, weak light intensity and the addition of amino -

acids, yeast, and soil extract promoted spore formation, whilst high temperature, strong light intensity, the addition of organic salts, and the dilution of the medium had the reverse effect. Erben (1959), working with the same diatom and using a 10 hour (day) photoperiod, reported that there was hardly any connection between spore formation and photoperiod, but gave no comparisons with other photoperiods. Holmes (1966) studied the effect of different photoperiods on male gamete and auxospore formation in Coscinodiscus concinnus. He found that the range of light intensity and temperature conditions under which these structures are formed is affected by photoperiod. Employing three photoperiods (16 hr.-, 12 hr.-, and 8 hr.- day) he found that the shortest photoperiod was conducive to the formation of male gametes and auxospores over a wide range of light and temperature conditions. Male gametes were developed and released over the temperature range of 9.0 - 26.7°C and the light flux range of 0.50 - 83.3 klux whereas auxospores occurred between 16.1° - 24.1°C and 0.33 - 83.3 klux. Male gametes appeared in maximum abundance a day or two before auxospores although concurrences were observed after the peak of abundance had passed. For the other two photoperiods the range over which they were formed was more restricted and auxospore formation took place in 76 hours or more, as compared with 38 hours for the 8 hour day. Moreover Holmes found that variation in the

amount of silicon, nitrogen and phosphate had no appreciable effect over the wide range of temperature and light intensity employed. This last finding confirms in part that of Erben (1959) who found that it was impossible either to inhibit auxospore formation in competent cells of Melosira nummuloides, or to initiate it in non-competent cells, by varying the concentration of nitrogen and phosphorus, or by modifying their metabolism with sublethal doses of organic inhibitors.

Allen and Nelson (1910), Braarud (1944) and Iyengar and Subrahmanyan (1944) observed that auxospores were regularly formed when marine diatoms were transferred to a lower salinity medium. Schreiber (1931), from his investigations of this phenomenon in Melosira nummuloides, stated that there is an artificial inhibition or promotion depending on whether the alga is transferred from dilute to concentrated sea water or vice versa. Bruckmayer-Berkenbusch (1955) refuted this statement on the grounds that Schreiber misinterpreted his results. From her experiments she showed that the highest employed concentrations and the greatest dilutions made with Erdschreiber solution reversed the initial spore formation to a partial or complete inhibition. Moreover, with dilutions of sea water she found that the resulting auxospores had rather small cell diameter. Quantitative data are lacking for these findings.

In 1937 Gross described what he regarded as auxospore formation in Ditylum brightwelli. Von Stosch (1965) contended that Gross did not find male gametes in his material. Gran and Angst (1931) had reported male gametesfor the same species of diatom from the North Pacific. From his own investigations von Stosch found that it was very difficult to bring about sexual reproduction in cultures of this diatom from the North Sea. The "resting spores" described by Gross appeared in his cultures and were considered by von Stosch to be the results of spontaneous or excited plasmolysis, obtained under unfavourable culture conditions, particularly unfavourable illumination. Prát (1934), Follman (1957), and Höfler (1963) have also confirmed those findings. Von Stosch obtained genuine "resting spores". Similar spores had already been observed by van Heurck (1880 - 1885), Gran and Angst (1931), and Cupp (1943), at the relatively low temperature of 12°C. What Gross described as "nuclearization" of the resting spores, von Stosch regards as nothing more than a recovery of the deplasmolysed condition. Von Stosch also described the attempts at shell building and the wide variation in size and shape as differences from the known methods of auxospore formation. He concluded that what Gross described was vegetative cell enlargement which he had observed taking place in nature in the European area. He defines vegetative cell enlargement as partial or complete extrusion of a protoplast

from its frustule, followed by regeneration of new valves using morphogenetic means to attain results (irregular shapes of valves, more variable and lesser size) different from those of auxospore formation. This finding is instructive for future researches on auxospore formation.

Few studies on the physiology of male gamete and auxospore formation have been made. In this regard Schreiber, Bruckmayer-Berkenbusch and Erben studied auxospore formation in an apparently autogamous diatom Melosira nummuloides whereas Holmes worked with an oogamous diatom Coscinodiscus concinnus producing male gametes and auxospores. Schreiber and Bruckmayer-Berkenbusch gave no details on the ratio of auxospores to vegetative cells produced under the various conditions of light intensity and temperature employed in their studies. Similarly Holmes did not give the proportion of male gamete forming cells, zygotes or vegetative cells produced in his experiments. Erben gave information relating cell size to auxospore formation and quantitative data on the relative abundance of auxospores. The treatment, however, in each case has been different. Consequently our knowledge of this aspect of diatom research is sadly wanting and demands more investigations using different diatom species from different geographical (temperate, tropical) areas.

3. The Physical and Chemical Conditions Affecting the Life of Diatoms in the Estuary

The standing crop in the Sierra Leone River Estuary usually includes a high proportion of large species belonging to the genera Coscinodiscus and Actinocyclus. Various factors affect the life of these diatoms. These factors include light, temperature, salinity, tidal and ocean currents.

Bainbridge (1960) found that during the dry season (November to April) the standing crop of phytoplankton was greater in the central area of the estuary than at the river mouth where turbidity was lowest. He suggested that this may be accounted for by the shallowness of the water in the estuary permitting the retention of cells near the surface. During the wet season, increased turbidity in the central area produced by tide and river discharge, resulted in an impoverishment of the standing crop, presumably in consequence of reduced light penetration. The daily illumination averaged over 24 hours during the dry season is of the order of 67 Klux on the surface of the water. The turbidity of the water reduces this amount of light so that the euphotic zone is very shallow. Bainbridge demonstrated that the lower limit of the euphotic layer is above the bottom of the estuary and the detritus content of the water reduces its transparency with depth. He inferred from this that the phytoplankton production is limited by suboptimal conditions of illumination.

During the period of occurrence of the large centric diatoms (Watts (1960) found that the average monthly temperature in the central area of the estuary rises from 28.1° to 29.3°C, and salinity from 28 to 32°/oo. The annual average monthly temperature and salinity lie between 26.4 to 29.3°C and 16.8 to 32.4°/oo respectively. The lowest monthly mean temperature (26.4°C) was recorded in August, and that of salinity (16.8°/oo) in September. The average temperature difference between the horizontal and vertical ranges is less than 1°C and the seasonal fluctuation in temperature is a little over 1°C. These small temperature differences alone are of little significance, but coupled with salinity changes they have a much increased limiting effect which is intensified in an upstream direction. Therefore during the wet season when salinity falls very sharply the combined effects of salinity and temperature appear to limit the distribution of the large centric diatoms. The author has observed that with the increased influx of sea water into the estuary after the rains, and the consequent rising values of salinity, coupled with a small rise in water temperature, the large centric diatoms reappear in the standing crop.

A study of the surface currents in the vicinity of the estuary indicates that onshore sets are most numerous during May to November, probably as a consequence of the greater influence of the Equatorial counter current (Bainbridge, 1960). From December to late July the flushing action of the river water

diminishes sufficiently to allow the development of a population of planktonic diatoms consisting predominantly of centric species, the pennate species being of only minor importance. Moreover this is the period of highest salinity and temperature when extensive vertical mixing is effected by the strong currents of spring tides. This results in a homogeneous column of water which becomes increasingly saline rather than brackish.

Watts (1958) found that the distribution of salinity in the estuary results from the circulatory current system. He observed a tendency towards a two layered transport system with an offshore movement of relatively brackish water compensated for by an onshore counter current of sea water close to the bottom of the estuary. As a result the water becomes stratified and the salinity increases with depth. Moreover the vertical salinity gradient is influenced by the changes in tidal range and the rate of river discharge. In the wet season the stratification is more marked at neaps than at springs whereas in the dry season it is generally not appreciable. The large dilution of the surface waters results in low salinity in the rainy season. The bottom waters nearest the mouth are relatively immune except during heavy floods when large changes in salinity become noticeable at neaps. This is not the case in the dry season when conditions are such that the vertical range of salinity is fairly uniform.

Bainbridge (1960) gave the results of a series of dissolved phosphate determinations carried out by P. Hansen from April to December, 1953. The dissolved phosphate content of the water was found to be slightly higher in the central region of the estuary (0.30 - 0.97 μ g-at. P/l) than in its mouth (0.25 - 0.72 μ g-at. P/l). These amounts were considerably higher than those Wattenberg (1957) obtained for the open Atlantic occan water just off the West African coast (0.0 - 0.36 μ g-at. P/l at 25m.; at the surface phosphate was not detectable). At the upper reaches of the estuary Watts (1958) found that the average concentration was 0.15 μ g-at. P/l. Bainbridge (1960) therefore concluded that the relatively high concentrations of dissolved phosphate in and off the mouth of the estuary may be due to the continual tidal mixing and frequent re-suspension of organic detritus derived from the river, the mangrove swamps and the plankton.

Single nitrate analysis carried out by Watts in November 1954 and reported in 1958 gave an average concentration of 5.29 μ g-at. N/l for both the central region of the estuary and its mouth. In the upper reaches he obtained an average of 10.13 μ g-at. N/l and suggested that river drainage must make a significant contribution to the nitrate concentration in the estuary.

Dissolved silicates are necessary for the growth of diatoms but few determinations have been made. Taylor (1966) found that dissolved silicates are higher in the upper reaches (11 μ g-at. Si/l)

than at the mouth (0.7 μ g-at. Si/l). In the central region the values range from 0.9 - 5.0 μ g-at. Si/l. He suggested that the major source of silicates is from the inflowing freshwater, and that its replinishment is dependent on the volume of fresh water entering the estuary. However, Bainbridge (1960) found vast numbers of broken and empty, heavily siliceous diatom frustules in the water samples collected in the estuary. He ascribed replinishment of silicates to the dissolution of these frustules.

Bainbridge also concluded that throughout the year grazing, which can be a factor of immense importance, has here no appreciable effect on the standing crop. He based his conclusion on the fact that there was no gradual increase in the concentration of zooplankton organisms when phytoplankton concentrations were consistently high. This conclusion needs further investigation as it seems at first sight somewhat surprising.

From the preceding review it is considered that the supply of phosphate, nitrate and silicate in the estuary is adequate for phytoplankton (diatom) growth throughout most of the year. This may largely account for the dominance of diatoms in the standing crop. However, the standing crop seems to be largely influenced by light, salinity, turbidity, tidal and surface currents. During the dry season their combined effect produces better conditions for development of the standing crop. In the rains the standing

crop is displaced seawards whilst as a result of the marked stratification in the estuary it may also settle into the bottom water. This may account for the principal diatoms in bloom during the dry season being absent during the rains. Therefore it can be inferred that it is during the rains that consolidation of the principal members of the phytoplankton takes place under sub-optimal conditions of light in the mouth of the estuary or in the bottom waters of the estuary. The resulting larger cells are re-suspended in the surface water during the dry season when vertical mixing eliminates stratification.

The genera Coscinodiscus, (to which the diatom under study belongs) and Actinocyclus are the principal members of the standing crop. It appears to the author that the foregoing explains the annual occurrence and apparent disappearance of the diatom in the standing crop of the river estuary.

4. MATERIALS AND METHODS

Collection of Material

Coscinodiscus pavillardii was isolated from water samples taken from the Sierra Leone River estuary within half a mile of the Corvette jetty, Kissy, on 23rd December, 1964. The collection of the samples was achieved using a fine silk plankton net. A specimen bottle of about 250 ml. capacity was tied on to the narrow end of the net. The wider end of the net was tied on to a wire loop fastened to a long stick. The whole assemblage was held by hand such that it was overhanging from midway along the side of a dinghy powered by a 6 H.P. outboard motor. The net was pulled through the water at a depth of about a foot below the surface at a cruising speed of 3 m.p.h. The towing time for each sample of plankton taken was about 15 minutes. Several such samples were taken. In the laboratory further concentration of the samples was made by decanting excess water. Several samples of the water were taken using salinity bottles for later estimation of the salinity over the area through which the net was pulled. A rough measurement of the temperature of the water was taken using a Negretti and Zambra thermometer.

Isolation and Culture

The diatom was isolated by the pipetting or washing method (Gross, 1937b; Pringsheim, 1946; Droop, 1954). This method essentially involves the withdrawal and transference of the diatom with a micropipette through many washings in sterile medium to get

rid of bacteria and other attached organisms. These manipulations were carried out aseptically under a dissecting microscope.

Although unialgal and axenic cultures were aimed at, the cultures obtained were unialgal but not axenic. Attempts to obtain bacteria free cultures of the diatom were made in the following:-

(a) 1% agar prepared with the synthetic medium.

(b) A mixture of penicillin and streptomycin (2:1) was added to flasks containing 25 ml. of the sterile synthetic medium to give resultant concentrations of 51, 102, 204, 306 and 408 units/ml. of both (after Spencer, 1952). The flasks were then illuminated for a week. Washed cells from each treatment were transferred to antibiotic free medium. At the same time similarly washed cells were tested for bacterial growth by inoculation into sterile antibiotic free peptone synthetic medium. The diatom, however, showed a reluctance to grow on subculture from the antibiotic treated culture to the ordinary synthetic medium.

The unialgal cultures used in this study were made from cells which had no antibiotic treatment, but which had been repeatedly washed and subcultured several times. These cultures showed no appreciable bacterial growth during the exponential phase of growth. The cultures were established in a synthetic medium of salinity approximate to that of the sample of sea water from which the diatom was isolated.

Identification

The supernatant liquid in a culture of the diatom was gently poured off so as not to disturb the diatom sediment. Concentrated hydrochloric acid was added to cover the diatom cells and the mixture heated until effervescence ceased. It was then cooled, diluted and centrifuged. The frustules were washed and boiled in concentrated sulphuric acid. The organic matter was oxidized with potassium nitrate and the washed frustules were mounted in Styrax.

Photographs of the acid-cleaned valves and the living material were taken on Kodak Panatomic X film using a Reichert microscope with 35 mm. camera attachment.

Culture media

The media used were U32, U36 and ASP2 (Provasoli et al, 1957). For the great bulk of the experiments the modified ASP2 medium (Parsons et al., 1961) was further modified and used (see below for composition of the media). This modification involved the use of the stated amounts for trace metals, minor elements and sodium silicate as for U32 and U36.

These media were chosen because they were nearly exact imitations of natural sea water and can provide the salinity range in the estuary. The only defect is that occasionally precipitates are formed during autoclaving (Provasoli et al., 1957). Light autoclaving was employed to remove this difficulty. The complete media were usually made up in one litre flasks and, after

adjusting the pH with hydrochloric acid, were autoclaved at 15 p.s.i. for ten minutes. It was observed that precipitates formed above a pH of 8.4. The inorganic salts were of B.D.H.AnalaR standard, with the exception of sodium metasilicate, which was of commercial grade supplied by Hopkin and William Limited. Vitamin B_{12} was obtained from Nutritional Bio-chemicals Corporation, United States of America. The other vitamins were of normal B.D.H. standard.

The complete medium, in each case, was made up of two major components - the basic inorganic salts, and the enrichments. The enrichments comprised the vitamin mixture, the trace elements mixture and the minor elements mixture. Separate stock solutions of the basic inorganic salts, the vitamin mixture, vitamin B_{12}, the trace elements mixture and the minor elements mixture were made. One litre of the complete medium usually was made from the stock solutions in the concentrations shown in the table. Ten different solutions of the complete medium corresponding to ten different salinities were used in this investigation. The ten different solutions were obtained by diluting the basic inorganic salts component ten times for the weakest and then nine times, eight times and so on, the strongest being the undiluted basic salts component. The concentration of the enrichment component was the same for all solutions of the complete medium. The salinities of the various solutions of the complete medium were determined by the

Table I. Compositions of synthetic culture media

(quantities are those used for making 1 litre)

The difference between the U32 and U36 media is that the U32 medium lacks sodium metasilicate.

A. Basic Inorganic Salts	U32/U36	ASP2 Modified
NaCl	24.5 g.	24.0 g.
$MgCl_2.6H_2O$	9.8 g.	10.9 g.
$CaCl_2$, anhydrous	0.4 g.	1.148 g.
Na_2SO_4, anhydrous	3.03 g.	4.0 g.
KCl	-	0.68 g.
$NaHCO_3$	-	0.195 g.
K_2SO_4	0.85 g.	-
H_3BO_3	-	0.026 g.

B. Enrichments - Common to all solutions of the complete medium		
KH_2PO_4	10.0 mg.	6.8045 mg.
KNO_3	100.0 mg.	50.55 mg.
Tris	500.0 mg.	500.0 mg.
$Na_2SiO_3.9H_2O$*	100.0 mg.	284.2 mg.
Vitamin B_{12}	0.0001 mg.	0.002 mg.
Minor elements(1)	10.0 ml.	10.0 ml.
Trace metals(2)	1.0 ml.	1.0 ml.
Vitamin Mix S3(3)	-	10.0 ml.
Thiamine	1.0 mg.	-

*In the tropics commercial grade sodium metasilicate cakes even when kept in air-conditioned room. The stated quantities never completely dissolved and therefore one cannot accurately estimate the amount in the media.

Compositions of synthetic culture media (cont.)

(quantities are those used for making 1 litre)

(1) Minor elements - 10 ml. of stock solution contain the following:-

Br (as KBr)	22.0 mg.
Sr (as $SrCl_2.6H_2O$)	3.8 mg.
Al (as $AlCl_3$)	0.028 mg.
Rb (as RbCl)	0.061 mg.
Li (as $LiCl.H_2O$)	0.006 mg.
I (as KI)	0.02 mg.

(2) Trace metals - 1 ml. of stock solution contain the following:-

Sodium EDTA	20.0 mg.
Fe (as FeNaEDTA)	0.1 mg.
Zn (as $ZnSO_4.7H_2O$)	2.3 mg.
Mn (as $MnSO_4.4H_2O$)	0.65 mg.
Mo (as $Na_2MoO_4.2H_2O$)	0.2 mg.
Co (as $CoSO_4.7H_2O$)	0.0063 mg.
Cu (as $CuSO_4.5H_2O$)	0.0013 mg.

(3) Vitamin Mix S3 - 10 ml. of stock solution contain the following:-

Thiamine hydrochloride	0.50 mg.
Nicotinic acid	0.10 mg.
Calcium pantothenate	0.10 mg.
Folic acid	0.002 mg.
p-animo benzoic acid	0.001 mg.
Inositol	5.0 mg.
Thymine	3.0 mg.

semi-micromethod of Kalle (1951) using silver nitrate and fluorescein sodium. The silver nitrate solution was standardized against 0.75 ml. of standard sea water of known chlorinity ("Eau de Mer Normale") supplied by the Laboratorie Hydrographique at Copenhagen. The ten different salinities were - 3.3, 6.7, 10.1, 13.3, 16.5, 19.9, 23.2, 26.7, 29.8, 32.9 °/oo.

The solutions of the complete medium used in the experiments on the vitamin requirements of the diatom were made by omitting the vitamin mixture from the enrichment component and adding the appropriate quantity of the vitamin(s) required. This operation gave six variations of the complete medium as follows:-

(1) Complete medium minus vitamin mixture and vitamin B_{12}, designated PLAIN.

(2) Complete medium minus Biotin and vitamin B_{12}, designated CM - B_{12} - BIOTIN.

(3) Complete medium minus Biotin, designated CM - BIOTIN.

(4) Complete medium minus vitamin mixture + vitamin B_{12}, designated PLAIN + B_{12}.

(5) Complete medium minus vitamin mixture + Biotin, designated PLAIN + BIOTIN.

(6) Complete medium minus vitamin mixture + Thiamine, designated PLAIN + THIAMINE.

The diatom culture was originally established in the 80 to 100% solutions of the complete medium. Before experiments on the influence of salinity on spore formation were carried out the diatom was gradually and progressively conditioned to the increasing dilutions of the medium. Thus cultures of the diatom were maintained at various dilutions. Transfer from a higher concentration to a lower concentration was made only after three to five subcultures with fresh medium during the exponential phase of growth.

The pH of the sea water of the estuary is usually within the range of 8.0 to 8.4, and the pH of the media for culturing was usually held within this range.

The media and stock solutions were all made up with deionised water of specific resistance greater than 4 megohms.

Culture apparatus and experimental conditions

Details of the culture apparatus used are shown in Fig. 1. Illumination was provided by three 2ft. 20W "tropical daylight" fluorescent lamps and strips of formica painted glossy white were used to reflect as much light as possible. The cultures received 5,500 to 8,000 lux at the level of their bases. A Lange Lux meter Type III reading directly in lux was used to measure the light intensity. This was achieved by filling the bath with distilled water to the level of the base of the culture flasks. A large petri dish of about the same thickness as that of the flask bases

Fig. 1. Culture apparatus

A. Row of culture flasks.

B. Constant temperature bath (perspex).

C. Glossy white cellotex light reflectors.

D. Block of wood for raising or lowering bath.

E. "Tropical Daylight" fluorescent tubes.

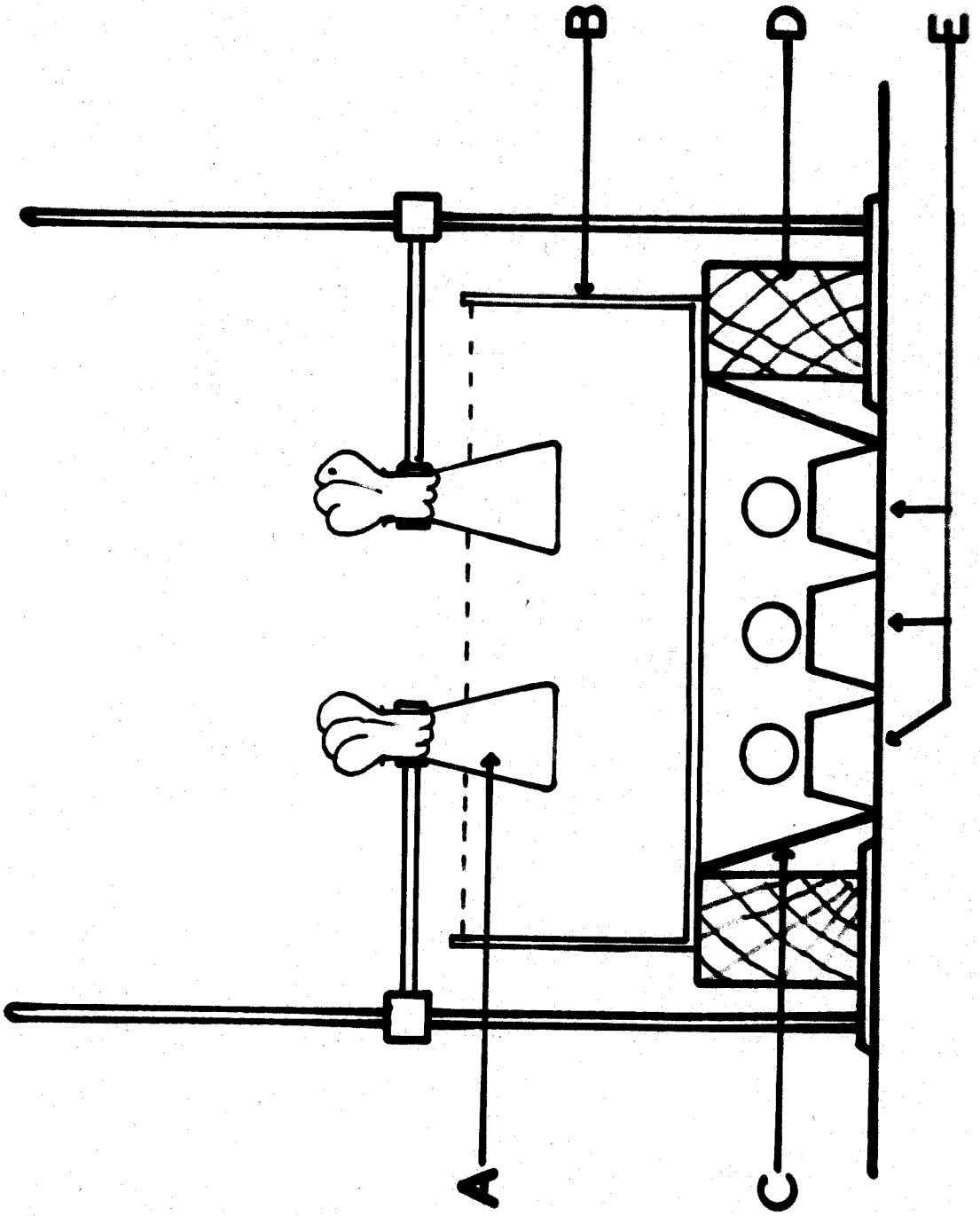


Fig.1

was placed just touching the surface of the water and carrying the photocell. An estimate of the light intensity over and along the length of the fluorescent tubes was made. The culture flasks were then arranged in two parallel rows of fives in the bath along the length of the fluorescent tubes. A qualitative comparison of the emission spectrum of the "tropical daylight" fluorescent tubes, with that of daylight, using a spectroscope, revealed that the red region of both spectra were similar and therefore there was need to supplement it by using tungsten filament lamps in the experimental set-up.

For high light intensity experiments the illumination was provided by three 4ft. 40W "tropical daylight" fluorescent tubes. Higher light intensities were obtained by covering the strips of formica with aluminium foil. The cultures were set up as above and they received 11,000 to 12,000 lux with the painted formica reflectors and 17,000 to 18,000 lux with the aluminium foil reflectors. Lower light intensities were achieved by attenuation with one to three layers of a black muslim cloth wrapped tightly round the culture flasks (two in each case). The illumination was measured by placing the photocell in a similarly wrapped petri dish at the level of the base of the flasks along the length of the fluorescent tubes.

In the temperature effect experiments a Kryomat cooling system was used to obtain constant temperatures of 25°C and below.

Stock cultures were grown in flasks in an air conditioned room on wire stands and illuminated from below. The temperature range was usually 25° to 28°C and the illumination range from 4,000 to 6,000 lux. The cultures were subcultured every five days. The experimental cultures were maintained at 28°C in a constant temperature water-bath made of perspex. This temperature was chosen because it is the average temperature noted for the estuary water over a five-year period of study (Watts, 1960).

For the quantitative experiments the diatom was cultured in 100 ml. Erlenmeyer Pyrex flasks containing 25 ml. of medium and plugged with non-absorbent cotton wool. Preliminary experiments were made using boiling tubes and the cultural technique of Kain & Fogg (1958). The cultures were illuminated from the side and were aerated with air which was not enriched with CO_2. This was done by means of a fine capillary tube passing to the bottom of the culture tubes and ending in a fine jet. Alternate cultures were aerated. A flow meter was used to control the rate of aeration. The aeration technique appeared to inhibit growth.

For the study of the methods of auxospore and male gamete formation the cultures were often observed in petri-dishes and watch glasses, which were frequently exposed for observations under a microscope or for photomicrography.

The experiments on the influence of salinity on male gamete and auxospore formation were carried out in $3\frac{1}{2}$" wide petri dishes standing on a supported wire mesh and illuminated from below in an air conditioned room. Three replicates were used in each case. The use of petri-dishes made observation under a binocular microscope easier and enough of the medium can be used to support the growth of a small inoculum (50 to 100 cells) whilst shading of the cells can be avoided.

Glassware was cleaned by soaking in chromic acid or 1% Haemosol solution followed by rinsing in running tap water and finally in hot distilled water. Pipettes were soaked in distilled water instead of receiving the last treatment. All culture vessels and pipettes were prepared and heat sterilized in an oven at 180°C for two hours as recommended by Pringsheim (1946).

The inoculum and the estimation of growth

Ten healthy looking cells were selected each time and washed in fresh sterile medium before inoculating the flasks. Aseptic techniques were always employed in these operations. The cultures were shaken vigorously once a day to assist diffusion of air. The use of an inoculum of ten cells got rid of the difficulty of shading, and also during exponential growth the pH of the medium was not affected. Various sizes of the diatom cells lying within the sexual induction size range were used in the experiments.

In the estimation of growth, 1 ml. samples were removed at a particular time each day after shaking the cultures vigorously. The cells were allowed to sediment in sedimentation tubes after treatment with Lugol's solution (Utermöhl, 1931; Lund et al., 1958). Counts were made on settled samples using an inverted microscope. The cells were too large for a haemocytometer to be used and the density of the cultures in the samples was so low that O.D. measurements would have been useless.

Usually growth is expressed as either the increase in cell numbers (cell concentration) or the increase of cellular material, (cell density). Since an increase in cell concentration is a measure of the true division rate (Monod, 1949), this has been adopted as the measure of growth. Therefore in this work the term growth rate is synonymous with cell division rate.

Growth is expressed as the number of doublings per day and is related directly to cell reproduction. The cell concentration is determined in terms of live and dead cells. The criterion for live cells is that of Lund (1949) where a cell is considered live unless it is empty or showing signs of lysis. The growth rate is determined during the exponential phase as it represents the maximum rate for the cultural conditions employed.

The growth constant is calculated from the integrated growth equation

$$K = \log_2 N/N_o \times 1/t$$

where N and N_o are the number of cells at the beginning and end of the time interval, t. Growth is depicted graphically on a plot of $\log_2 N/N_o$ against time, t, in days. This gives the growth constant K as the number of doublings per day. The mean generation time or division rate can easily be calculated from K.

The experimental set-up permitted the use of parallel duplicate cultures. It was observed that the light intensity along the length of the fluorescent tubes was not uniform but increased from the ends towards the centre. Therefore it was necessary to ascertain whether there was any significant difference in growth amongst the five cultures depending on their position along the length of the fluorescent tubes. The students t-test and the F test were used to assess the statistical significance of the differences in growth rates.

5. RESULTS

1. MORPHOLOGY AND TAXONOMY OF THE CLONE OF THE DIATOM, COSCINODISCUS PAVILLARDII

Morphological Characters.

Examination of the living material revealed that the cells of the diatom are usually free and solitary; disc shaped in valve view (Plate I) and rectangular in girdle view (Plate II). Two cells may be occasionally found attached after division (Plate II). The valves are flat with a slight central depression. The nucleus is large, conspicuous and usually seen at the centre of the cell. It is held in position by cytoplasmic strands extending to it from the lining layer of cytoplasm inside the cell wall (Plate II). The chromatophores are numerous small rounded bodies rather uniformly scattered throughout the cell (Plate III).

Light micrographs as well as camera lucida diagrams have been used to illustrate the nature of the acid-cleaned frustules. The valve surface is finely areolate (Plate IV). The areolae are hexagonal (Fig. 2b) and arranged in radiating lines, running from a clear central hyaline space which is fairly large (Plate IV). The areolae range from 6 in 10 µ near the central area to 5 in 10 µ towards mid-valve and edge of valve (Fig. 2a - c). The valve surface is dotted all over with a number of small spinulae (Plate IV and V). The valve margin is narrow and furnished with a circlet of small spinulae which may be close together or separated by about ten radii of areolae (Fig. 2b and Plate VI). In some valves of a frustule there

Legend to Plates I - VI

Plate I - disc shaped cells with prominent central nucleus, x 350.

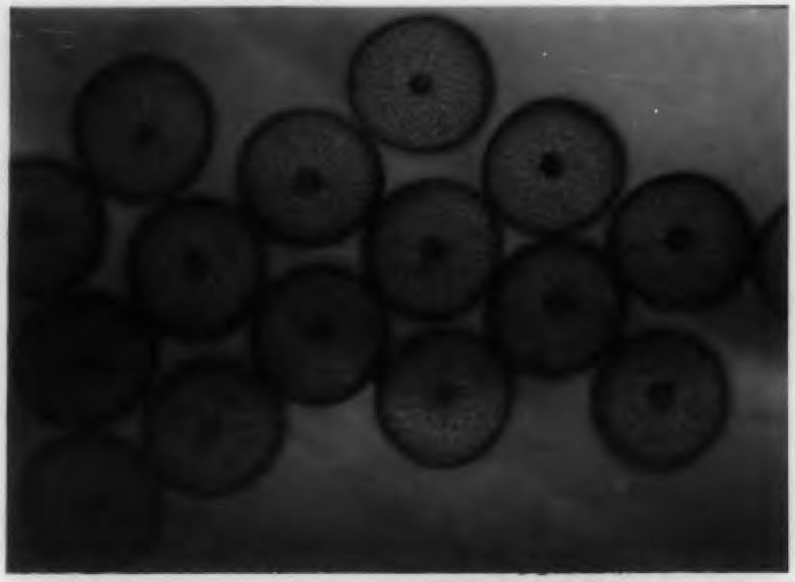
Plate II - rectangular shape of cell (girdle view); nucleus suspended centrally by cytoplasmic strands, x 910.

Plate III - cell showing numerous chromatophores, x 975.

Plate IV - cleaned valve showing radiating rows of areolae and central hyaline space, x 975.

Plate V - hexagonal shape of areolae; white specks are spinulae, x 8,750.

Plate VI - circlet of spinulae among radiating areolae, x 3,250.



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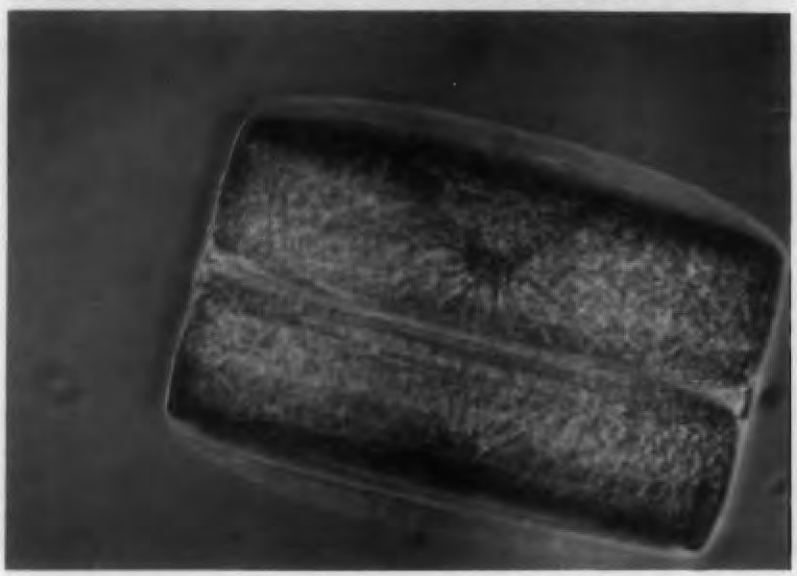

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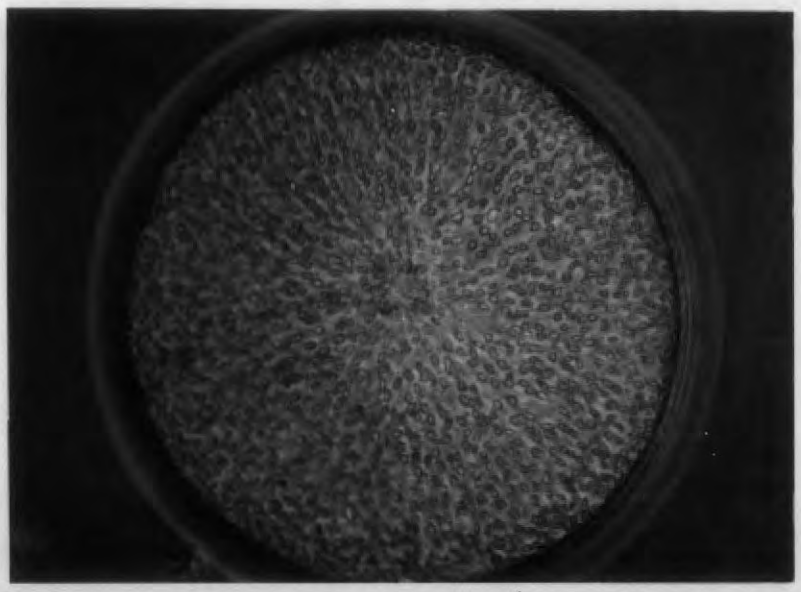

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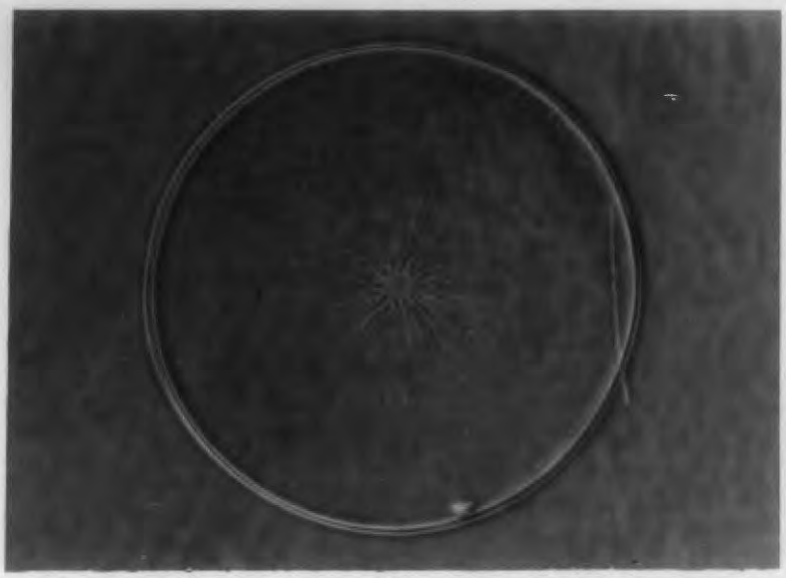
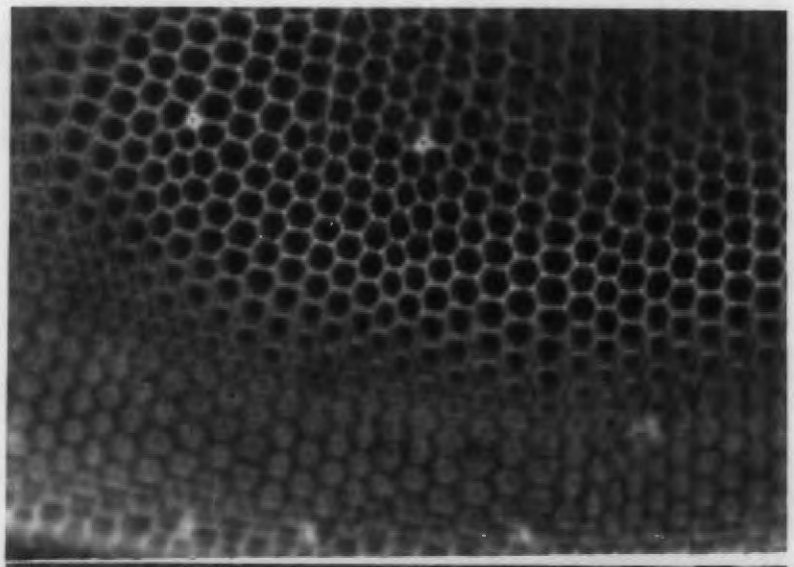


IV



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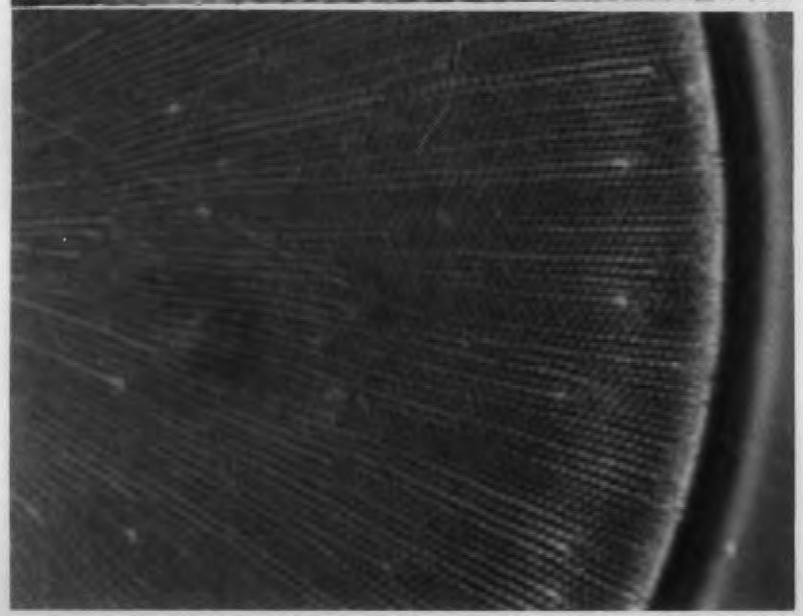


VI

Fig. 2. Diatom frustule structure drawn with the aid of a camera lucida.

a. Middle region and margin.

b. Margin

c. Area near central hyaline space.

(black dots are spinulae)

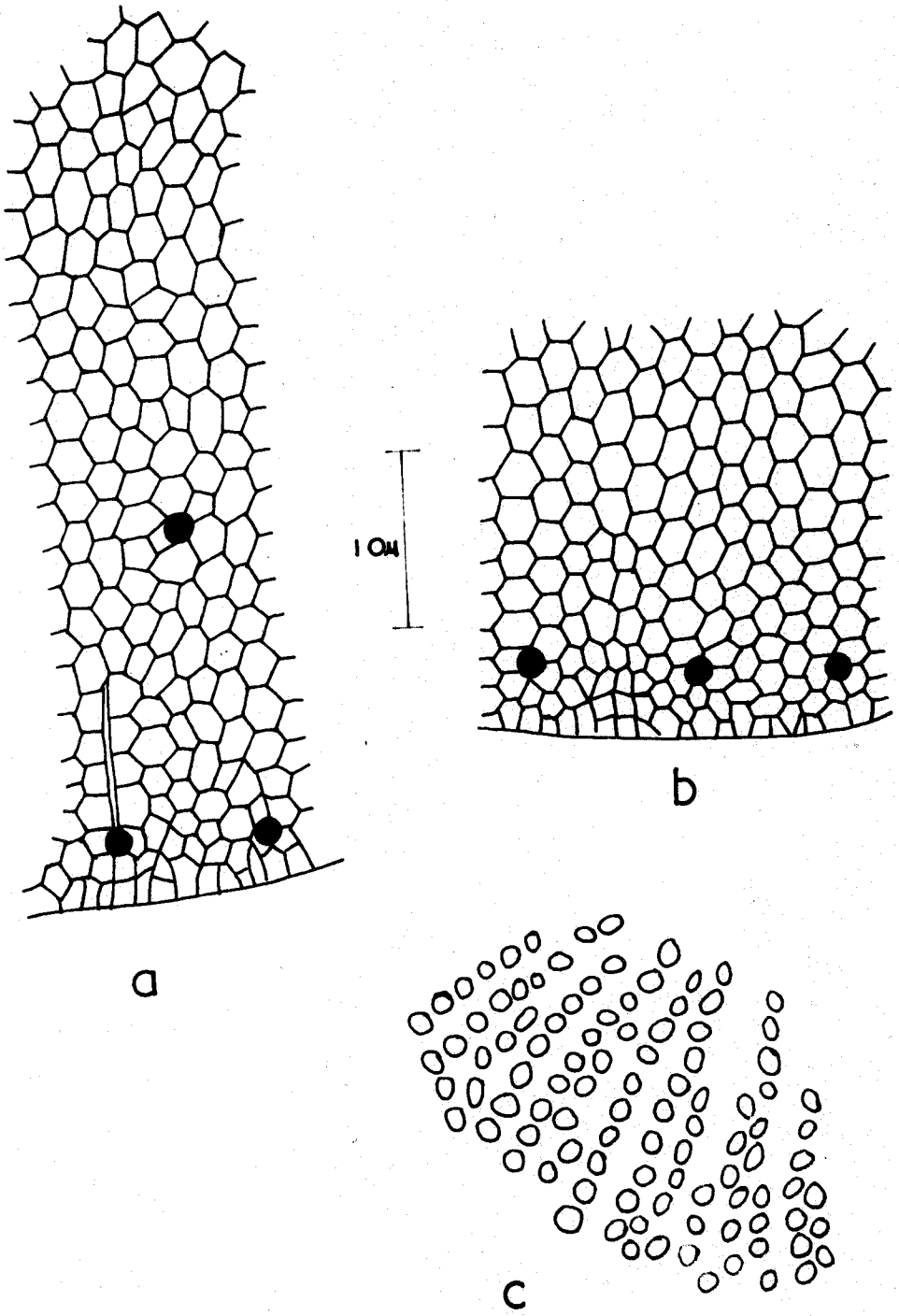


Fig. 2

may be another circlet of spinulae in mid-valve. There are two larger spines (apiculi) set widely apart on the valve margin. The girdle is a single, non-perforated band. The outer poroid membrane is extremely fine and can be seen on the areolar margins (Plate V).

The diatom forms auxospores and the cells arising from them have a maximum cell diameter of $464 \pm 44\ \mu$. The mean width of the pervalvar axis of recently divided free cells is 127 μ and that of cells recently divided but stuck together is 255 μ. The mean minimum diameter of the cells obtained in cultures at a size below which further growth was impossible, was 64 μ.

Taxonomic problems

The determination of some of the many species comprising the genus Coscinodiscus Ehrenberg is extremely difficult. The wide range of variability in shell morphology among the species is the cause of the difficulty. Hence two independent determinations of the species of the clone of the diatom under study were sought - N.I. Hendey, England and Professor H.A. von Stosch, Germany.

Hendey (private communication) determines the clone as a member of the taxonomically complex gigas - janischii - pavillardii group. He writes "no one knows the limits of each phase and no doubt intermediates exist which make nonsense of taxonomic divisions. Ehrenberg's type of C.gigas no longer exists and the illustrations

in Schmidt's Atlas (1874) and those of Hustedt (1930) only mislead." He contends that in the typical "gigas" the areolae are larger and the circular cover pores are very clearly seen, whereas in the author's specimens the cover spores seem absent and the sieve plate, i.e. outer poroid membrane, is extremely fine. Moreover spinulae are not present in C.gigas. Similarly von Stosch (private communication) dismisses C.gigas on the grounds that it has no apiculi which are present in the author's specimens.

Hendey places the clone closer to C.janischii as the specimens agree very well with some specimens he took from Southampton Water, England. He states that "the central area (fairly constant in the author's specimens) can be very variable and may be almost closed up with areolae. Often valves of the same frustule may differ, one being 'open' with a fairly large central area as in your specimens, while the other is covered all over with areolae." Lebour (1930) gives the latter as a description for C.pavillardii. Hendey observed that the specimens have a number of small spinulae dotted over the valve surface - a specific character of C.pavillardii. However, Hendey rules out C.pavillardii as a possible determination because of the size and much finer areolation of the author's specimens. On the other hand von Stosch considers that the specimens should be called C.pavillardii, for C.janischii has a heavy areolation at

the margin as against the very slight one of the author's specimens. Moreover, C.janischii has no apiculi as shown in Schmidt's Atlas (1874). Also von Stosch contends that from Janisch's paper his "C.marginatus, which is not identical with C.marginatus Ehrb., is meant to be C.janischii of Rattray and Hustedt." He questions the description of C.marginatus Janisch as "it seems to have puncti in the figure of Janisch (which he did not describe) and has no very marked difference between the marginal and the inner areolae (which he mentioned in his description)". Von Stosch therefore argues that in no case is it sufficiently similar to the author's specimens, and being not so well described by Janisch, the specimens cannot be considered as C.janischii. From the description and figures of C.pavillardii given by Pavillard (1925) von Stosch considers that Hustedt (1930) seemed to imply that the interstitial meshes are not provided with apiculi. Pavillard however described them, and von Stosch observed them in the author's specimens. He therefore concludes that the Sierra Leone material conforms well to the Mediterranean species, C.pavillardii.

From the foregoing discussion the author accepts von Stosch's identification of the diatom as C.pavillardii. Pavillard's description of C.pavillardii gives its maximal diameter as 350 μ. It is considered that Pavillard may not have seen young auxosporic material (which was obtained in culture by the author)

and therefore the species was not erected on the dimensions of cells immediately developing from auxospores.

It appears that the diatom under study is the one Bainbridge (1960) called *C.gigas*.

2. PRELIMINARY EXPERIMENTS

RESULTS ON CULTURING THE DIATOM.

1. Preparation of bacteria-free culture of the diatom

(a) The diatom failed to divide in 1% agar prepared with the synthetic medium. The cells died after a couple of days.

(b) In attempts to prepare bacteria-free cultures with antibiotics it was found that in the presence of 408 units and above, of the mixture of penicillin and streptomycin no bacterial growth was observed after repeated washings and final inoculation of a few cells in the synthetic medium prepared with peptone. The cells, however, failed to divide. Other similarly treated and washed cells were inoculated into the plain synthetic medium. In this case, no apparent turbidity developed but the cells divided rather slowly (about one division in three days) and erratically.

Several attempts were made to stimulate growth in such cultures but all were unsuccessful. These included the addition of a small quantity of (a) bacterized medium (1 ml. to 25 ml. of culture), (b) Seitz filtered bacterized medium, (c) sintered glass filtered bacterized medium, and (c) a tiny grain of sodium sulphide. The latter was done as Droop (1961) had reported stimulation of growth in Skeletonema costatum with small quantities of sodium sulphide. In all the attempts the diatom remained alive for up

to two weeks but showed hardly any stimulation of their vegetative growth rate.

It was therefore decided to use cells which had no previous antibiotic treatment, but which had been repeatedly washed and further washed between several subcultures. Cultures obtained from these cells showed no appreciable bacterial growth during the exponential phase of growth. Moreover, when grown in peptone synthetic medium for a week, no appreciable growth of bacteria occurred.

Examination of the bacteria closely associated with the diatom cells revealed that they were rod-like.

2. Choice of culture medium

The growth rates in the U32 and U36 media were the same (one division in about 40 hours), but faster in the modified ASP2 medium (one division in about 30 hours), under the same conditions of light and temperature.

Since the diatom cells appear to be heavily silicified, it was necessary to determine where the cells got their silica from during vegetative growth in the U32 medium - a medium which lacked silicate. On testing the medium for silicate a positive result was obtained with ammonium molybdate and reducing metol sulphite-oxalic acid solution (Strickland and Parsons, 1960). Almost the same intensity of colour was obtained as for U36.

3. Cultural conditions

In employing the culture technique of Kain and Fogg (1958) the cultures were aerated at the constant rate of 10 ml./min. in one experiment and 5 ml./min. in another experiment. Both experiments were run simultaneously. The use of air unenriched with carbon dioxide for aeration was merely intended to keep the cells in suspension. In the aerated cultures growth was inhibited. This was more marked at the higher aeration rate, as there was scarcely any growth at all. Growth in the alternate stagnant cultures, which were aerated for five minutes every day, was slow but continued (one division every sixty hours).

When conical flasks were used instead of boiling tubes the cultures were illuminated from below and shaken vigorously once a day. Good growth of the diatom was achieved. Consequently culturing in flasks was preferred.

4. Culturing the diatom at various salinities

Cultures were established at the following salinities - 16.5, 19.9, 23.2, 26.7, 29.8 and 32.9 $^{o}/_{oo}$. The cultures were originally established with cells of average diameter 381 μ. The cultures were repeatedly subcultured every five days. It was observed that the average size of the cells decreased with time. In four months of culture the average diameter of the cells was 309 μ. This is accounted for by a decrease in average diameter of 18 μ per month.

Comparison of the rate of decrease in average cell diameter in nature and in artificial synthetic medium revealed that it was of the same magnitude under both conditions.

Cells of average diameter 309 μ began to form spores in all the salinities used except 16.5 °/oo. Spore formation at the 32.9 °/ooS was not as plentiful as at the other salinities in which it occurred. Salinity, therefore, appears to have an influence on spore formation.

5. The effect of an illumination range of 5,500 to 8,000 lux on the growth rate

Along the length of the fluorescent tubes used it was found that the light intensity was not uniform. It increased from the ends towards the centres. It was therefore necessary to determine whether there was any significant difference in growth in the cultures placed at points along the length of the tubes. The results of one such experiment are given in Table II.

The F test was used to determine how significant the differences were among the live and total cells respectively (Tables II - IV). There was no significant difference in growth among the treatments both for live and total cells at the 0.1% level. Therefore the variation of light intensity along the length of the tubes did not affect the growth rate of the diatom. Consequently it was decided to use the average figures for the ten flasks in estimating the growth rate.

TABLE II. Log_2 values for the average figures obtained from parallel replicates

(L = live cells; T = total cells)

Time (days)	Flasks (i)&(vi) at 5,500 lux		Flasks (ii)&(vii) at 7,300 lux		Flasks (iii)&(viii) at 7,800 lux		Flasks (iv)&(ix) at 7,800 lux		Flasks (v)&(x) at 7,200 lux	
	L	T	L	T	L	T	L	T	L	T
3	4.1	4.1	2.9	3.3	4.3	4.3	4.1	4.1	4.1	4.3
4	5.1	5.1	4.6	4.6	5.6	5.6	5.7	5.8	5.5	5.6
5	6.5	6.6	6.2	6.3	7.0	7.0	7.1	7.1	6.6	6.6
6	7.1	7.1	7.2	7.3	8.3	8.3	8.2	8.2	7.9	7.9
7	8.3	8.3	8.4	8.5	8.9	9.0	8.9	9.0	8.8	8.9
8	9.0	9.1	9.4	9.6	9.0	9.1	9.1	9.3	9.4	9.5
9	9.4	9.6	9.6	9.9	9.1	9.4	9.1	9.5	9.3	9.6
10	9.3	9.5	9.6	10.0	8.7	9.3	8.9	9.5	9.2	9.7
11	9.3	9.8	9.7	10.5	8.7	9.5	8.9	9.6	9.4	9.9
12	9.1	9.9	9.5	10.4	8.6	9.6	8.6	9.6	9.1	9.9

TABLE III. Analysis of Variance for Live Cells

Source of Variance	Sum of Squares	Degrees of freedom	Mean Square
Between treatments (columns)	0.3518	4	0.08795
Residual	169.746	45	3.772

$$F \text{ ratio} = \frac{0.08795}{3.772} = 0.0233$$

Not significant at 0.1% level

TABLE IV. Analysis of Variance for Total Cells

Source of Variance	Sum of Squares	Degrees of freedom	Mean Square
Between treatments (columns)	0.5152	4	0.1288
Residual	209.512	45	4.6558

$$F \text{ ratio} = \frac{0.1288}{4.6558} = 0.02766$$

Not significant at 0.1% level

DISCUSSION

The failure of diatom cells treated with antibiotics to grow on subculture to antibiotic-free medium has been reported by Spencer (1954). He proposed that the antibiotics must have either directly affected the algae; or that the antibiotics, in controlling the bacterial flora, have removed the source of the essential nutrients or growth factors of the algae. In the case of the diatom under study it is considered that the concentration of the antibiotics used in the elimination of the bacteria was toxic to the diatom, and therefore inhibited its growth. It is not likely that the diatom was dependent on the bacteria nutritionally. For attempts at stimulating growth of the diatom with bacterized medium were unsuccessful.

The growth of the diatom in the U32 medium which lacked silicate can only be explained in terms of a dissolution of silica from the walls of the culture flasks during autoclaving. The amount of silicate which may be contained as impurities in the salts can be considered negligible as the salts were of analytic reagent quality. Silica is soluble in alkaline solutions (Jørgensen, 1955). Since the culture medium is slightly alkaline it is felt that significant amounts of silica are released from the flasks during autoclaving. This is in agreement with the findings of Armstrong as reported by Spencer (1954).

The cause of inhibition of growth observed in the aerated cultures can only be guessed at. The pH of 8.4 of the medium is considered beneficial because at such a pH carbon dioxide is avidly absorbed and forms a reservoir of bicarbonate. This provides increased carbon dioxide without having to employ bubbling or shaking (Provasoli et al., 1957)

The low rate of growth of the stagnant cultures in boiling tubes, as compared with the rate of those in conical flasks, may be the result of self shading and the direction of illumination. The cells of the cultures in the boiling tubes settled to the bottom of the tubes in a heap. Consequently fewer cells could receive the same amount of light. This disadvantage was increased as the cultures were illuminated from one side only. In the case of the cultures in the conical flasks the cells were dispersed in the medium such that each cell received the same amount of light which was directly below them.

The unmodified ASP2 medium is designed such that the nitrate and phosphate content are kept low to suppress excessive bacterial growth. This makes such a medium suitable for bacteria-free cultures. In the modified ASP2 medium the nitrate content is reduced to about one twelfth of that of the unmodified medium

(i.e. from 5882 μ g-at. N/l as $NaNO_3$ to 500 μ g-at. N/l as KNO_3). The phosphate is increased from 29 μ g-at. P/l as K_2HPO_4 to 50 μ g-at. P/l as KH_2PO_4 - a concentration which was found adequate for phytoplankton growth (Parsons et al., 1961; Jitts et al., 1964). However Provasoli et al. (1957) found that the concentration of 29 μ g-at. P/l is perfectly adequate for stagnant cultures and the possibility of precipitation is limited. Therefore an increase of 21 μ g-at. P/l would greatly enhance the possibility of precipitation during autoclaving. This was found to be the case if the pH of the medium was not adjusted to 8.4 before autoclaving. However, with light autoclaving, it was found that the possibility of precipitation was much less - a finding also of Provasoli et al. (1957).

3. SEXUALITY AND CELL SIZE

From the preliminary experiments it was observed that as the cells attained a certain size in certain salinities spores were produced. It was therefore decided to investigate over what range of cell size spore formation occurred in a wider range of salinity. Consequently the diatom was repeatedly subcultured in the various salinities until it seemed impossible for further cell multiplication to occur. This investigation lasted over a year.

The cultures used had previously been established at the different salinities (see Materials and Methods). No cultures could be established in salinities of 6.7 °/oo and below. In a salinity of 6.7 °/oo little or no growth took place and after about three days the cells died. Immediately after inoculation in salinities of less than 6.7 °/oo the cells burst and died. The author suggests that the bursting is the result of an osmotic effect, the culture medium being hypotonic to the cell contents of the diatoms.

Fig. 3 illustrates the relation of vegetative cell diameter to the diameter of the cells developing from auxospores. It can be observed that the rate of decrease in the average vegetative cell size was uniform down to about 180 µ. Thereafter there was a slowing down of the rate. The average size of cells developing from the

Fig. 3. The relation of vegetative mean cell diameter with time; and the mean diameter of cells developing from auxospores produced by vegetative cells within the sexual induction size range.

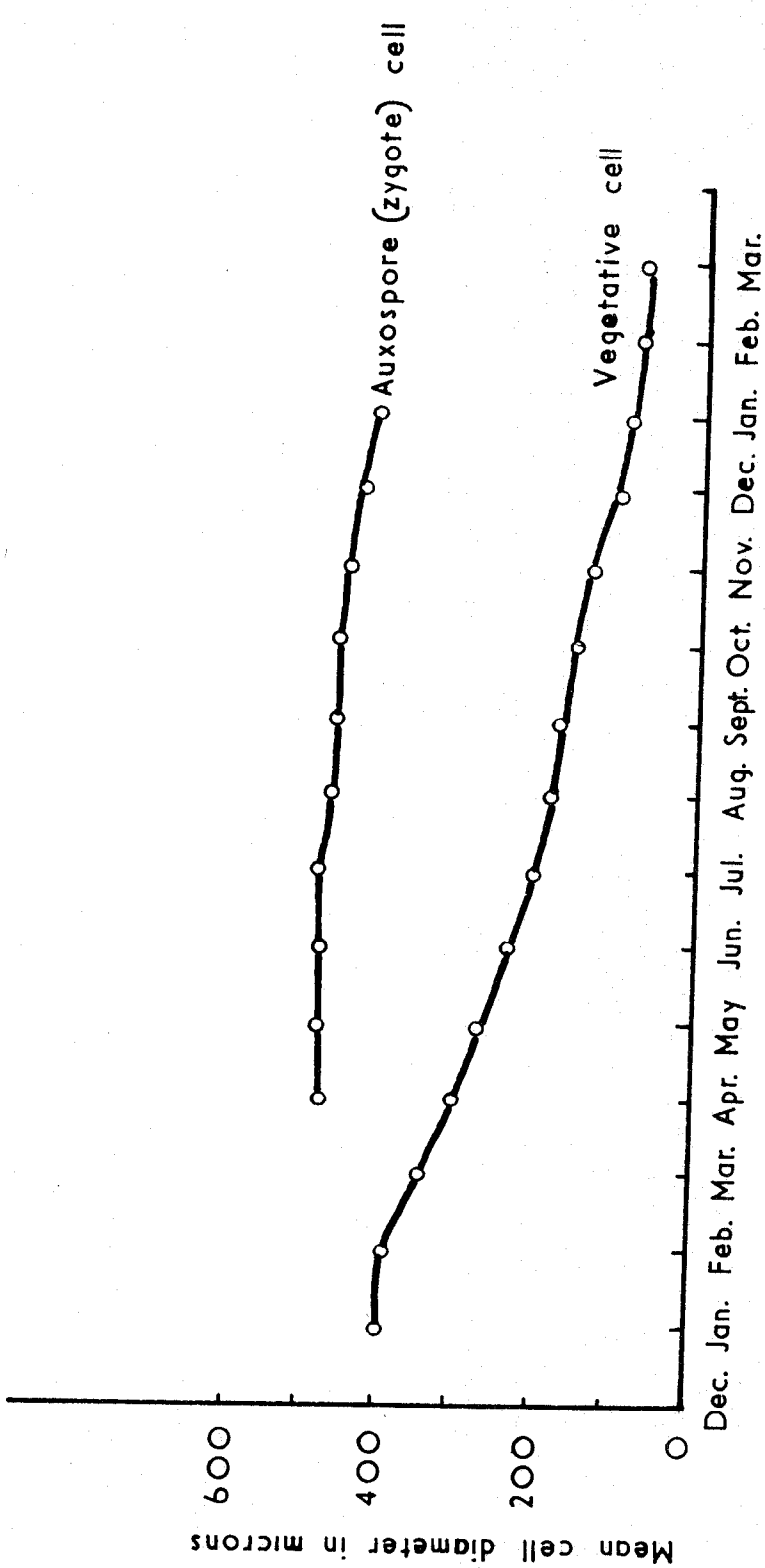

Mean cell diameter in microns
600
400
200
0
Dec. Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar.
Auxospore (zygote) cell
Vegetative cell

Fig. 3

auxospores is nearly the same in all cases. The maximum cell diameter attained was 510 μ and the minimum was 450 μ. Spore formation occurred within the average cell diameter range of 91 to 309 μ. This is about 20 to 63% of the mean maximum cell diameter. Optimal production of spores occurred within the range of about 30 to 55% of the mean maximum cell diameter i.e. 145 to 273 μ. Within this size range the cells developing from the auxospores were more viable than those produced at the upper limit of the sexual induction size range.

The cells developing from the auxospores can be from about one and a half to four and a half times the average diameter of the cells producing them. There was no apparent difference in size between the male gamete or zygote mother cells, as the cultures used contained only cells of fairly uniform diameter. Fewer male gamete mother cells were observed in the upper limit of the sexual induction size range, but the number increased progressively with decreasing cell diameter. In the case of the auxospore mother cells the opposite tendency was observed. Consequently the proportion of male gamete and auxospore mother cells varied with their size or diameter.

Sexuality may not occur in all cells within the sexual induction size range. It was observed that some cells continued dividing vegetatively and their products became still smaller until their size was reduced to about 15% of the mean maximum

cell diameter, i.e. 64 µ, after which division ceased and death ensued.

The salinity and temperature range within which sexuality can be induced was found to be 19 to 33 °/oo and 24 to 28°C respectively.

4. GROWTH RATE OF CELLS OF DIFFERENT DIAMETERS AT 28°C AND UNDER CONTINUOUS ILLUMINATION

During the course of the preliminary experiments on the growth of the diatom in a range of salinities it was observed that as the cells multiplied they became smaller and smaller in size. The smaller cells appeared to multiply faster than the larger cells. It was therefore decided to investigate whether the different growth rates of the various sizes of cells bear any relationship to each other. The growth rate was determined during the exponential phase of growth.

The investigation was carried out at two salinities - 13.3 °/oo and 19.9 °/oo, because in cultures at these salinities male gamete and auxospore formation are inhibited. The following diameter sizes of cells were used at the different salinities:-

(i) 13.3 °/oo - 73 - 91 μ; 91 - 109 μ; and 327 - 345 μ.

(ii) 19.9 °/oo - 127 - 145 μ; 145 - 164 μ; 236 - 254 μ and 327 - 345 μ.

The experiments were separated in time as the sizes could not be obtained at the same time. The results are shown graphically in Fig. 4(b) and (a) at 13.3 °/oo and 19.9 °/oo respectively. The growth rate (≡ mean generation time) are presented for total cells (i.e. live and dead cells) in Table V.

Fig. 4a, b. Growth curves of different sizes of cells at two salinities, 13.3 °/oo and 19.9 °/oo.

(i) & (ii)	327 - 345 µ
(iii)	236 - 254 µ
(iv)	145 - 164 µ
(v)	127 - 145 µ
(vi)	91 - 109 µ
(vii)	73 - 91 µ

4c. The relationship between total cell surface area and division rate.

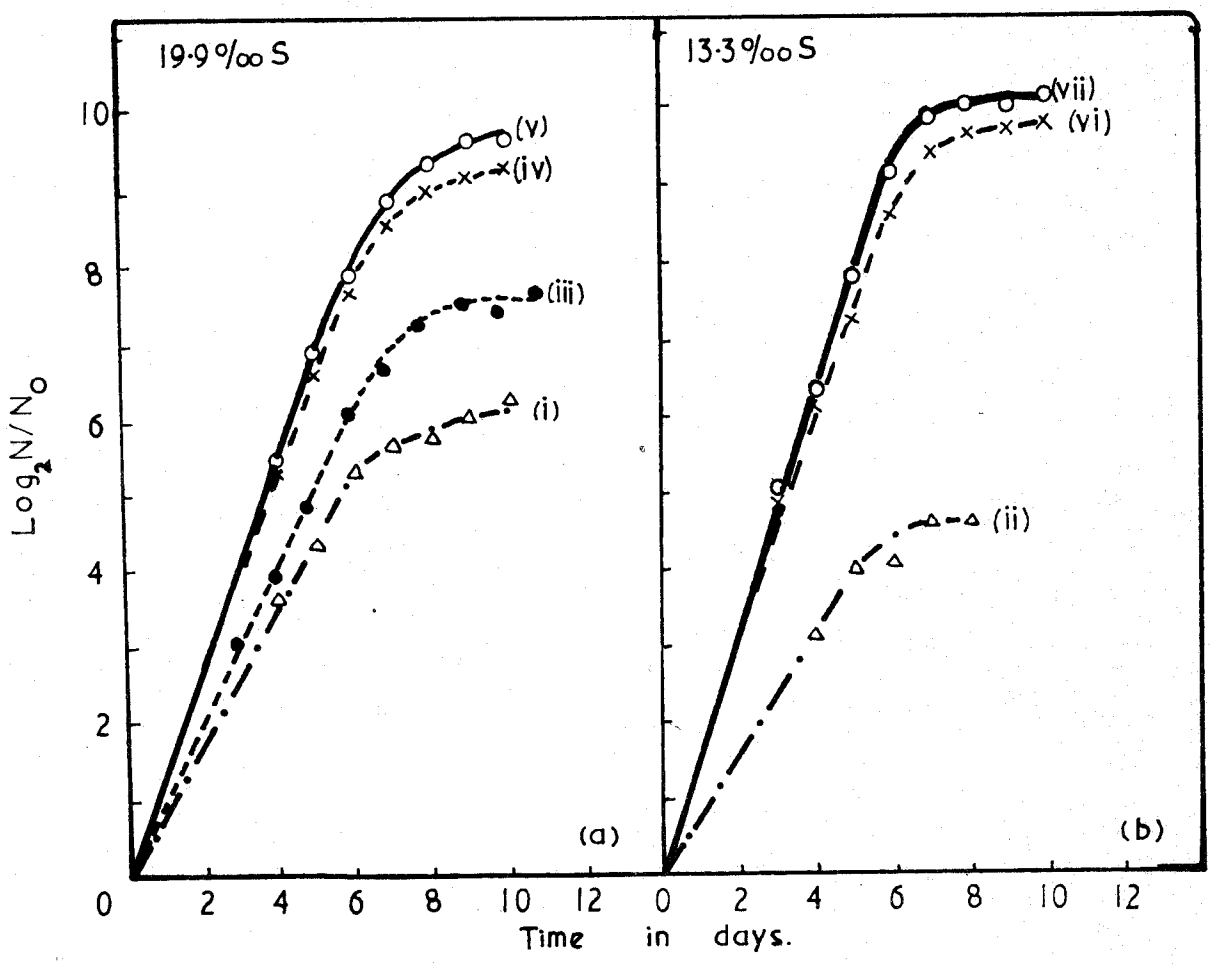

19·9 ‰ S
13·3 ‰ S
(i)
(ii)
(iii)
(iv)
(v)
(vi)
(vii)
(a)
(b)
$Log_2 N/N_0$
Time in days.
0
2
4
6
8
10
12

Fig. 4

TABLE V. Relation of cell diameter to mean generation time at the different salinities

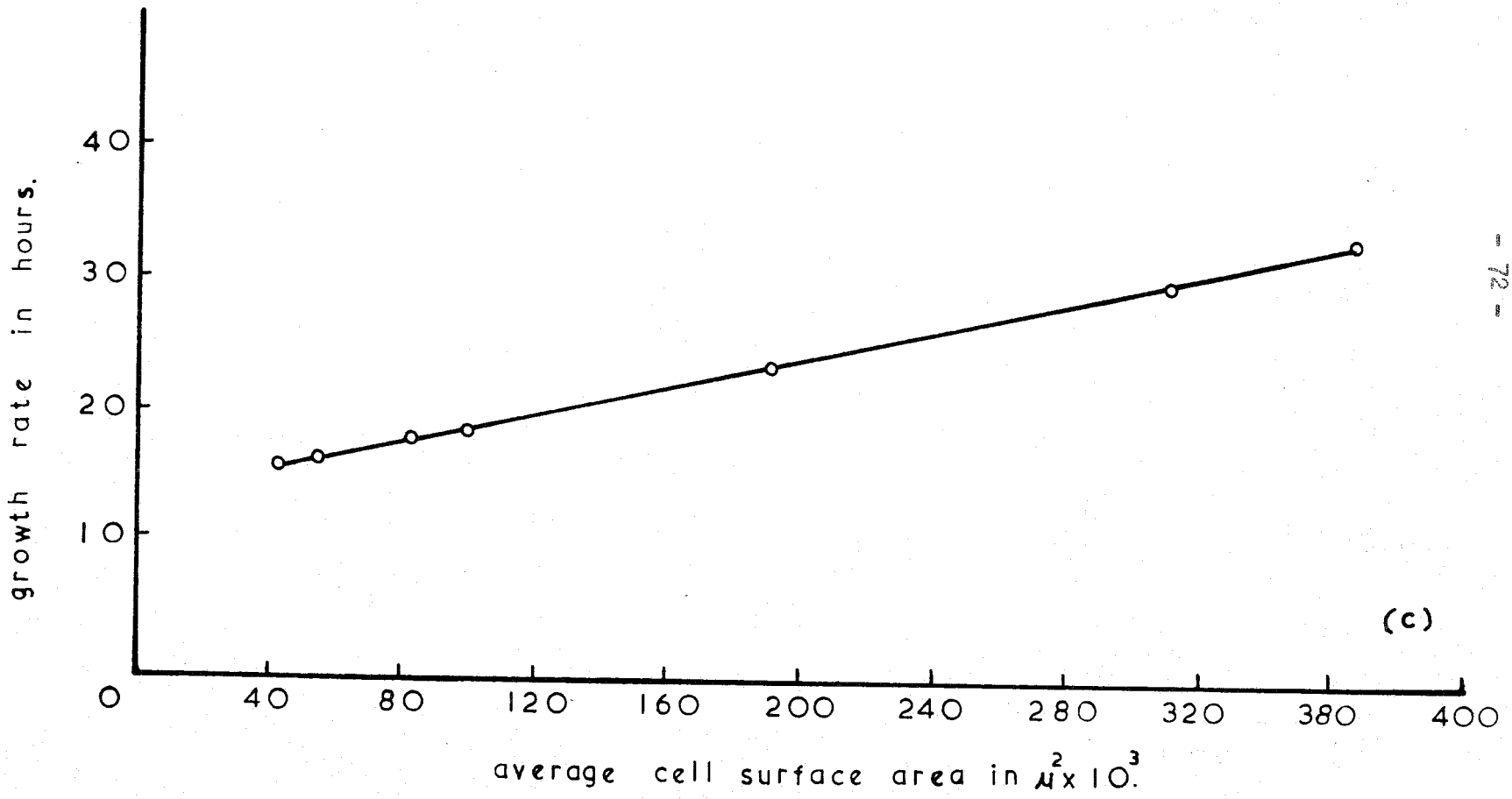
Salinity (°/oo)	Diameter range (microns)	Mean generation time (hour)
13.3	73 - 91	15.8
	91 - 109	16.4
	327 - 345	30.0
19.9	127 - 145	18.0
	145 - 164	18.5
	236 - 254	23.5
	327 - 345	30.0

TABLE VI. Relation of average cell surface area to mean generation time

Average cell surface area (sq. microns)	Mean generation time (hour)
43,280	15.8
55,600	16.4
83,320	18.0
99,150	18.5
192,100	23.5
311,800	30.0
(i) 367,400	33.5

(i) computed from Table VII

Fig.4c


growth rate in hours.
40
30
20
10
0
40
80
120
160
200
240
280
320
380
400
(c)
average cell surface area in $\mu^2 \times 10^3$.

The relationship between total cell surface area and division rate is expressed graphically in Fig. 4(c). Cell surface area* was found to be a more useful statistic than cell volume since it gives the effective measure for the absorption of nutrients. It is assumed that total cell surface area varies with cell volume.

From Table V it is obvious that the smaller cells divided more rapidly than the larger ones. In the two categories of smaller diameter range of cells in both salinities the differences in the growth rate are slight.

In Table VI the mean generation time decreased with reduction of average cell surface area. Expressed graphically, a straight line is obtained, Fig. 4(c), indicating that the relative growth constant is the same for all cell sizes.

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*Average cell area = average total surface area, calculated by using the formula $2\pi r^2 + 2\pi rl$. (l = mean length of pervalvar axis) See Results Section 5.1. for value.

5. THE EFFECT OF EXTERNAL FACTORS ON THE GROWTH RATE OF THE DIATOM AT 28°C AND UNDER CONTINUOUS LIGHT

Since smaller cells grow faster than larger ones it was decided to use cells of the same average size in any one set of experiments. Thus the growth rate under the varying conditions of one factor can be compared. The external factors investigated were salinity, light intensity and temperature. All cells used were those which cannot be induced to form spores. In the case of the experiments to determine the effect of light intensity and temperature the cells used belonged to the second generation of auxospore cells derived from the original clone. Although the sizes of the cells fall within the sexual induction size range yet it was difficult to induce spore formation in them.

1. Salinity

At salinities of 13.3, 16.5, 19.9 and 26.7°/oo the diameter range of cells used was 327 to 345 µ and at 32.9°/oo it was 364 to 382 µ. These cells belonged to the original clone.

The results are set out in Table VII(a).

The growth rate was approximately the same at the salinities 13.3 to 26.7°/oo but slightly less at the 32.9°/oo S in which a different size range of cells was used.

The growth curves at the different salinities are shown in Fig. 5 (a to c). It can be seen that the number of total and of live cells obtained at the stationary phase had some relationship to the salinities used.

TABLE VII(a). Growth rate (≡ mean generation time) at different salinities

Salinity (°/oo)	Mean generation time (hour)
13.3	29.8
16.5	30.0
19.9	29.8
26.7	30.2
32.9	33.5

Fig. 5. Growth curves at different salinities.

a. 32.9 °/ooS; cell diameter 364 - 382 μ.

o—o—o total cells,

o--o--o live cells.

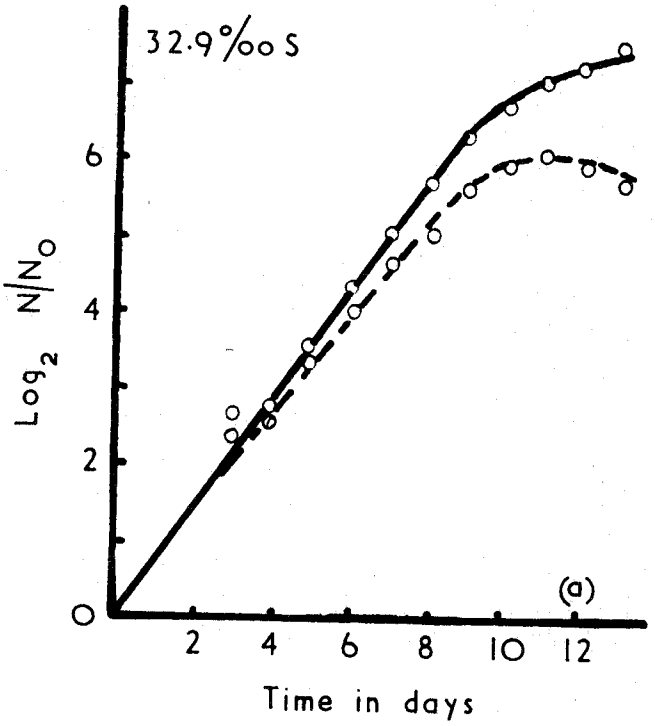
b. Total cells - 13.3, 16.5, 19.9 and 26.7 °/ooS;

cell diameter 327 - 345 μ.

c. Live cells - same details as for (b).

d. Total cells - at 10.1 and 13.3 °/ooS;

cell diameter 73 - 91 μ.


32.9‰ S
Log_2 N/N$_o$
0
2
4
6
(a)
2
4
6
8
10
12
Time in days

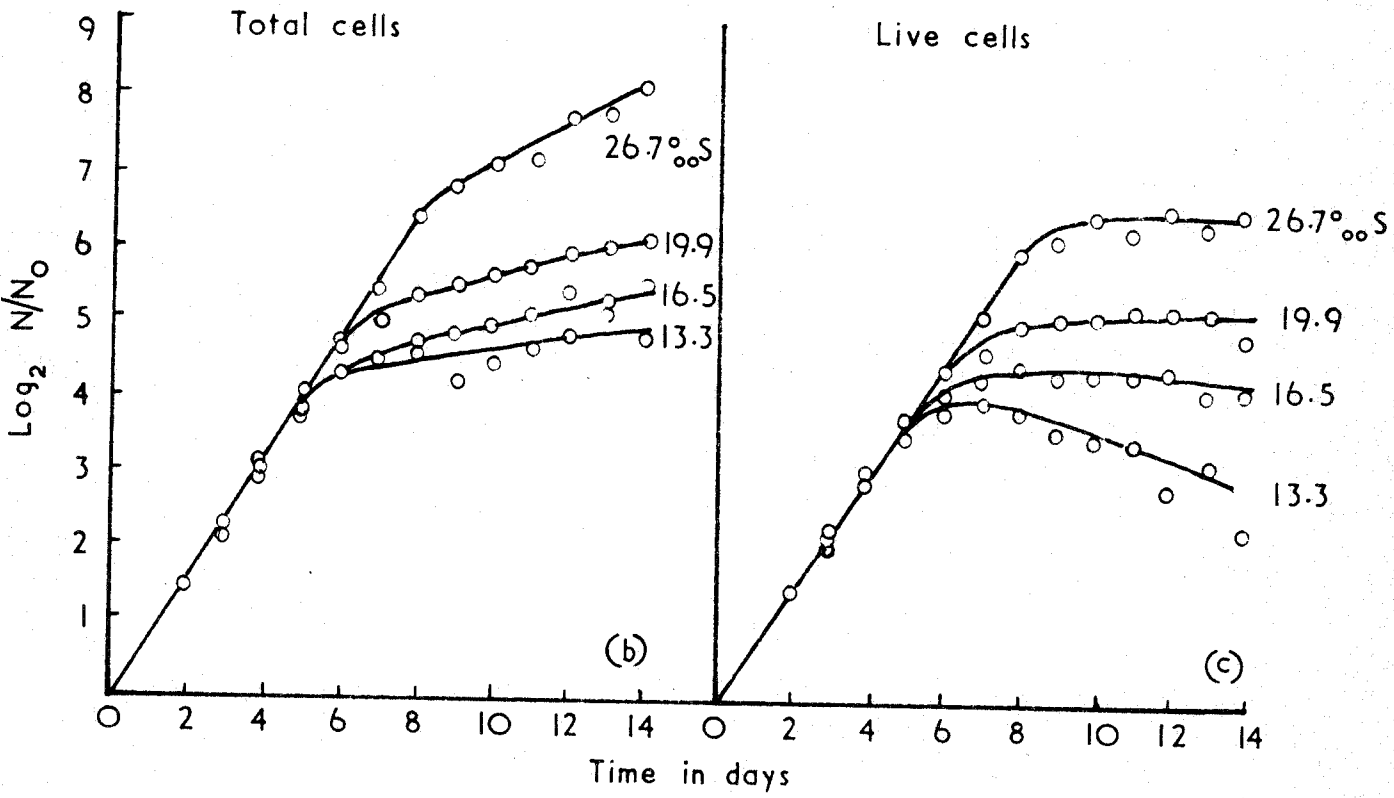

Total cells
Live cells
Log_2 N/N$_o$
26.7‰S
19.9
16.5
13.3
(b)
(c)
Time in days

Fig. 5

TABLE VII(b). Growth rate (mean generation time) at different salinities

Salinity (°/oo)	Mean generation time (hour)
10.1	28.0
13.3	15.8

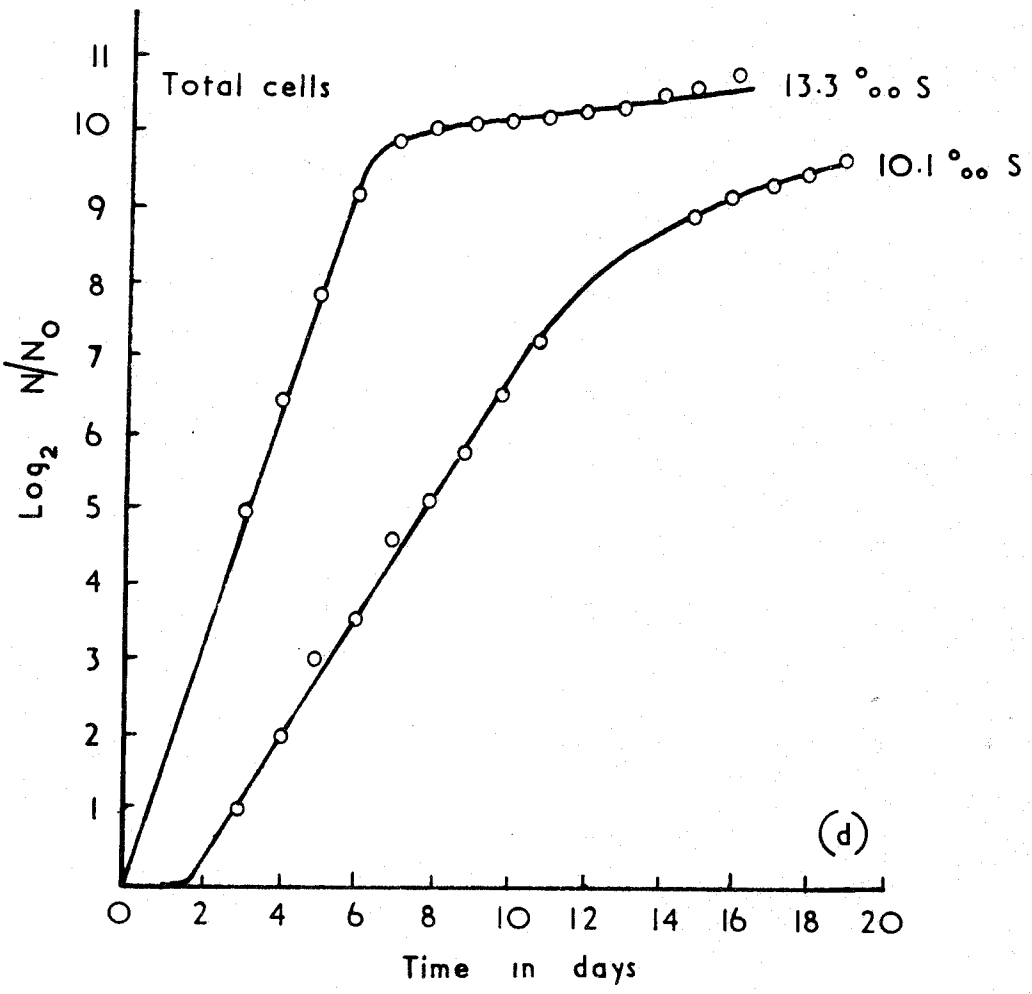

Total cells
13.3 °oo S
10.1 °oo S
(d)
Log_2 N/N$_0$
Time in days
0 2 4 6 8 10 12 14 16 18 20
1 2 3 4 5 6 7 8 9 10 11

Fig. 5

In a later investigation cells of the diameter range 73 to 91 μ were used at the salinities of 10.1 and 13.3°/oo. These cells also were the products of the vegetative multiplication of cells of the original clone. The growth rate of the cells at 13.3°/oo was slightly more than twice that of cells at 10.1°/oo S (Table VII(b)). Moreover at the latter salinity a lag phase of about two days was observed (Fig. 5(d)). In that time a few cells died and the rest became adapted.

In grouping the results of both investigations together it can be said that the growth rate of the diatom is similar over the salinity range of 13 to 33°/oo, and below the range it falls appreciably. More precise information requires that all the experiments be conducted simultaneously at the various salinities with the same size of cells. This was impracticable owing to an unavoidable limitation on the number of experiments that could be done at the same time.

2. Light Intensity

This investigation was carried out with cultures at 26.7 °/ooS and cells of diameter range 200 to 218 μ.

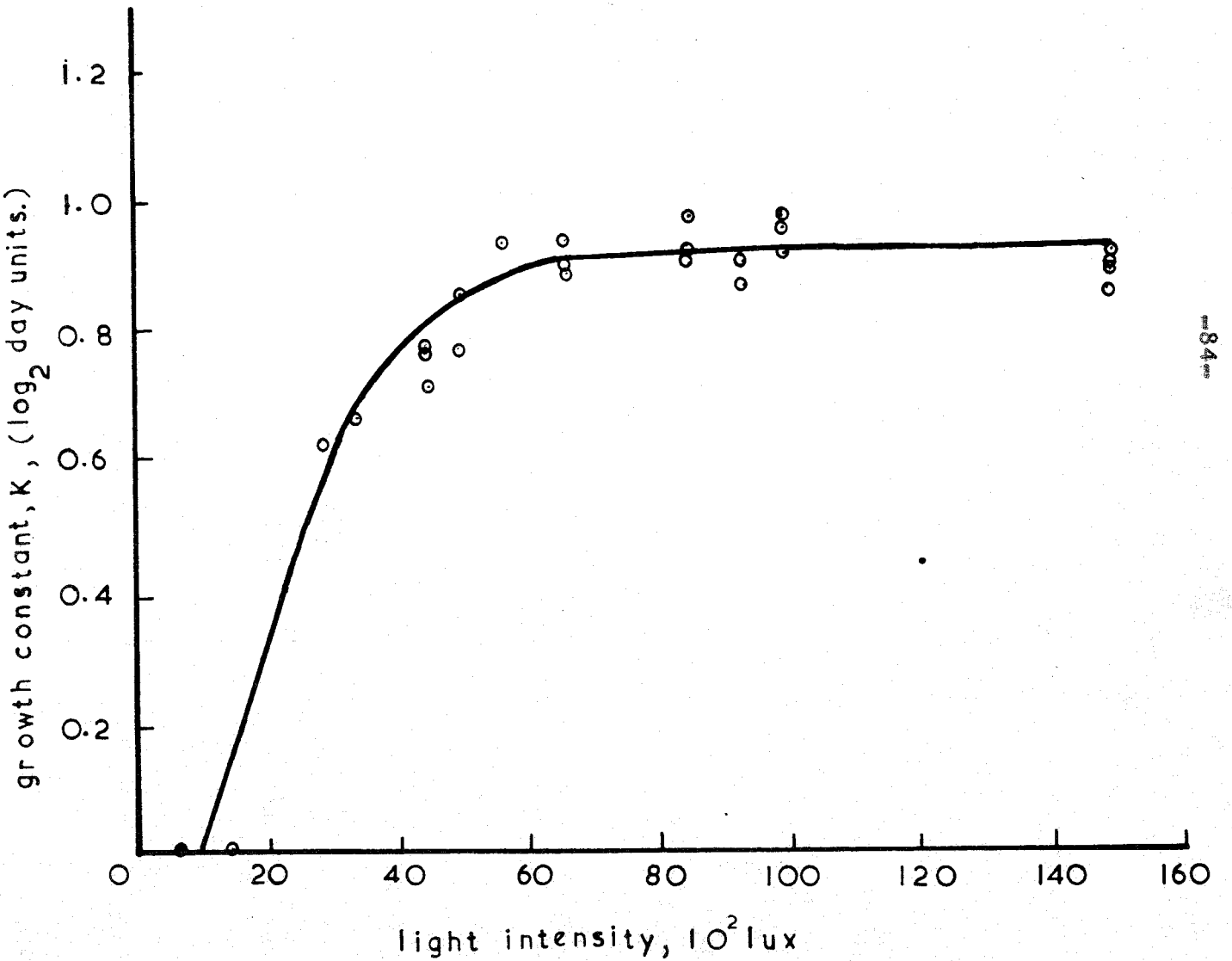
The relative growth constants for the various light intensities are presented in Table VIII. The relative growth constant for each light intensity was slightly variable, but it appears that there was saturation at about 6,000 lux and no inhibition at nearly 15,000 lux (Fig. 6). Each point on the growth curve is the average rate for two cultures.

TABLE VIII. Relation of light intensity to relative growth constant

Light intensity (lux)	Relative growth constant K, ($\log_2$ day units)
2,850	0.63
3,400	0.66
4,500	0.71, 0.76, 0.77
5,000	0.77, 0.85
5,700	0.93
6,600	0.88, 0.93
8,550	0.89, 0.98
9,400	0.86, 0.86, 0.90
10,000	0.95, 0.91, 0.97
15,000	0.86, 0.86, 0.91, 0.91, 0.86, 0.89, 0.89, 0.90

Fig. 6. Relative growth constant, K, ($\log_2$ day units) as a function of light intensity.

Fig. 6


1.2
1.0
0.8
0.6
0.4
0.2
0
20
40
60
80
100
120
140
160
growth constant, K, (log_2 day units.)
light intensity, 10^2 lux

3. Temperature

The growth rate was examined at the following temperatures - 15°, 20°, 25° and 28°C. Cultures at a salinity of 26.7 °/oo and containing cells of diameter range 182 to 200 μ were used.

The growth of the diatom at 15°C was erratic. The growth rates at the other three temperatures are given in Table IX. It is obvious that the growth of the diatom at 20°C is significantly lower than at the other two temperatures. The difference in growth rate of two hours at 25° and 28°C cannot be considered as significant since similar variation has been shown to occur within the illumination range used (5,500 to 8,000 lux) in the light intensity experiments.

While some stock cultures were in an air-conditioned room on stands (as described in the Materials and Methods) the air-conditioner blew up overnight. Consequently the air cooled cultures became heated up to a temperature of 33 to 35°C. They remained in this condition for about a day. On examination of the cultures it was found that the cells had died. It would seem therefore that a temperature of about 33°C inhibits the growth of the diatom.

TABLE IX. The growth rate at different temperatures

Temperature ($^{o}_{C}$)	Mean generation time (hour)
20	27.8
25	22.9
28	20.9

6. EFFECT OF SPORE FORMATION ON THE GROWTH OF THE DIATOM UNDER CONTINUOUS ILLUMINATION AND AT 28°C

It was suspected that spore formation may have an effect on the growth rate of the diatom. Consequently it was decided to investigate if there was such an effect.

This investigation was carried out at three salinities - 23.2 °/oo, 26.7 °/oo and 29.8°/oo. Two kinds of cells were used - (i) cells arising from the original clone isolated from the estuary having a diameter range of 200 to 218 μ; (ii) cells arising from the auxospores of the latter having diameter ranges of 291 to 309 μ and 182 to 200 μ. These sizes of cells lie within the sexual induction size range.

The initial percentage of spore formation and mean generation time are shown in Table X.

It was observed that male gamete formation usually began within 72 hours, and auxospore formation within 120 hours, after inoculation. Their induction usually took place during the exponential phase of growth.

Another investigation was made to determine the percentage of cells involved in male gamete and auxospore formation, as well as vegetative division, at two salinities - 23.2 °/oo and 26.7 °/oo - using cells in the diameter range 109 to 127 μ. The diameter range of the cells fall towards the lower limit of the sexual induction

TABLE X. The relation between spore formation and growth rate at different salinities and for different sizes of cells

	Salinity (°/oo)	Mean generation time (hour)	% Initial Spore formation	Diameter range (μ)
A.	26.7	27.3	15	200 - 218
	3 days later	26.0	10	
B.	29.8	18.2	5	
	26.7	21.5	10	291 - 309
	23.2	18.2	5	
C.	26.7	16.9	10	182 - 200
	23.2	15.9	7	

Fig. 7. Percentage spore formation and of vegetative cells at two salinities.

o—o—o total spore formation,

o--o--o male gamete formation,

o-·-o-·-o zygote formation.

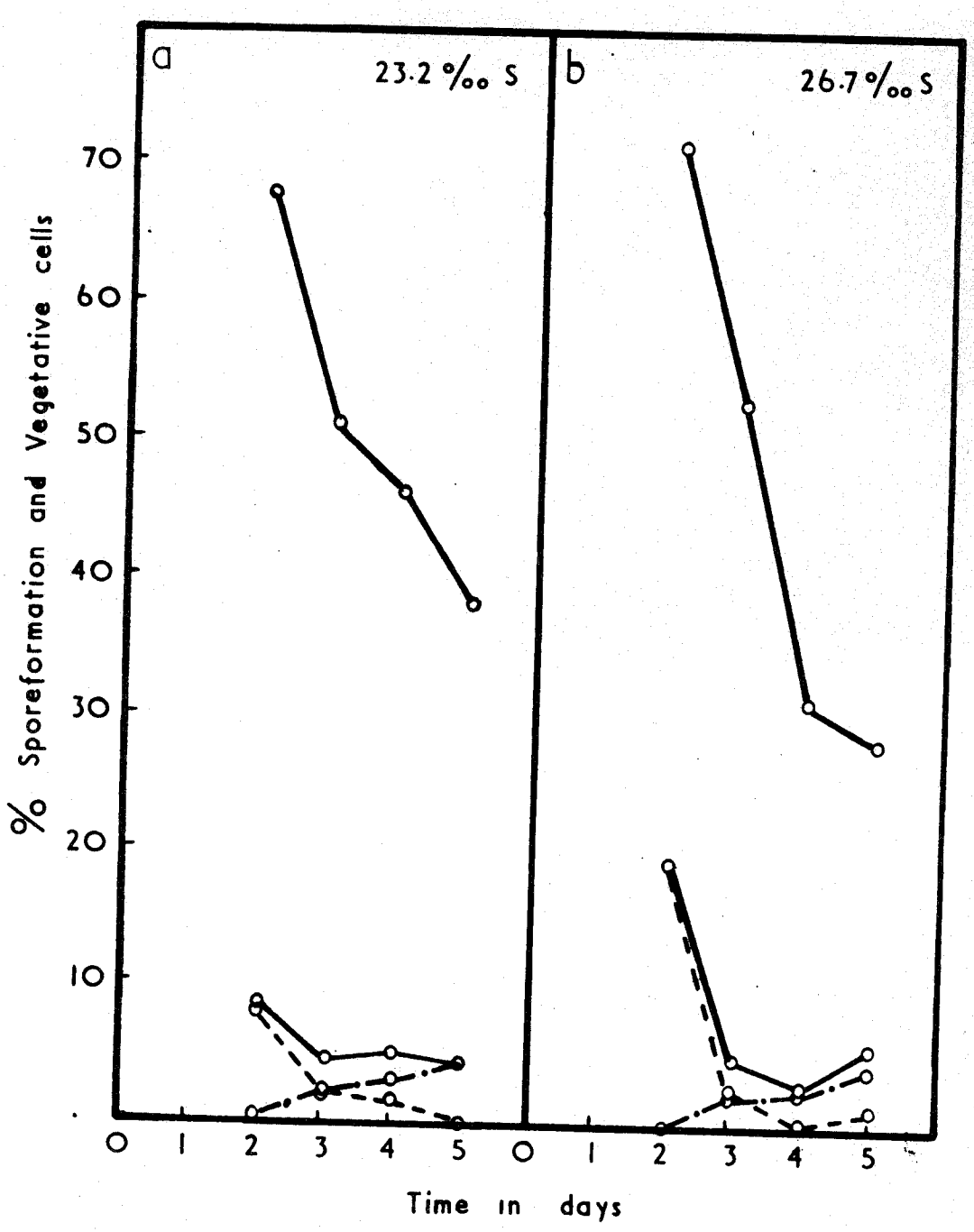

a
23.2 ‰ S
b
26.7 ‰ S
% Sporeformation and Vegetative cells
70
60
50
40
30
20
10
0
1
2
3
4
5
Time in days

Fig. 7

size range. The inoculum was large (about 100 cells). The observation was made during the period of exponential growth and the results are expressed graphically in Fig. 7 (a) and (b).

It was observed that although twice as many male gamete mother cells were produced initially at 26.7°/oo S than at 23.2°/oo S, about the same percentage of auxospores were formed. The initial induction of male gamete formation and consequently their release was a little earlier at 23.2°/oo S than at 26.7°/oo S. Early stages of developing auxospores were observed on the second day at the lower salinity. Male gamete formation took place throughout the exponential phase but the percentage of cells involved decreased with time, whereas developing auxospores were on the increase until the end of the exponential phase when their numbers declined rapidly. The percentage of "vegetative" cells dropped rapidly with the inception of male gamete formation and the drop was more pronounced when more cells were involved.

7. EFFECT OF EXTERNAL FACTORS ON SPORE FORMATION

Preliminary observations on the growth of the diatom indicated that salinity has an effect on spore formation. It was decided to investigate this factor further and also the effect of other factors such as temperature, light intensity and photoperiod. In the experiments on photoperiodic effect a 12 hour photoperiod was chosen as this is approximately the ecological photoperiod of the diatom in the estuary.

1. Salinity

The production of male gametes and auxospores was first examined in all the established cultures in the different salinities of the medium and then in subcultures prepared by transferring cells from one salinity into another. The cells used were those of diameters which lie within the sexual induction size range. The working temperature range was 24 to 28°C.

Spore formation only occurred within the salinity range of 19 to 33 °/oo. None occurred in the 16.5 °/oo salinity(S) and below. Relatively greater spore formation occurred in cultures of salinity 26.7 °/oo (Tables XI and XII). Usually within 72 to 96 hours some 10% of the cells would have started male gamete formation, depending on the diameter of the cells. This percentage then decreased with time, as the percentage of cells commencing auxospore formation increased.

TABLE XI. Male Gamete Formation

Transferred to (°/ooS)	Original Salinity (°/ooS)			
	32.9	26.7	19.9	16.5
32.9	X	+	+[1]	-
29.8	++	+	+	+
26.7	+++	X	++	++
23.2	++	++	+	+
19.9	+	+	X	+
16.5	-	-	-	X

[1] Did not proceed further than spermatogonia stage

+++ 10 - 15%

++ 5 - 10%

+ less than 5%

- no male gamete or auxospore formation

X no transfer made

TABLE XII. Auxospore Formation

Transferred to (°/ooS)	Original Salinity (°/ooS)			
	32.9	26.7	19.9	16.5
32.9	X	-	-	-
29.8	++	+	+	+
26.7	+++	X	++	++
23.2	++	++	+	+
19.9	+	+	X	+
16.5	-	-	-	X

+++ 10 - 15%

++ 5 - 10%

\+ less than 5%

\- no male gamete or auxospore formation

X no transfer made

The results obtained in subculturing experiments in which the cells were transferred from one salinity level to another are summarized in Tables XI and XII. The diameter range of the cells used was 255 to 273 μ.

From the results it can be seen that there was no inhibition to spore formation in subculturing cells from a higher to a lower salinity level except at the 16.5 °/ooS level, where spore formation did not occur normally. Some inhibition was noted on transferring cells from a lower to a higher salinity level especially at the 32.9 °/ooS level. At the 32.9 °/ooS level spermatogenesis usually took place, but very few male gamete mother cells were produced. Whether the male gametes were released or not, no auxospore formation was observed. It therefore appears that spore formation occurs within a given salinity range and is not induced by mere dilution.

Cells showing various stages of male gamete and auxospore formation were transferred to fresh medium of 16.5 °/ooS. Spermatogenesis usually proceeded until the male gametes were released and auxospore development was completed. When some cells, which included various stages of male gamete formation, were transferred from cultures actively producing male gametes and auxospores, into fresh medium of 16.5 °/ooS, auxospores were produced. These germinated to give cells with diameters ranging

from 400 to 450 μ. It would seem, therefore, that when gametogenesis had begun the production of male gametes and auxospores was not affected by changing salinity.

2. Temperature

Spore formation in cells of diameter range 291 - 309 μ was investigated at five temperatures and two salinities. The temperatures were 15°, 20°, 25°, 28° and 29°C; the salinities - 23.2 °/oo and 26.7 °/oo. The cells were from those arising from the auxospore of the original clone. The size of cells and the salinities lie within the sexual induction size and salinity ranges.

The results are shown in Fig. 8 (a - d) and Table XIII. At 15°C the growth of the diatom was erratic and no spore formation occurred.

Spore formation was more pronounced in cultures at 26.7°/oo S (Salinity) than in those at 23.2°/oo S. At the 26.7°/oo S it was slightly better at 25° than at 28°C (Table XIII). No spore formation occurred either at 20° or 29°C. At 23.2°/oo S developing auxospores appeared on the fifth day after inoculation at 25° and 28°C. At 26.7°/oo S they appeared on the fourth and fifth day at the two temperatures respectively.

Cultures of 23.2°/oo S grew at the same rate in temperatures of 25°, 28° and 29°C even though spore formation occurred at 25° and 28°C. In those of 26.7°/oo S the growth rate was about the same at 25° and 28°C, the temperatures at which spore formation occurred, but slower than at 29°C. It is apparent that the slower growth rate is a result of the pronounced spore formation at this salinity. The growth rate was the same in both salinities at

TABLE XIII. Effect of temperature on the growth rate and spore formation at two different salinities

Salinity (°/oo)	Temperature (°C)	Mean generation time (hour)	Initial Spore formation (%)
23.2	20	22.5	Nil
	25	17.8	5
	28	18.2	5
	29	18.0	Nil
26.7	20	23.4	Nil
	25	20.4	12
	28	21.5	10
	29	18.3	Nil

Fig. 8. Growth curves at two salinities and at different temperatures.

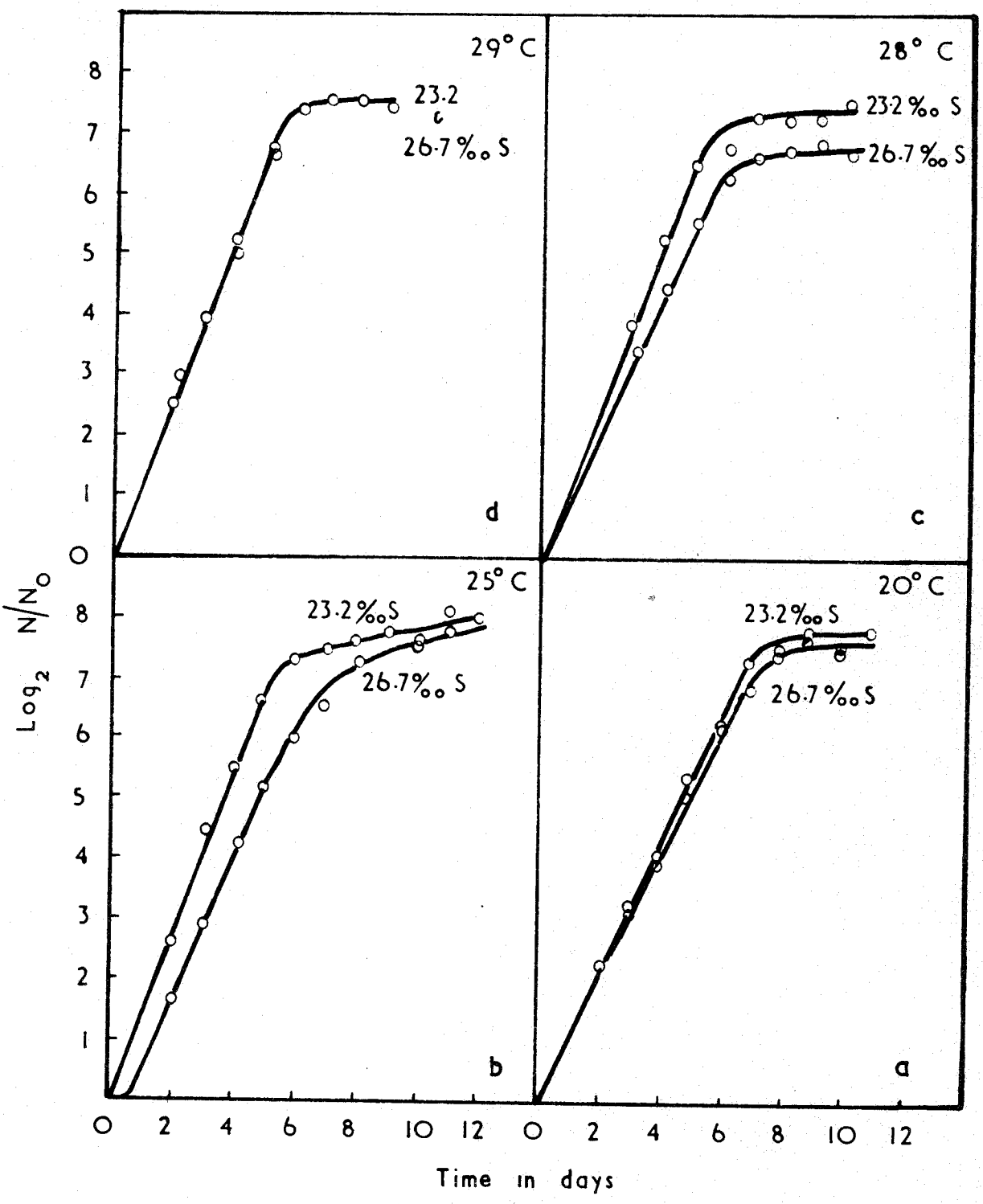

29° C
23.2 ‰ S
26.7 ‰ S
d
28° C
23.2 ‰ S
26.7 ‰ S
c
25° C
23.2 ‰ S
26.7 ‰ S
b
20° C
23.2 ‰ S
26.7 ‰ S
a
Log2 N/No
Time in days
0 2 4 6 8 10 12
1 2 3 4 5 6 7 8

Fig. 8

20° and 29°C respectively.

It appears that the temperatures, 25° and 28°C, lie within the optimum range for spore formation in the diatom. Also the maximum temperature for spore formation seems to be 28°C as it did not occur at 29°C. Thus a difference of one centigrade degree above the maximum temperature was enough to inhibit spore formation.

3. Alternating Light/Dark Cycle and Continuous Illumination

The salinity level of 26.7°/oo was chosen because it had been found that cultures of this salinity at 28°C produced more spores than those within the sexual induction salinity range. Cells (originating from auxospores of the original clone) of diameter range 255 - 273 µ were used. The mother culture, which provided the inocula for the cultures, was obtained from subcultures which had received the light/dark treatment for over a fortnight.

The alternating light/dark cycle was a twelve hour light period followed by a twelve hour dark period. Two series of experiments were carried out at this light treatment. One of them was sampled every twenty four hours and the other every twelve hours. The latter samples were taken in order to investigate the effect of the individual light and dark periods. A control experiment was carried out simultaneously under continuous light and sampled every twenty four hours.

The results are shown in Fig. 9 (a - c) and the mean generation time and percentage initial spore formation in Table XV.

It was found that many more male gametes and auxospores were produced under the light/dark cycle treatment than under continuous light. During the exponential phase of growth, the mean generation time under the light/dark cycle treatment was slower by about four hours than that under continuous light (Table XV). Developing

TABLE XV. Comparison of the influence of two light treatments on male gamete and auxospore formation at 28°C

Light treatment	Mean generation time (hour)	% initial Spore formation
Continuous light	12.8	10
Alternating light/ dark cycle	16.7	20

Fig. 9. Growth curves of the diatom in

a. continuous light sampled every 24 hours,

b. alternating light/dark cycle sampled every 24 hours

c. alternating light/dark cycle sampled every 12 hours

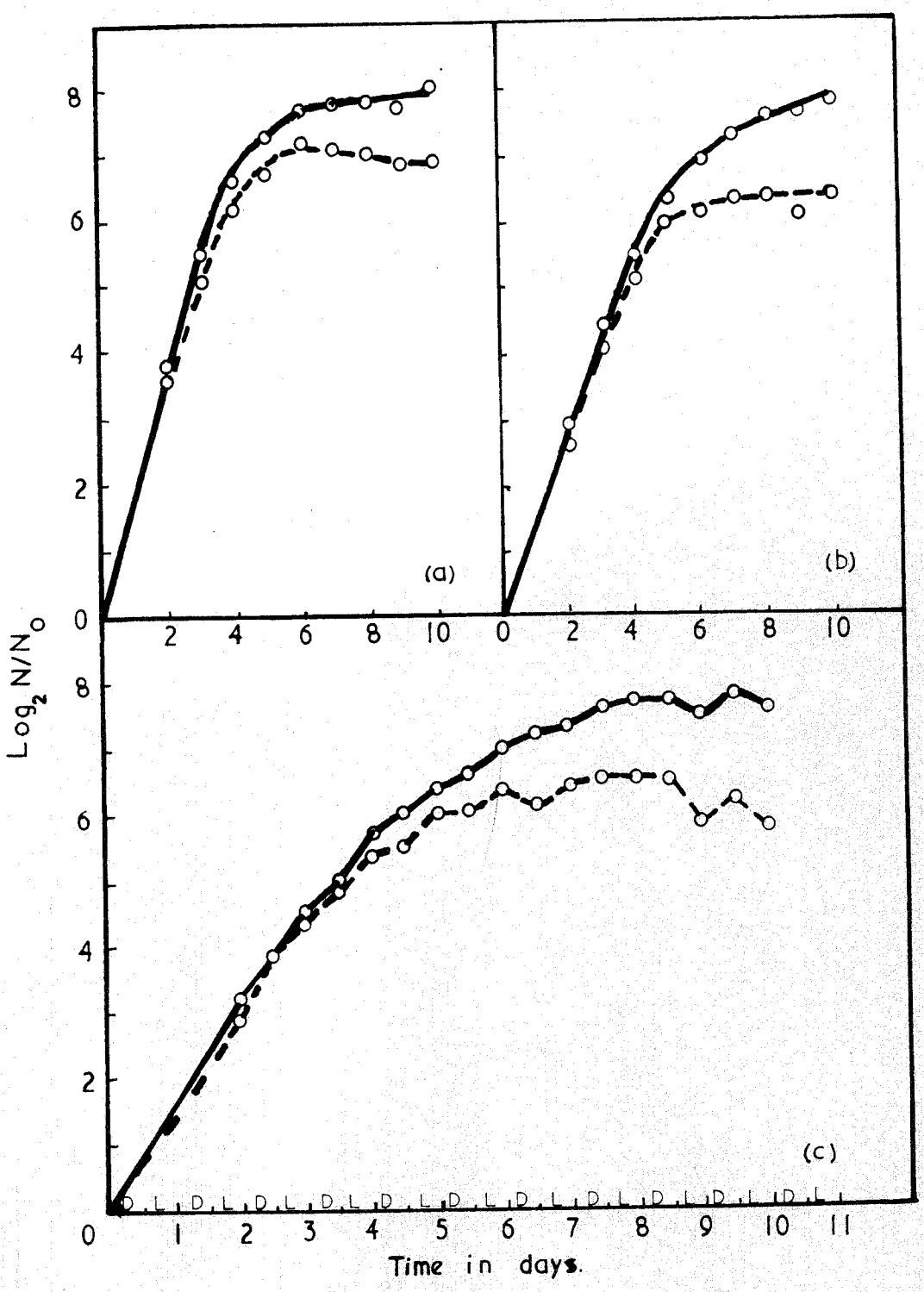

8
6
4
2
0
(a)
(b)
2
4
6
8
10
$Log_2 N/N_o$
(c)
D L D L D L D L D L D L D L D L D L D L D L
0
1
2
3
4
5
6
7
8
9
10
11
Time in days.

Fig. 9

auxospores appeared earlier under continuous light than in the alternating light/dark cycle treatment (Fig. 9a, b).

For the first five days during the exponential phase of growth the number of cells in the cultures under continuous light was about twice that under the light/dark cycle. Thereafter the number of cells in both sets of cultures approached each other. Thus the light treatments had different effects on the diatom during the exponential phase of growth, though their subsequent effects on growth were the same. The 12 hour photoperiod was more conducive to spore formation than the continuous light treatment.

When sampling was done every twelve hours (Fig. 9c), it was observed that during the first four days of growth under the light/dark cycle, the normal division rate was promoted by both the light and dark periods. Thereafter the dark periods produced a decrease in the number of live cells, until the onset of the stationary phase.

4. High Light Intensity

It has been shown that when cultures were subjected to an alternating light/dark cycle of twelve hours light and twelve hours darkness, more spores were produced than under continuous light. Consequently it was decided to determine the effect on spore formation when the light intensity was doubled (i.e. 11,000 - 12,000 lux). The experiment was carried out at the salinity of 26.7°/oo and cells of the diameter range 255 - 273 μ were again used. The results are set out in Table XVI.

The mean generation time was the same for the continuous low light and alternating high light treatments, and no spore formation took place under the alternating high light treatment. The difference in mean generation time between the alternating low light and alternating high light treatments was about four hours. The same difference in mean generation time was observed between the continuous low light and alternating high light treatments. Although the same amount of light energy was received by the cells in the two latter treatments, and the growth rate was the same, yet no spore formation occurred. Therefore the inhibition of spore formation in the alternating high light treatment was a direct effect of the duration of illumination. When about half the high light intensity was used in the same period of time, the growth rate was slower and spore formation promoted. Therefore it is suggested that the high light intensity, whilst promoting a growth

TABLE XVI. Comparison of the effect of three light treatments on growth rate and spore formation

Light treatment	Mean generation time (hour)	Spore formation
Continuous low light	12.8	Yes
Alternating low light/ dark cycle	16.7	Yes
Alternating high light/ dark cycle	13.2	No

rate in which spore formation was possible, also inactivated those metabolic processes which would lead to spore formation.

8. THE EFFECT OF VITAMINS

1. Growth

In the course of the investigations the author ran out of biotin and a biotin-free medium was prepared and used. Good growth of the diatom was obtained as in the case of the complete medium. This indicated that biotin was not required by the diatom for growth. In general diatoms have seldom been shown to require biotin for growth. Consequently the result was not surprising. In spite of this, it was decided to investigate whether the same result would be obtained by the presence or absence of one or some of the vitamin component of the complete medium.

It was realized that since the cultures of the diatom were not necessarily bacteria free the results would be difficult to interpret. But since bacterial growth was not appreciable during the exponential growth of the diatom it was felt that any observed effects can be interpreted as a direct action on the diatom.

Since vitamins are only required in small quantities the inoculum was prepared such that there was a minimum of carry-over of the vitamins.

The cells used in this investigation were taken from cultures in the complete medium of salinity 26.7°/oo. They were washed five times in sterile plain medium of the same salinity before inoculating into the six variants of the complete medium (see Materials and Methods) which were also of the same salinity. Six

TABLE XVII. Successive subculture experiments in variants of the complete medium

(-, no growth; +?, doubtful growth; + to ++, slight to moderate growth; +++, abundant growth)

SUBCULTURE NO.	PLAIN	CM - B_{12} - BIOTIN	PLAIN + THIAMINE	PLAIN + BIOTIN	PLAIN + B_{12}	CM - BIOTIN	CM
1	++	++	++	++	+++	+++	+++
2	+	+	+	+	+++	+++	+++
3	+	+	+	+	+++	+++	+++
4	+?	+?	+?	+?	+++	+++	+++
5	+?	+?	+?	+?	+++	+++	+++
6	-	-	-	-	+++	+++	+++

TABLE XVIII. The growth rate in three vitamin media

Medium	Mean generation time (hour)
PLAIN + B_{12}	27.4
CM - BIOTIN	20.2
CM	22.3

successive subcultures were made in each case under continuous light (5,500 - 8,000 lux) and in a temperature range of 24 - 28°C. The diameter range of the cells was 327 - 345 μ. The growth rate of the diatom in those variations of the complete medium which supported abundant growth was subsequently determined.

The results indicated that the diatom required vitamin B_{12} for growth but not biotin or thiamine. In the media where growth was inhibited the cells were pale, and with increasing inhibition to vegetative growth they became markedly elongated (the length of the pervalvar axis was more than twice the value in a recently divided cell) and failed to divide.

The biotin free medium produced the best growth rate. However, the growth of the diatom in the CM medium was better than in the PLAIN + B_{12} medium.

The results are outlined in Tables XVII and XVIII.

2. Spore formation

It was shown in the last section that the vitamin media CM, CM - BIOTIN and PLAIN + B_{12} favoured the growth of the diatom. As a result it was decided to determine the influence of these media on spore formation. Cells, of diameter range 255 - 273 μ, were obtained from cultures of 16.5°/oo S (a salinity in which spore formation does not occur), washed several times and inoculated into the three media at 26.7°/oo S. The percentage of each type of spore was determined during the exponential phase of growth and shown in Table XIX and Fig. 10 (A - C). The inoculum of 1 ml. contained about 100 cells.

Spore formation was earlier in the CM - BIOTIN medium and better than in the other two vitamin media. With the decline in the percentage of male gamete formation the percentage of auxospore formation increased. The latter observation indicated a definite relationship between the two kinds of activity. Biotin seemed to influence spore formation adversely. Vitamin B_{12} was necessary and it appears that the other constituents of the vitamin mixture had some influence on spore formation.

TABLE XIX. Percentage spore formation in three vitamin media.

Type of Spore formation	Type of Medium	% Spore formation per day			
		2nd	3rd	4th	5th
Male Gamete	PLAIN + B_{12}	22.5	2.6	0.8	0.4
	CM	17.4	2.6	-	0.7
	CM - BIOTIN	17.2	2.8	-	3.0
Zygote	PLAIN + B_{12}	-	-	0.3	2.8
	CM	-	-	0.9	2.3
	CM - BIOTIN	-	6.5	6.4	10.2

Fig. 10. Percentage spore formation in three vitamin media

o——o——o male gamete formation,

o---o---o zygote formation.

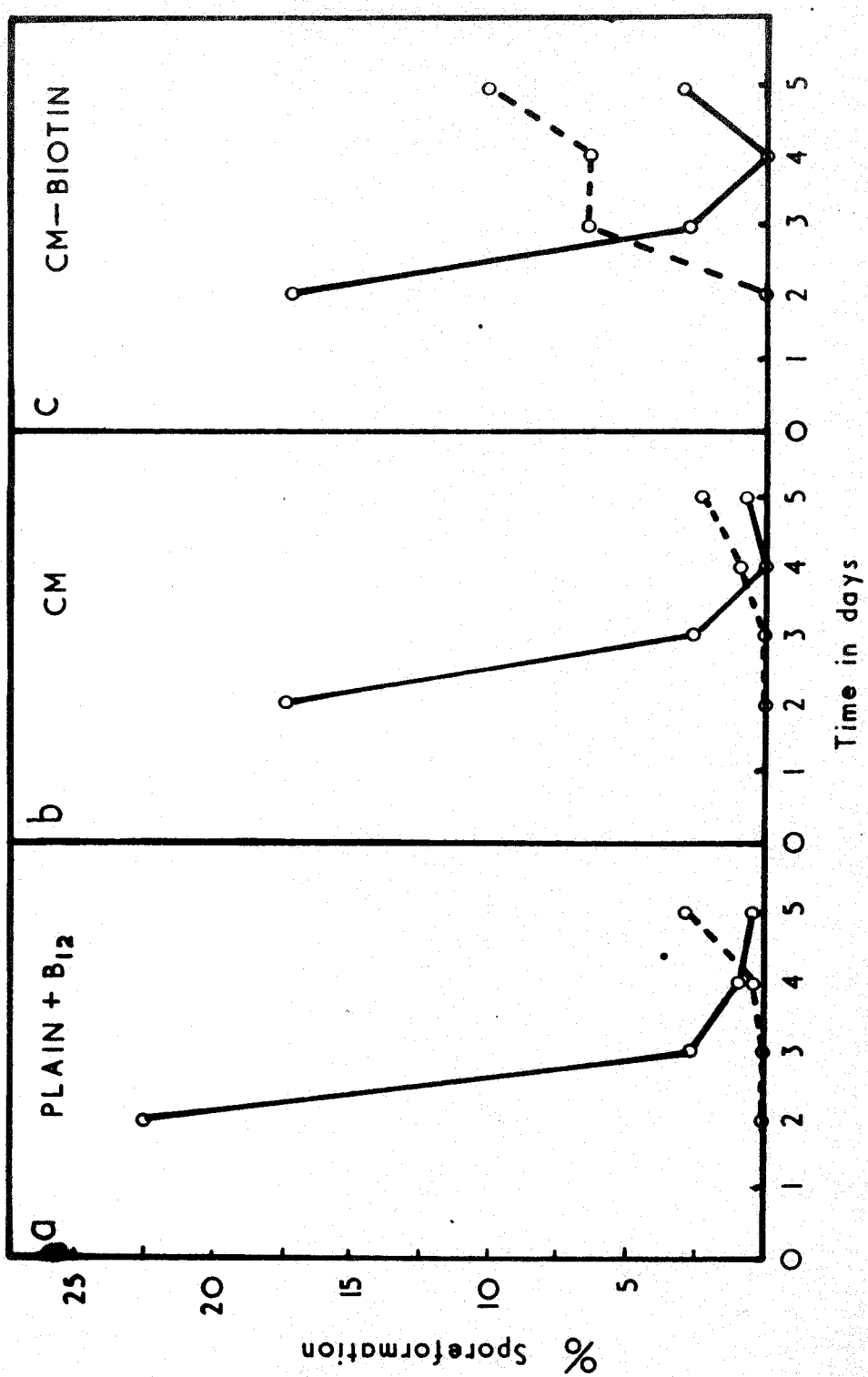

a
PLAIN + B_{12}
b
CM
c
CM—BIOTIN
% Sporeformation
25
20
15
10
5
0
1
2
3
4
5
Time in days

Fig. 10

6. OBSERVATIONS ON THE LIVING MATERIAL

THE METHODS OF SPORE FORMATION IN THE LIVING CELLS OF THE DIATOM UNDER CONTINUOUS ILLUMINATION

Whilst spore formation was occurring in cells within the sexual induction size range, the methods of formation were investigated. Accounts of the methods for male gamete and auxospore formation and development are given below. Photomicrographs of the sequence of the events as well as drawings made from the photomicrographs are used as illustrations of the text.

The method of male gamete formation, as related below, was followed in one cell. However, the sequence of events is essentially the same in other cells in which it was observed. The account of auxospore formation is based on a number of observations made on many cells undergoing this type of spore formation.

1. Male Gamete Formation

It was not possible to determine exactly the initiation of male gamete formation as the cells usually rest on the flat surface of their frustules and the first division often took place parallel to the flat surface. However, occasionally the first division of the nucleus can be observed in valve view (Plate VII). The commencement of the second division could be recognised by the slight shrinking away of the protoplasts of the products of the first division from the frustule wall, the presence of two nuclei, and the first signs of constriction of the protoplasts.

Legend to Plates VII - XVIII and Fig. 11

Plate VII - Two daughter nuclei in undivided protoplasm, x 1,050.

Plate VIII - IX and Fig. 11 (a - d) - 2nd division of protoplasm

Plate X and Fig. 11e - Completion of 3rd division, x 875.

Plate XI and Fig. 11f - 4th division in progress, x 875.

Plate XII and Fig. 11g - 5th division in progress, x 910.

Plate XIII and Fig. 11h - 6th division in progress, x 910.

Plate XIV - 6th division nearing completion and commencement of 7th division, x 910.

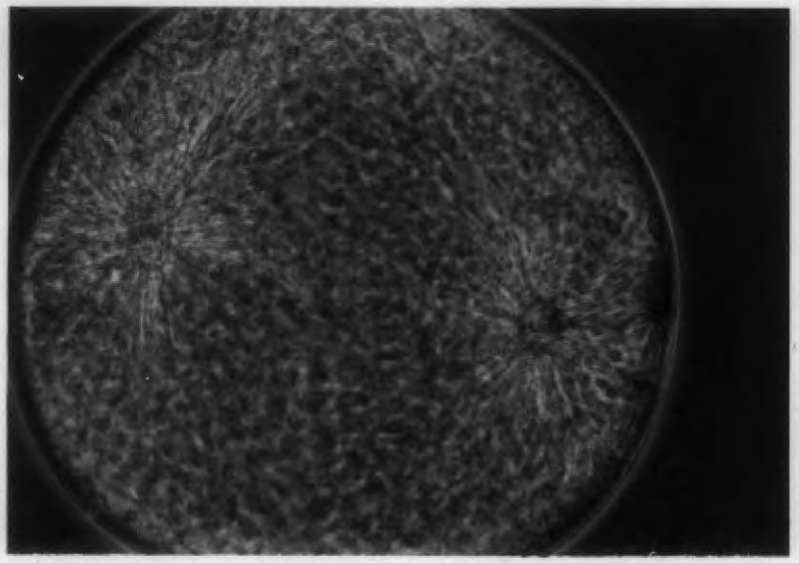
Plate XV and Fig. 11i - Completion of 7th division and formation of Spermatogonia, x 910.

Plate XVI - XVII - Production of actively swimming male gametes from spermatogonia, x 800

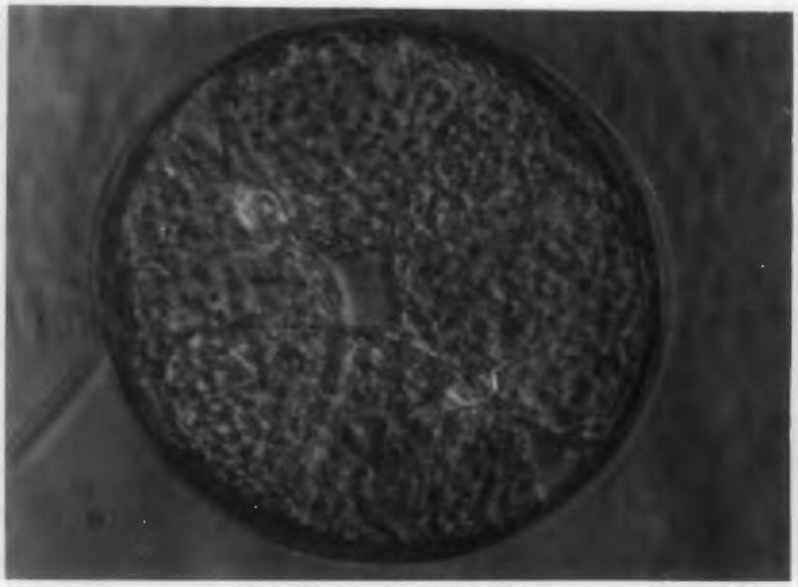
Plate XVIII a and b - Liberation of male gametes, a - x 1,575.
b - x 560

Plate IX a, b - x 875

Plate IX c - x 910



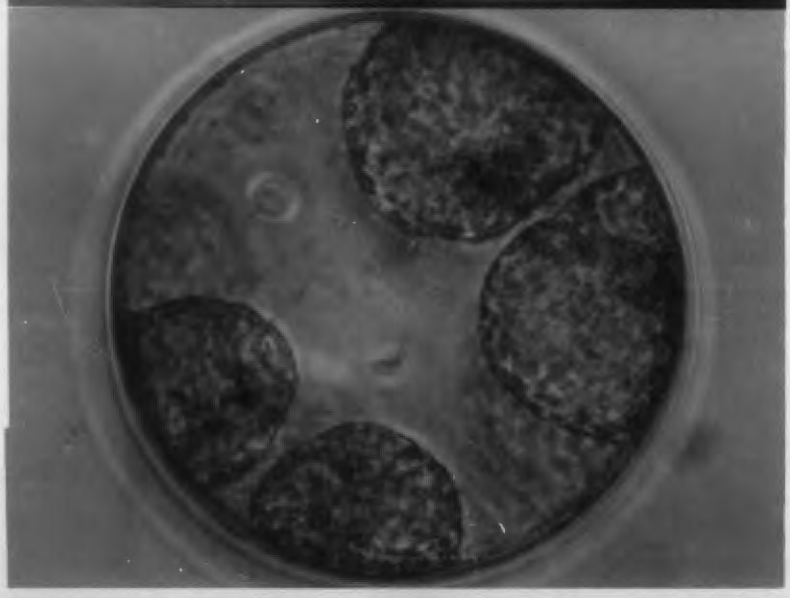
VII



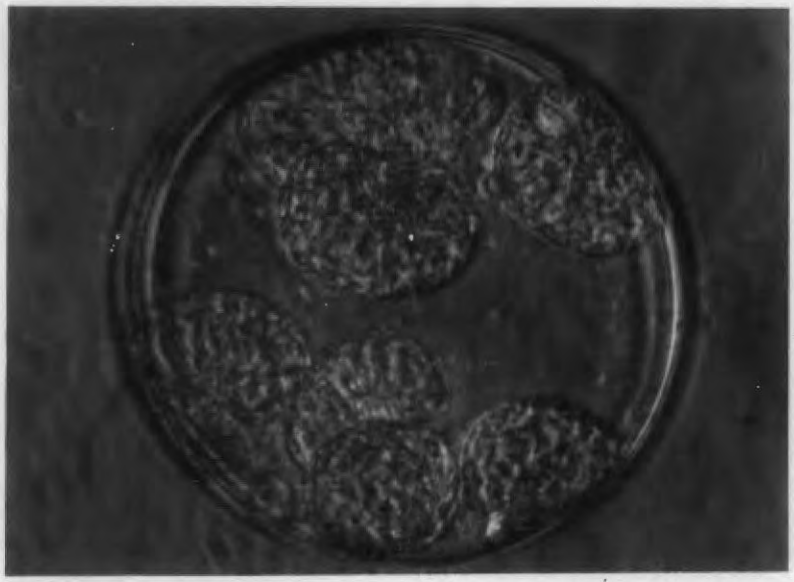
VIII

a

b IX

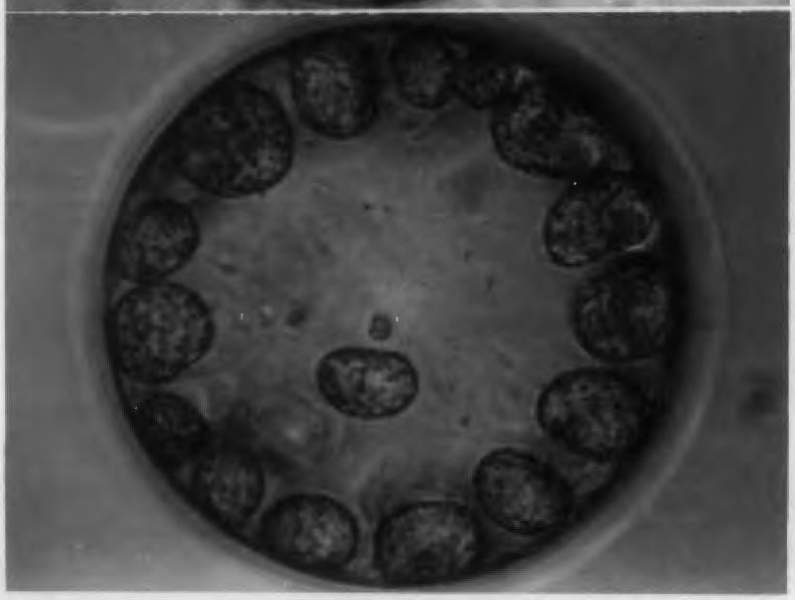


c



X

XI



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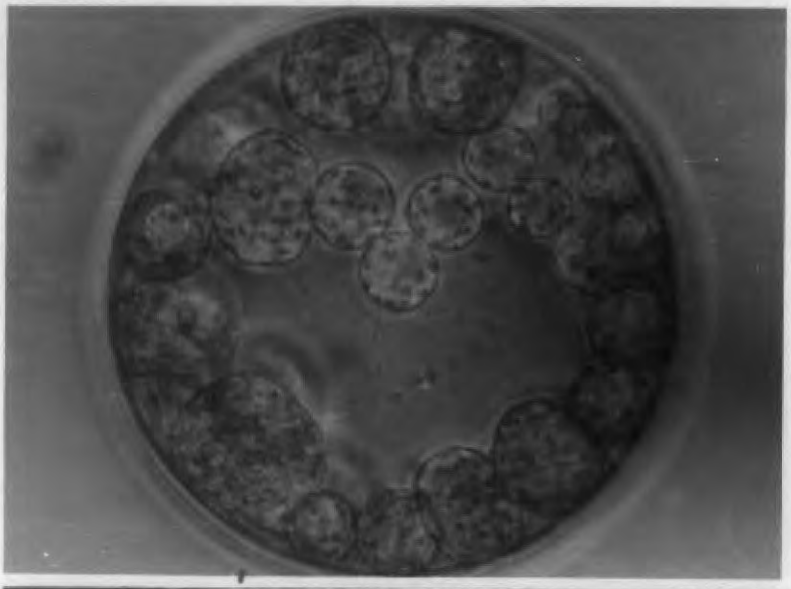

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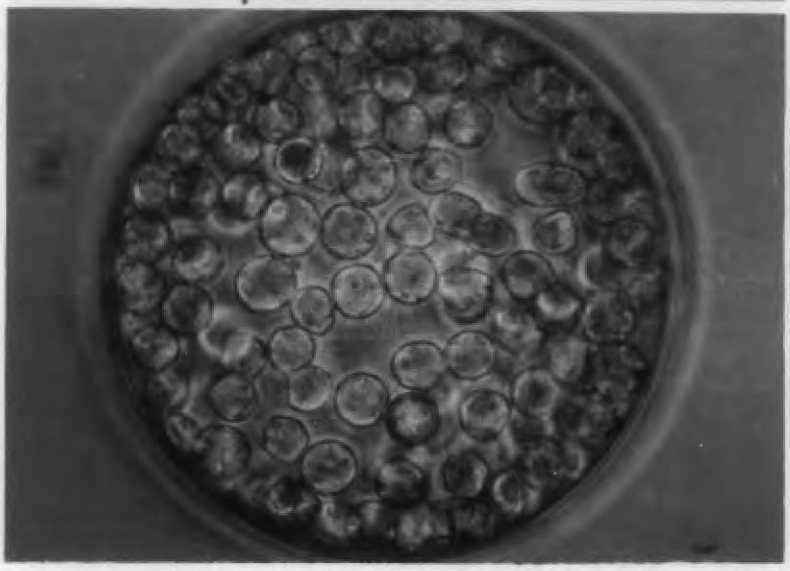

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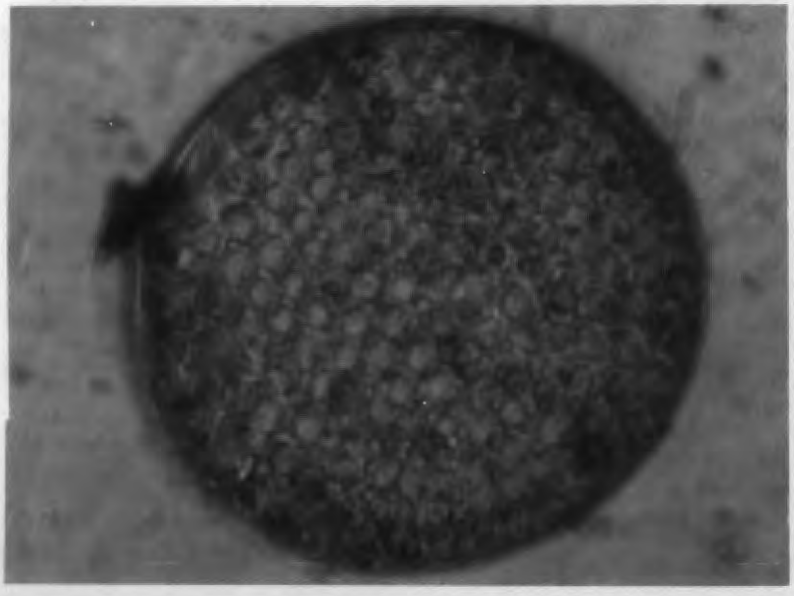
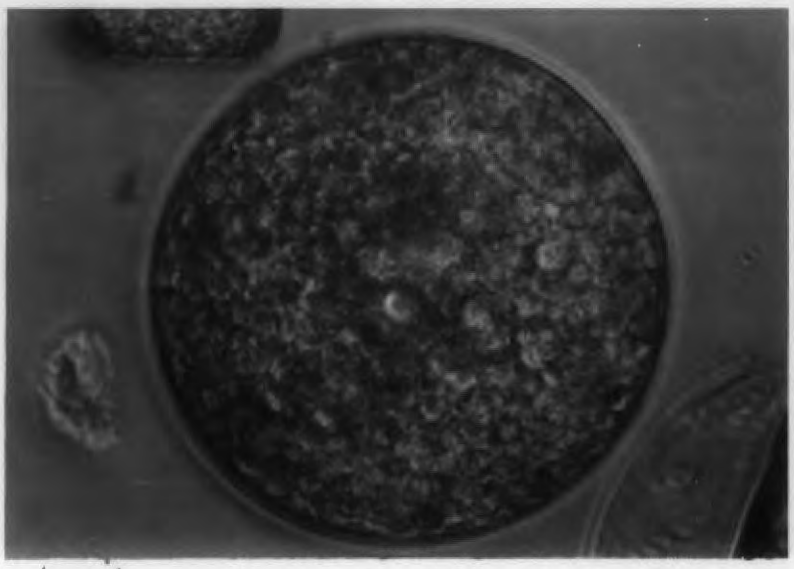
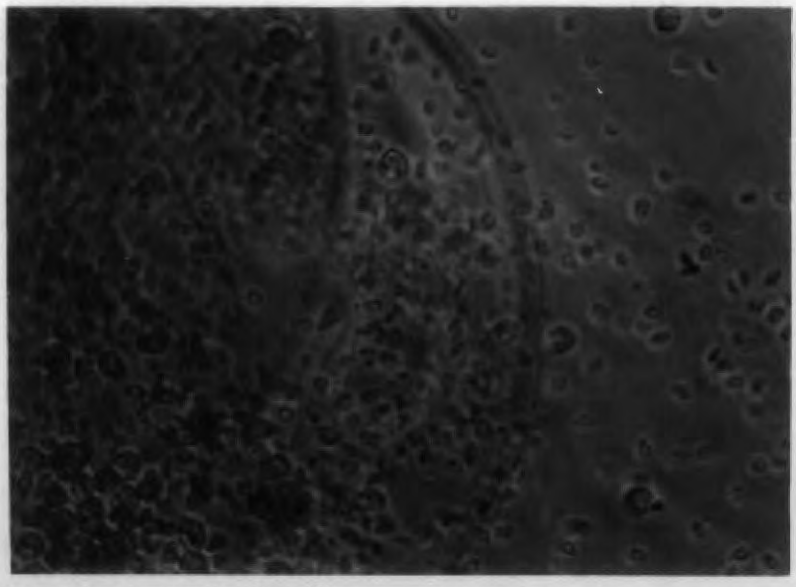


XV

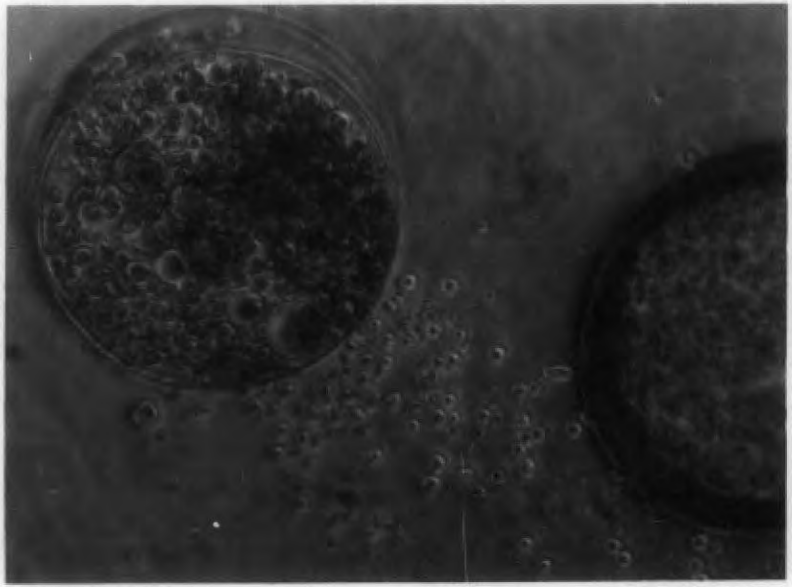


XVI

XVII



XVIIIa



XVIII b

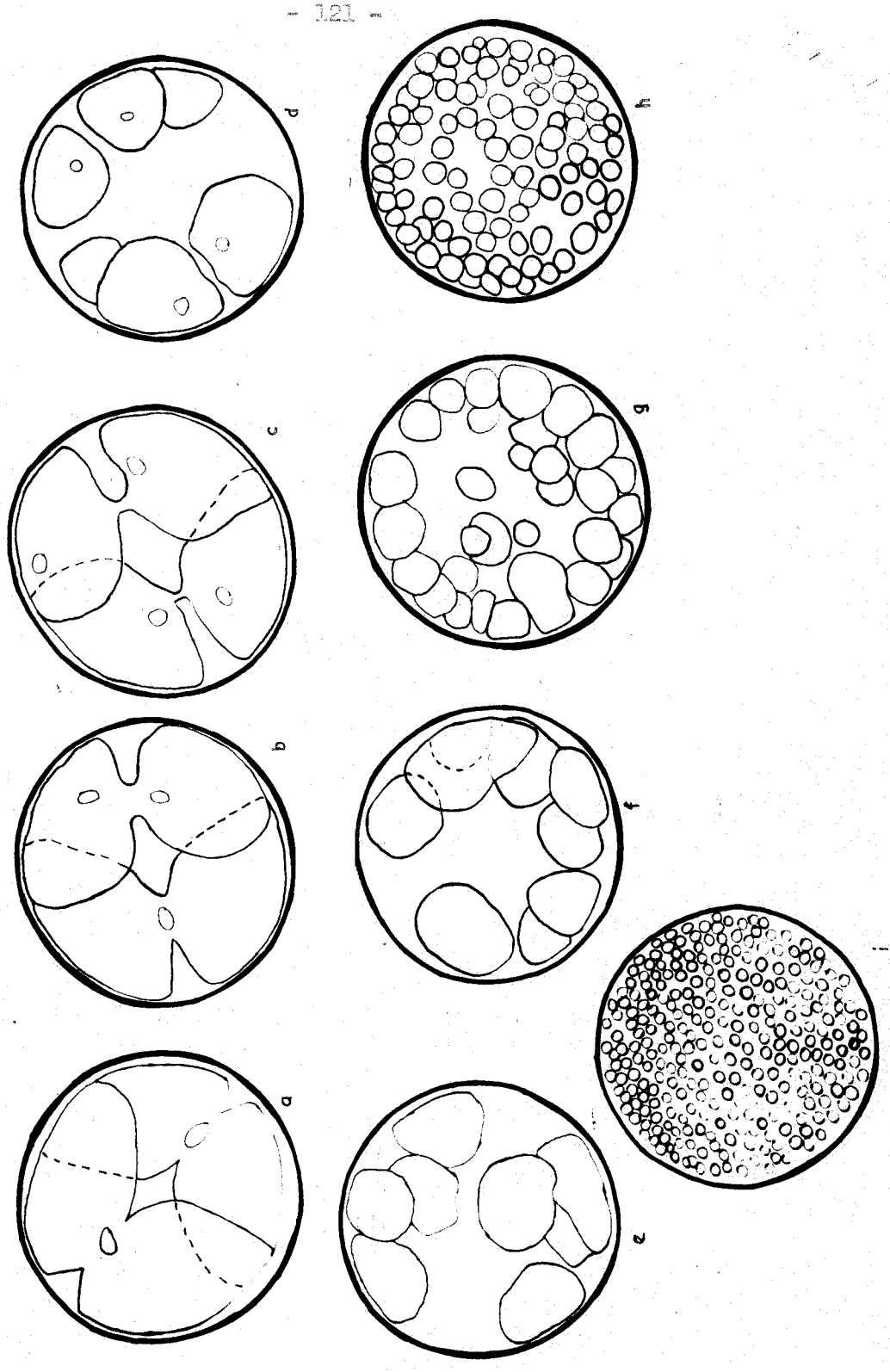


Fig. II

The account of male gamete formation as seen in one cell now follows. At 10.30 a.m. on 10.7.65 it was observed that the protoplast of a cell had divided once, parallel to the flat surfaces of its frustule and that each of the resulting division products were undergoing further division (Plate VIII). This second division took about an hour and a half to be completed (Plate IX a to c and Fig. 11 a to d). After a further two hours it was observed that a third division had been completed and a fourth division was in progress (Plate X and Fig. 11e). After this observation the cell was transferred from the original medium into fresh medium in a watch glass by means of a mircopipette and its course of development followed. At 2.50 p.m. the fourth division was still in progress (Plate XI and Fig. 11f) and it was completed in about an hour and a half. By 3.50 p.m. the fifth division had commenced (Plate XII and Fig. 11g). By 4.50 p.m. the diatom cell was almost filled with non-flagellated rounded protoplasts and this was believed to be a division phase towards the sixty-four "celled" stage (Plate XIII and Fig. 11h). By 5.50 p.m. the diatom cell was entirely filled (Plate XIV), and a further division had begun. By 7.00 p.m. the 128 "celled" stage had been completed (Plate XV and Fig. 11i); this being adjudged by the reduced size of the rounded protoplasts. There followed a period of about three to four hours when no further division was observed. Suddenly

at about 10.30 p.m. there was much activity. The presumptive spermatogonia were in constant motion and division was taking place producing uniflagellated bodies (male gametes) which were seen moving amongst the spermatogonia (Plates XVI and XVII). By 11.00 p.m. the valves of the diatom frustule were slightly pushed apart and the motile male gametes were liberated in large numbers into the medium (Plate XVII). During the liberation of these gametes it was observed that not all of the rounded protoplasmic bodies had divided (Plate XVIII). Many of them of various sizes never escaped from the diatom cell except when the valves were completely freed from each other. The male gametes were apparently not viable for more than a day after liberation. The rounded protoplasts usually possessed many chromatophores and the male gametes only a few.

Each motile male gamete possessed a single long flagellum; they were pear-shaped and about 8 μ long. Two kinds were observed; one had a "sheath" arising from the rather rounded anterior end enveloping about a quarter of the length of the flagellum; the other type had no such "sheath" (Fig. 12). The proportions of each kind could not be determined owing to their small sizes, large number and were also actively swimming within the cell. The formation and liberation of the male gametes probably took place in twelve hours. No fusion between two male gametes has been observed after their liberation from the mother cell.

Fig. 12 A. & B. Two kinds of male gametes; B. male gamete with "sheath" towards anterior end of gamete body; x 125, 000.

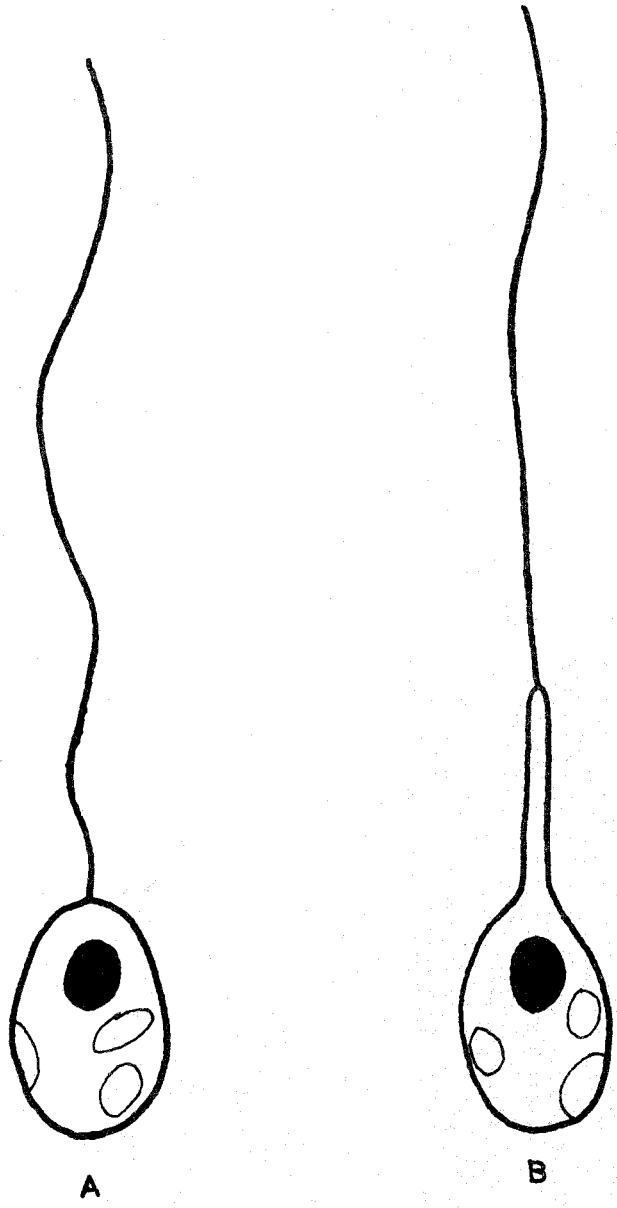


Fig. 12

Male gamete formation did not seem to take place at any particular time of day. Usually various stages of their formation could be seen at the same time in the same culture. Male gamete mother cells were morphologically similar to the vegetative cells and spermatogenesis occurred entirely within the mother cell, without the appearance of siliceous or membraneous partitions within it. Although in this observation some spermatogonia fail to undergo meiosis, it is presumed that the number of gametes produced per cell is 4 x 128.

From previous observations of cells extruding their protoplasm, which had been transferred into fresh medium, it had been noted that the protoplasm usually shrank into the frustule before gradual extrusion was recommenced. This is thought to be simply an example of the common osmotic effect which occurs when plant cells are transferred to fresh solutions isotonic with the original old solution. This behaviour must have slowed down the process during the fourth division stage as the cell contents were becoming adjusted to the new conditions. The loss in time during this period of adjustment was confirmed by observations on other cells which were not removed from the original medium after the initiation of male gamete formation.

2. Auxospore (Zygote) Formation and Development

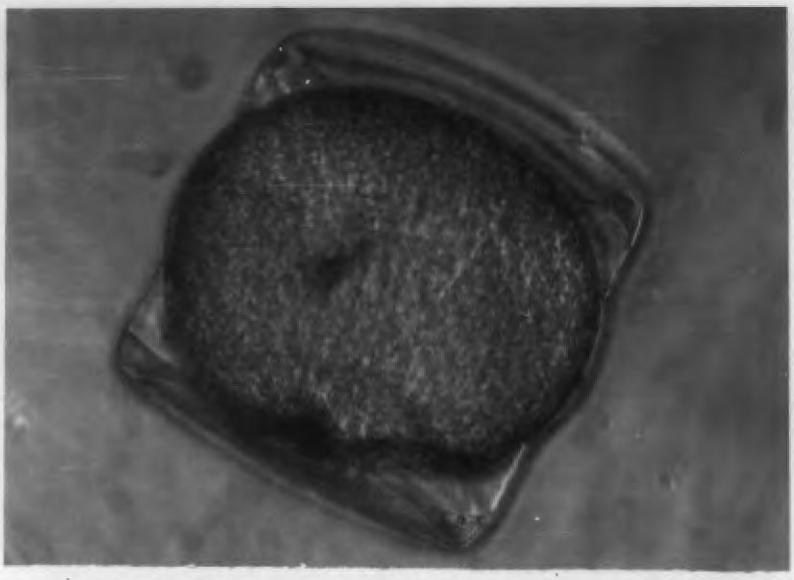
Auxospore development begins with the protoplasm of the auxospore becoming detached from the frustule walls of the mother cell (Plate XIX and Fig. 13a). The two valves, comprising the frustule, are severed along the girdle band and the auxospore gradually either enlarges between the two valves, thus pushing them further apart before freeing itself from them, or emerges directly into the medium. The former is the usual way in which development commences. Meanwhile the auxospore is enveloped in a delicate non-silicified membrane which is capable of expansion and known as the perizonium. The auxospore usually assumes a spherical shape in the medium and has a single nucleus suspended by several protoplasmic strands attached to the periphery. The nucleus is not usually centrally placed but is found more to one end of the auxospore (Plate XX and Fig. 13b). The protoplasm contains numerous chromatophores.

Plates XXI and XXII show the emergence of an auxospore from one of two attached daughter cells. It should be noted that neither daughter cell has had time to develop a second valve and separation has not yet occurred. Plates XXIII and XXIV and Fig. 13c, d show the developing auxospore attached to the two valves of the original cell.

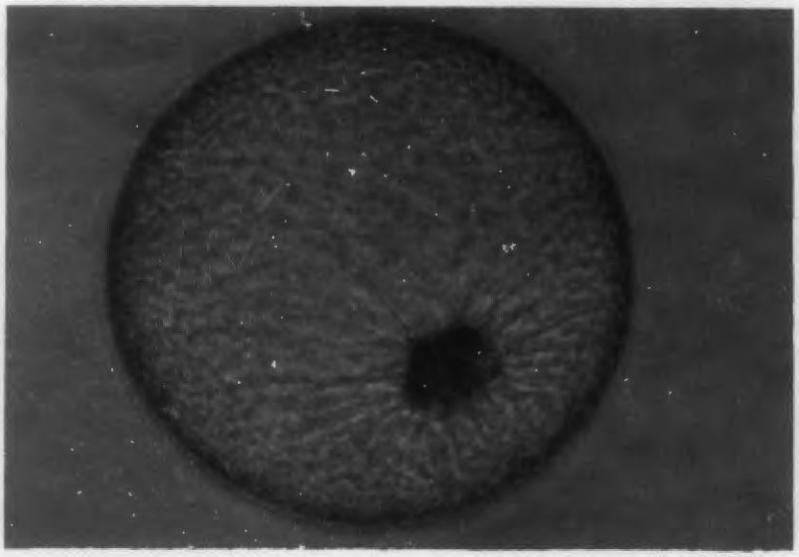
When the auxospore is liberated directly into the medium, development proceeds until a large size is attained. An auxospore developing between two valves of the mother cell frees itself from them in one of two ways. It can completely free itself from them

Legend to Plates XIX - XXXV and Fig. 13

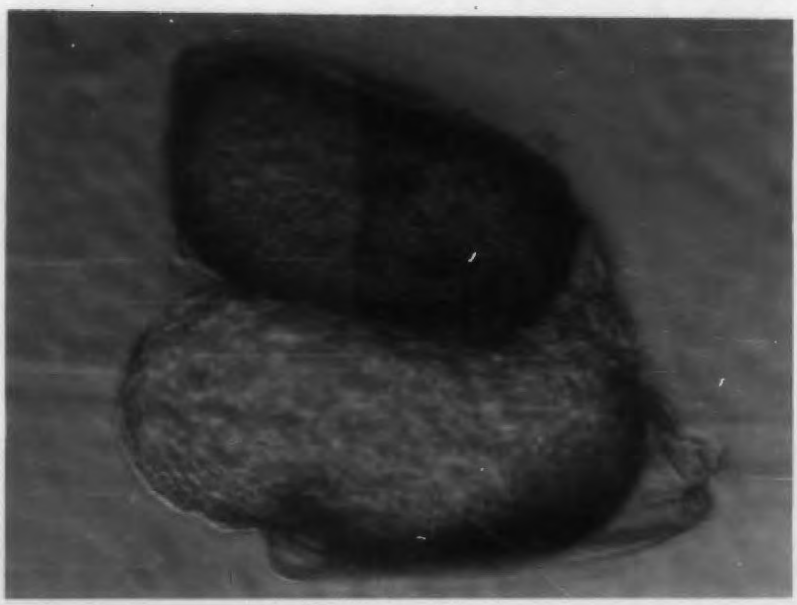
Plate XIX and Fig. 5a	enlargement of zygote within diatom frustule, x 1,250.
Plate XX and Fig. 5b	spherical zygote liberated into medium with distinct nucleus, x 1,250.
Plates XXI and XXII	emergence and liberation of zygote into medium from one of the attached daughter cells, XXI- x 1,060 XXII - x 875.
Plates XXIII and XXIV and Fig. 5c - d	developing zygote attached to the two valves of the parent diatom frustule, x 875.
Plate XXV	detachment of zygote from both valves of parent frustule, x 875.
Plates XXVI - XXVIII and Fig. 5e - f	complete detachment of one valve and secretion of new valve on the side where the nucleus is situated; XXVI & XXVII - x 875 ; XXVIII - x 1,250.
Plates XXVIII - XXX and Fig. 5i - j	shedding of part of perizonium at end of secretion of new valve; XXIX & XXX - x 875.
Plates XXXI - XXXII and Fig. 5g - h	secretion of second new valve, x 875.
Plate XXXIII	zygote mother cell and new cell (larger) arising from zygote, x 560.
Plate XXXIV	partial extrusion of protoplast into a thin transparent sphere; sphere is about twice diameter of cell, x 560.
Plate XXXV	complete detachment of sphere, which floats in the medium, x 560.



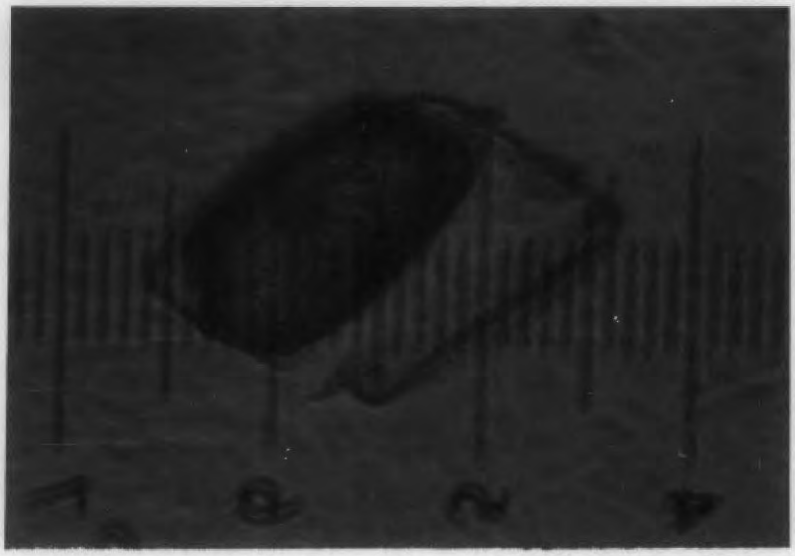
XIX



XX



XXI



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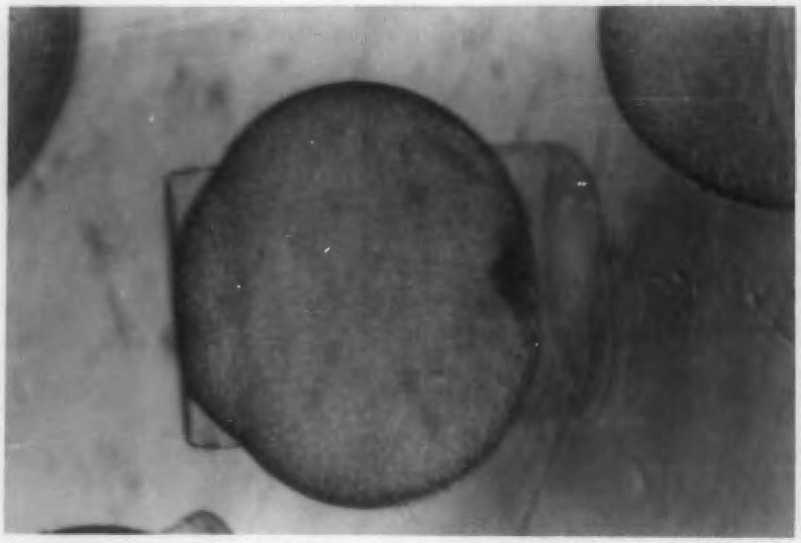
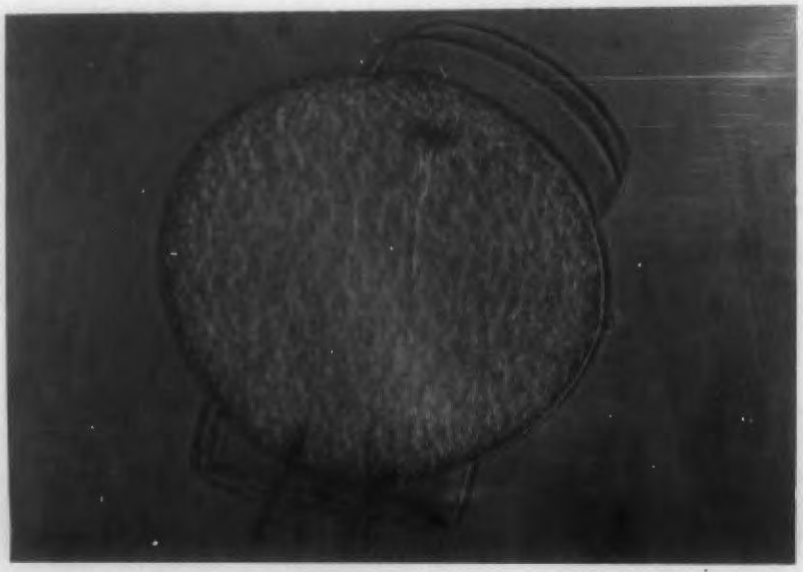


XXIII



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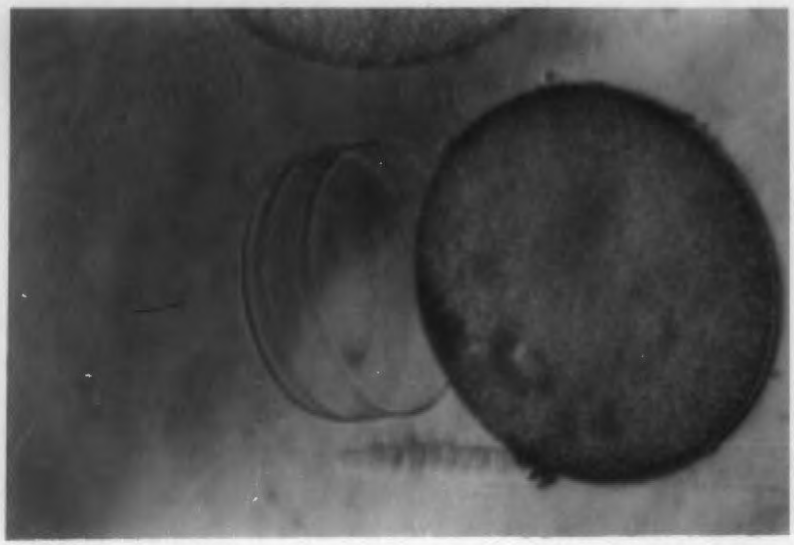
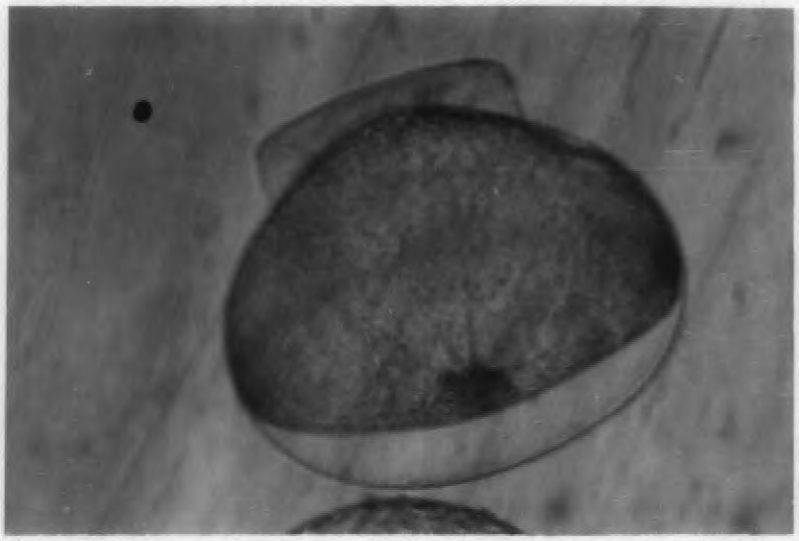
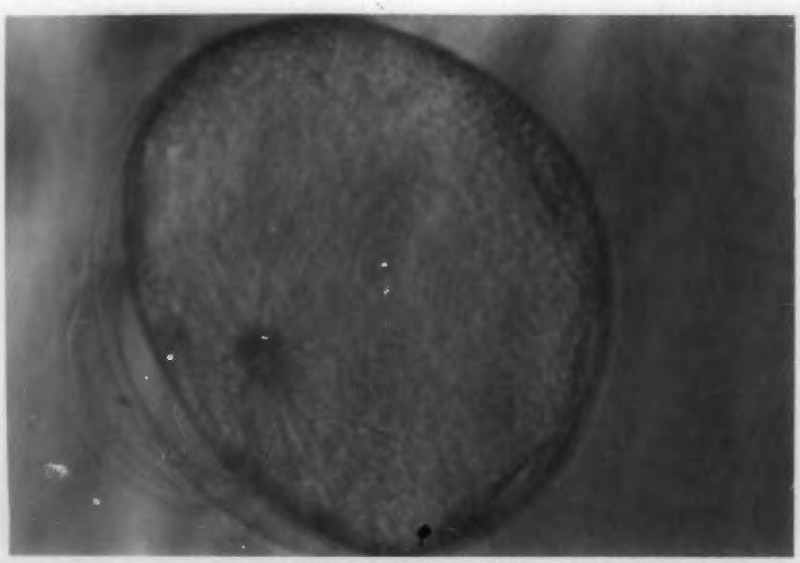


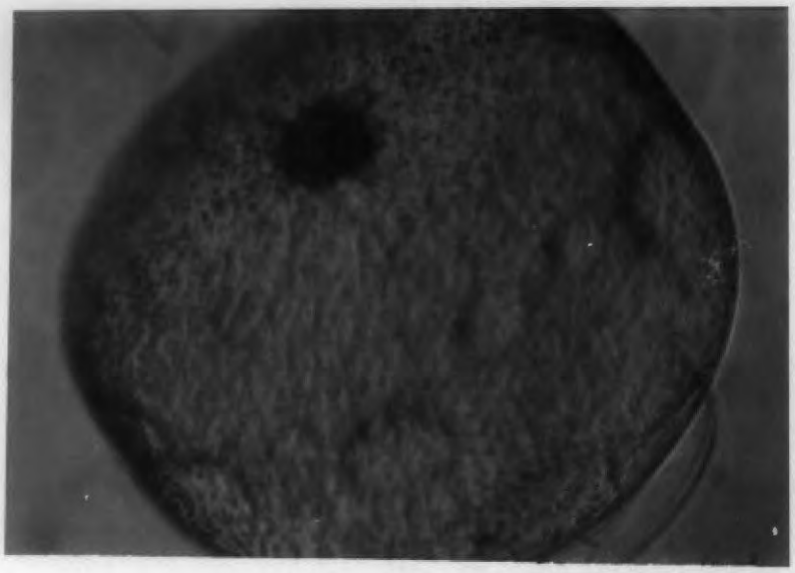
XXV



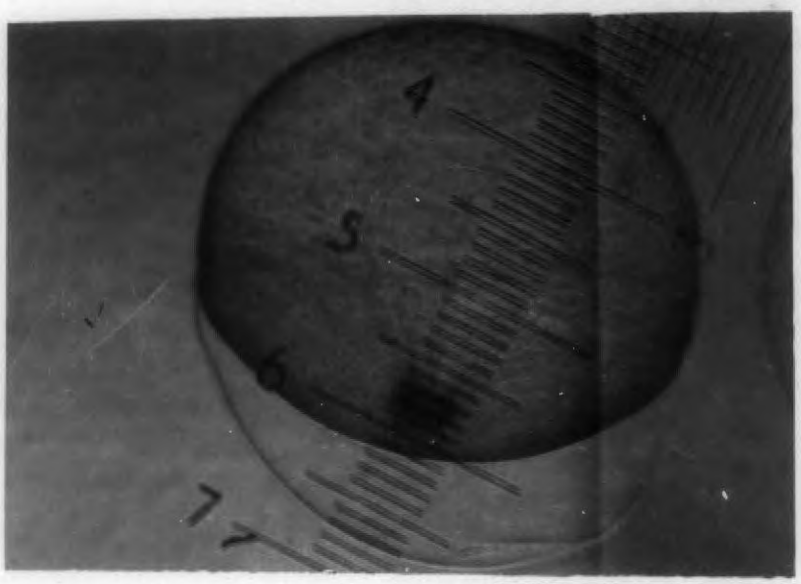
XXVI



XXVII

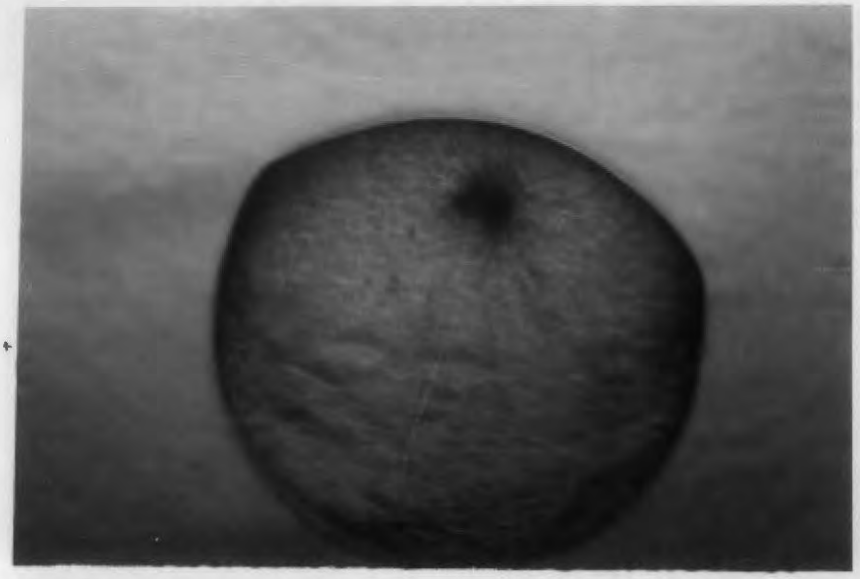


XXVIII



XXIX

XXX



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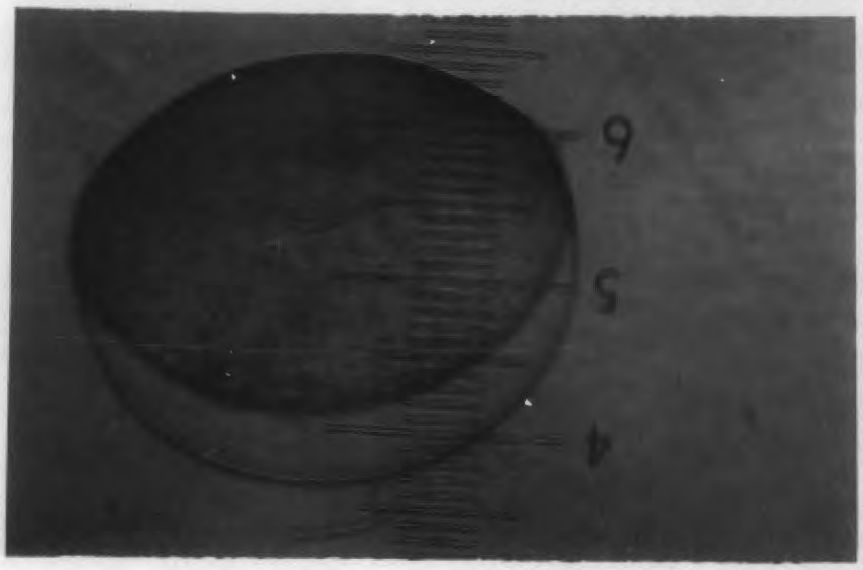
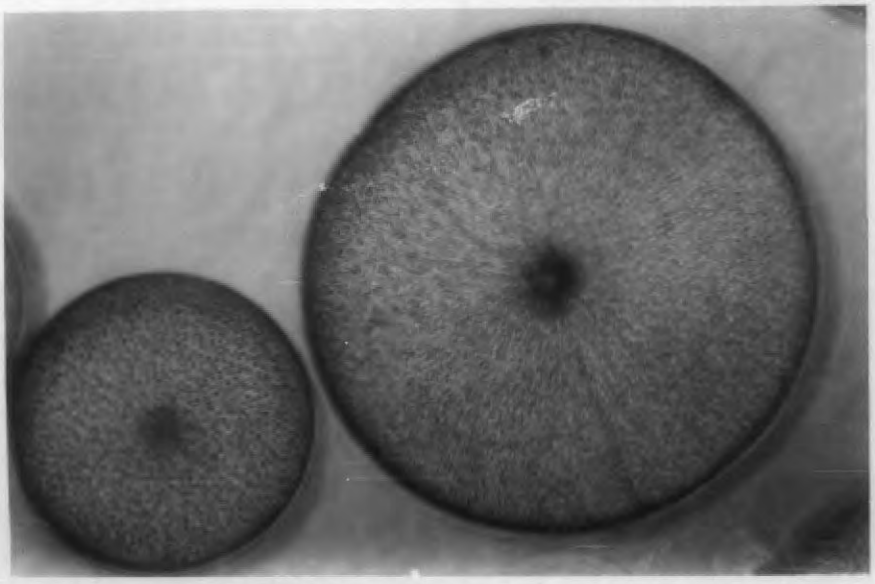


XXXII



XXXIII

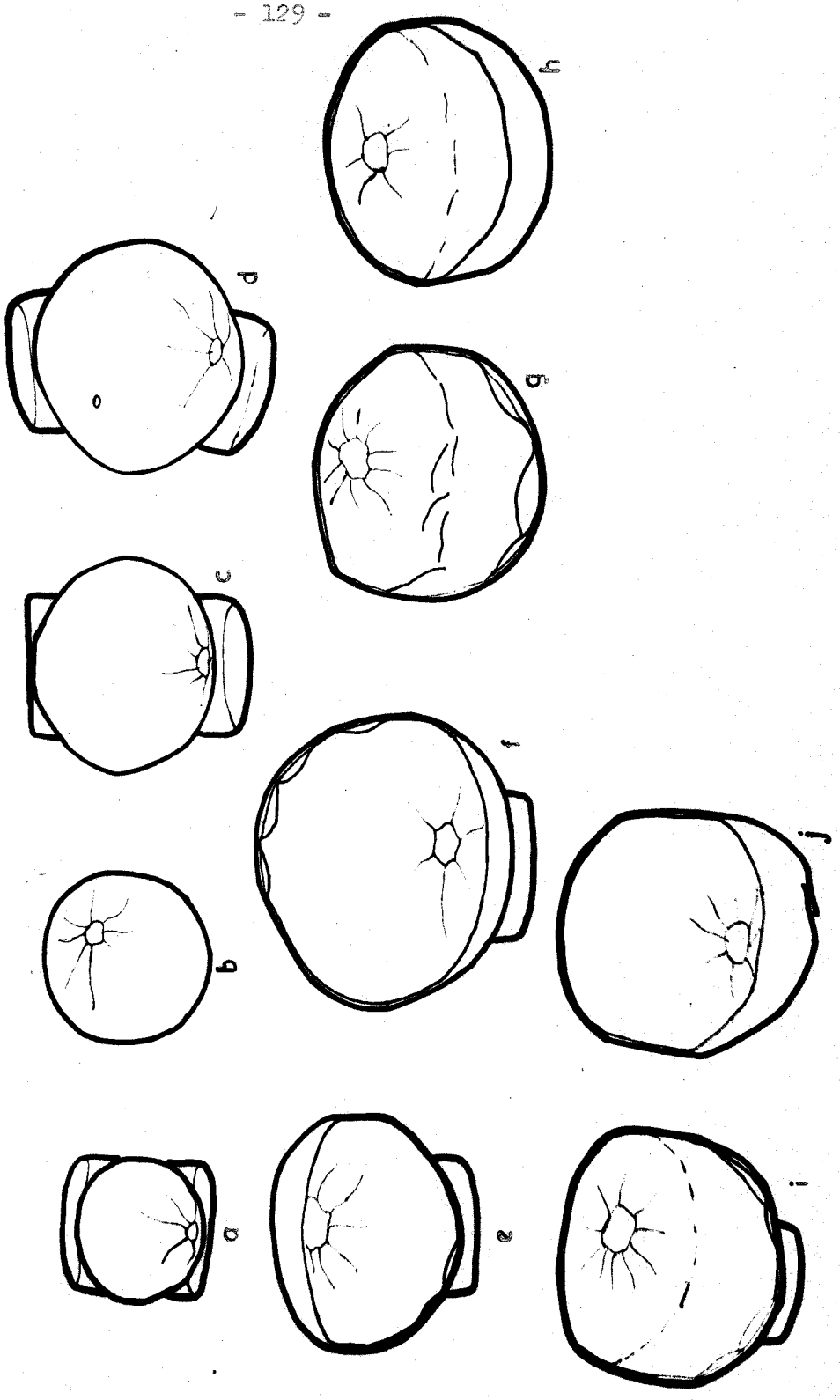
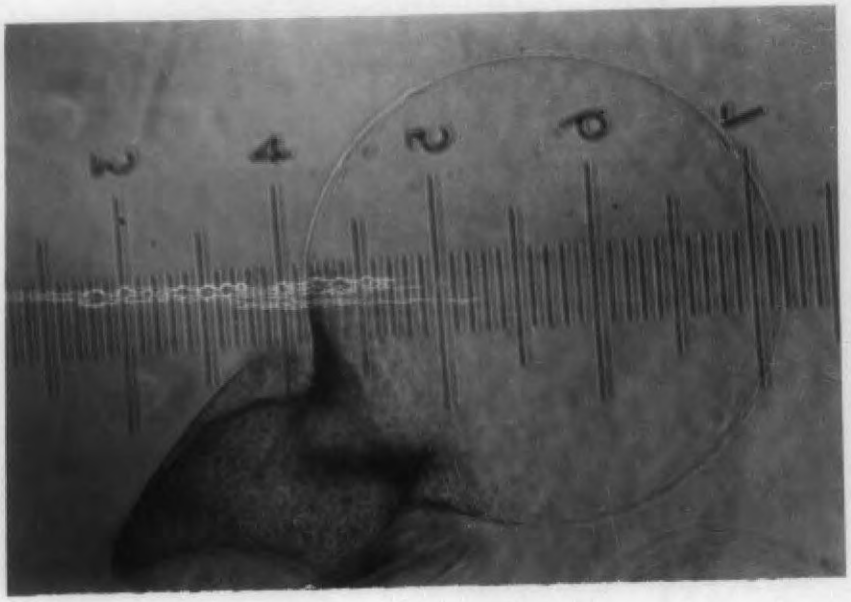


Fig. 13

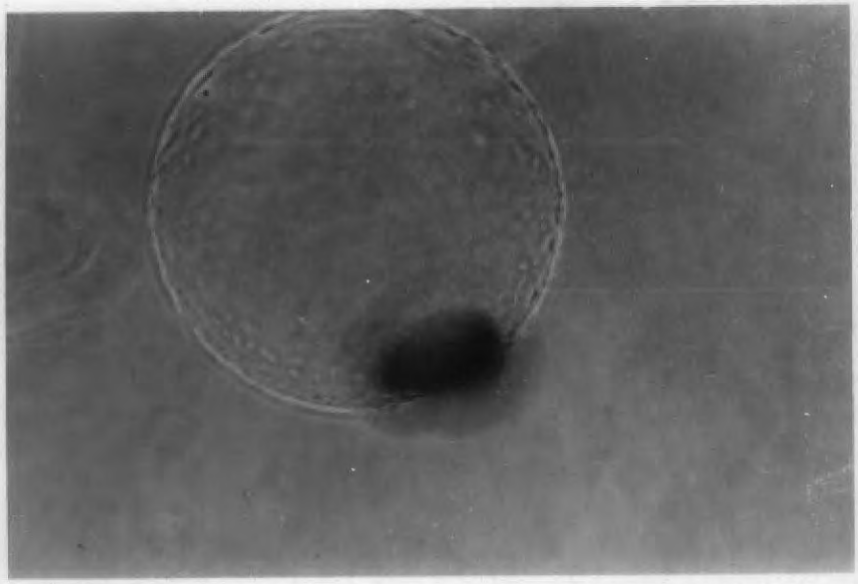
before it secretes its first valve within the perizonium (Plate XXV), or from one of them after the secretion of its first valve (Plates XXVI and XXVII), and from the other after the secretion of its second valve.

When the auxospores have attained their maximum possible size, the contents then contract from one side of the perizonium and secrete a new valve. Usually this valve is secreted on the side where the nucleus is situated (Plates XXVI, XXVIII and Fig. 13e, f, i). The valve is almost flat and has the markings and shape of the species. When the first valve is completely formed, the part of the perizonium under which it has formed is shed (Plates XXIX, XXX and Fig. 13j). The contents then contract from the perizonium on the opposite side to the newly formed valve, and the second valve is secreted (Plates XXXI, XXXII and Fig. 13g, h). The perizonium may be shed in halves when the valves are completely formed, or may envelop the newly formed cell before it finally ruptures and is liberated. Plate XXXIII shows a cell which has developed from an auxospore and the auxospore mother cell. The whole process of an auxospore developing into a new cell of the maximum diameter range of the species usually lasts for about two to three days.

Usually one or two days after the liberation of the male gametes, auxospore development is observed. This observation was made after transferring a cell undergoing spermatogenesis,



XXXIV



XXXV

together with a few other vegetative cells, into fresh medium in a watch glass. The course of events was then followed after the male gametes had been liberated. It has not been possible to determine optically which cells are male gamete or egg mother cells or normal vegetative cells. Occassionally cells with two nuclei in the undivided protoplast have been observed using phase contrast objectives. Probably this type of cell becomes the egg mother cell.

At no time has a male gamete been observed to penetrate another diatom cell, although many have been seen to swim, directly after release, to a cell and become attached to the girdle region of its wall. Auxospore development has never been observed to occur before male gamete formation. Some cells have been seen to extrude all or part of their cytoplasm into a sphere which does not develop frustules. These spheres may remain attached to the cell (Plate XXXIV) or become completely detached or liberated (Plate XXXV) into the medium. These sphere producing cells are not associated with zygote formation, for they have been seen in old cultures; in cultures with cells outside the sexual induction size range; in cultures of salinity outside the sexual induction salinity range; and in cultures where no male gamete formation has occurred. They may or may not possess chromatophores, depending on whether the cytoplasm is completely or not completely extruded. A nucleus is never readily seen by means of phase contrast light microscopy. On occasions the spheres can reach comparatively large dimensions

(500 to 1,000 μ in diameter). Probably these are abnormal products as surmised by Holmes (1967) for similar structures in his cultures.

The cultures used in these observations contained normal vegetative cells of similar size with a diameter difference of 18 u (i.e. 255 to 275 μ). There was no apparent gradation in size within this range. About 80% of the cells were of the smaller diameter. Both sizes of cells produced male gametes and zygotes but many more of the smaller diameter cells were prone to do so than of the larger diameter ones.

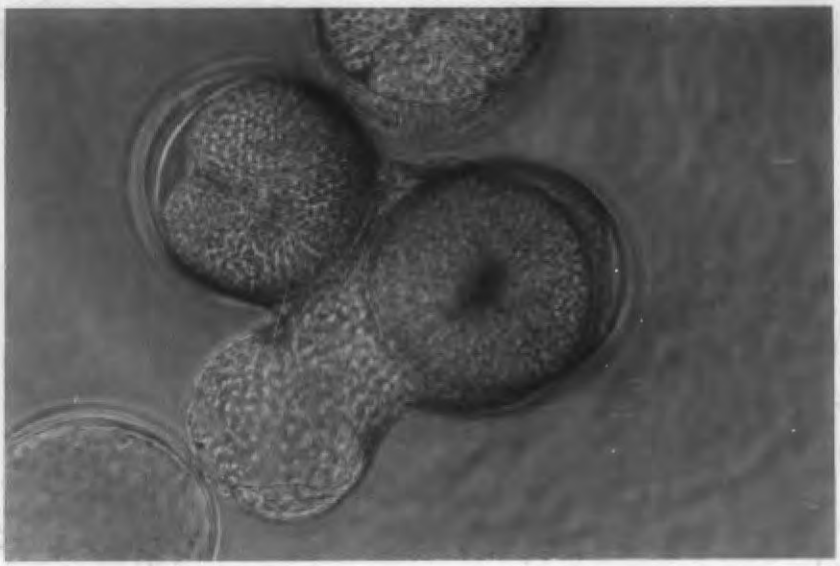
Many attempts were made to follow nuclear divisions during male gamete and zygote formation using acetocarmine and Feulgen staining techniques, but without success. Staining was not satisfactory and features of nuclear activity were indistinct.

3. A Possible Case of Isogamy in the Diatom

Two cells were seen joined together along the girdle region and also showing protoplasmic extrusion into a sphere (Plate XXXVI). This observation was made in a culture under continuous illumination in which male gamete and zygote formation were occurring. The protoplasmic extrusion appeared to have originated principally from one of the cells. Protoplasmic streaming was also evident. Two hours after the observations were started the thin transparent sphere grew larger, apparently at a faster rate than that at which the protoplast streamed into it (Plate XXXVII). Marked shrinkage of the protoplasm from one side of one of the cells was also noticed. A side view revealed that the protoplasts of both cells were in intimate connection and the nuclei of each could be seen clearly (Plate XXXVIII). After two further hours (Plate XXXIX) it was observed that the protoplast of the cell from which the sphere did not appear to originate was moving into the other, though there was not an obvious increase of protoplasm in the sphere. At this stage the cells were carefully transferred into fresh medium to make further observations. Immediately after this the sphere shrank (Plate XL). An hour later (Plate XLI) the sphere was seen enlarging again and the migration of the protoplast resumed until after a further three hours the condition shown in Plate XLII was reached. The cells were then left overnight and the condition shown in Plate XLIII

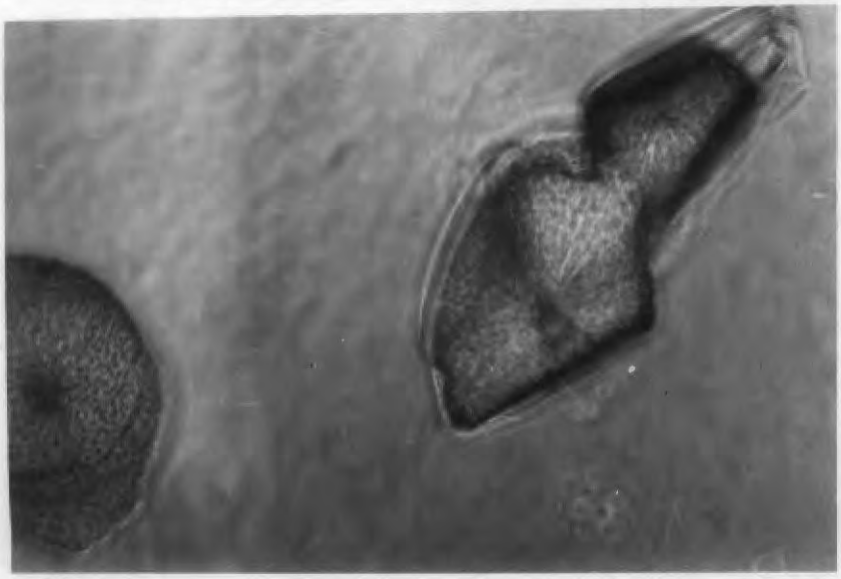
Legend to Plates XXXVI - XLIV

Plate XXXVI	two cells joined together; extrusion of protoplast of one cell into a thin, transparent, and spherical body, x 560.
Plate XXXVII	enlargement of spherical body, x 560.
Plate XXXVIII	intimate connection of both cells, x 560.
Plate XXXIX	migration of protoplast of one cell into the other, x 560.
Plate XL	shrinkage of spherical body when transferred into fresh medium, x 560.
Plate XLI	enlargement of spherical body in fresh medium, x 560.
Plate XLII	further enlargement of spherical body and partial migration of protoplast of one cell into the other; x 560.
Plate XLIII	almost complete migration of protoplast; x 560.
Plate XLIV	speck of protoplast remaining in cell whose protoplast has completely migrated into the other cell; and partial extrusion of "coalesced" protoplasts into spherical body, x 350.

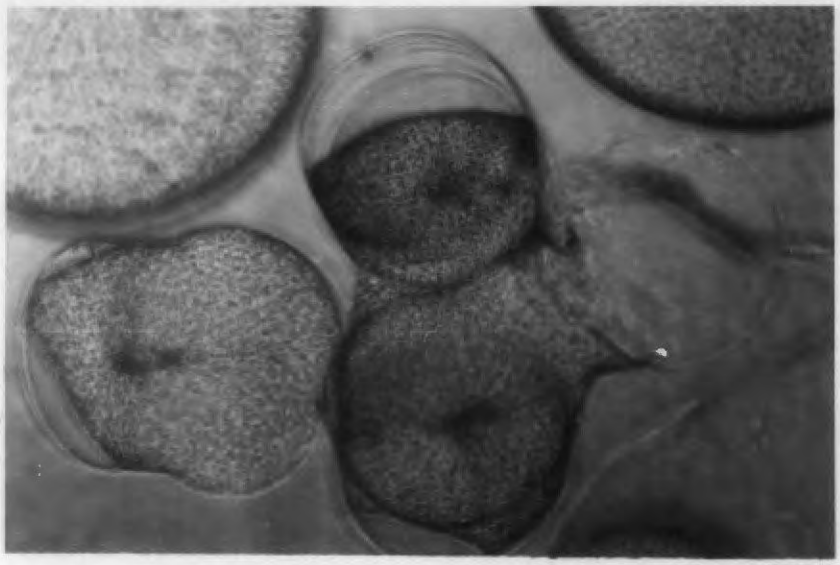


XXXVI

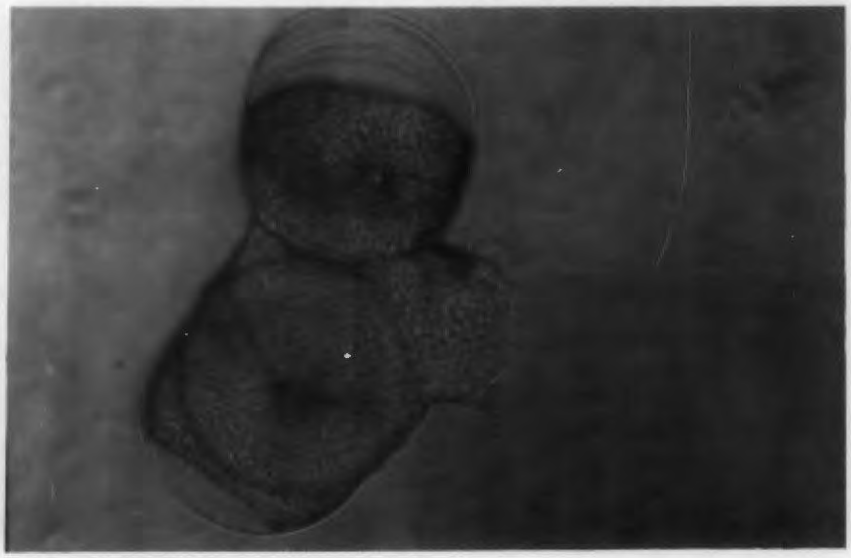
XXXVII



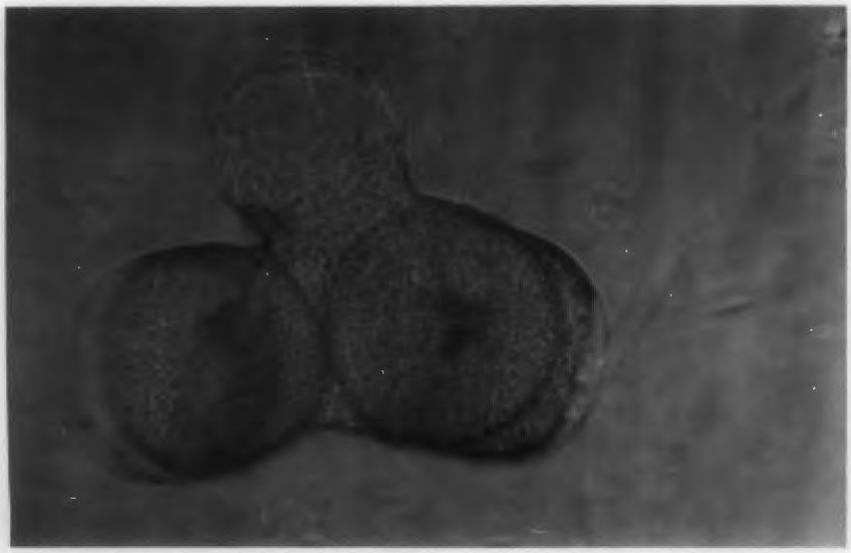
XXXVIII



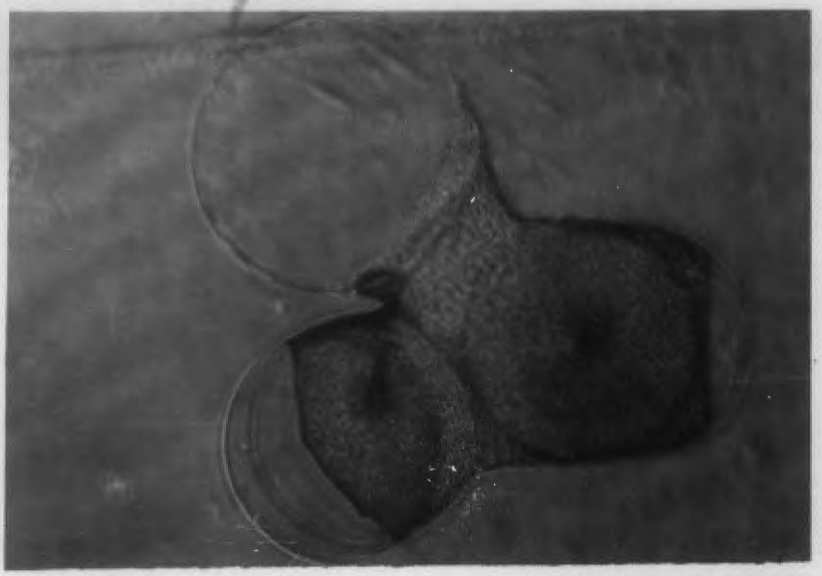
XXXIX



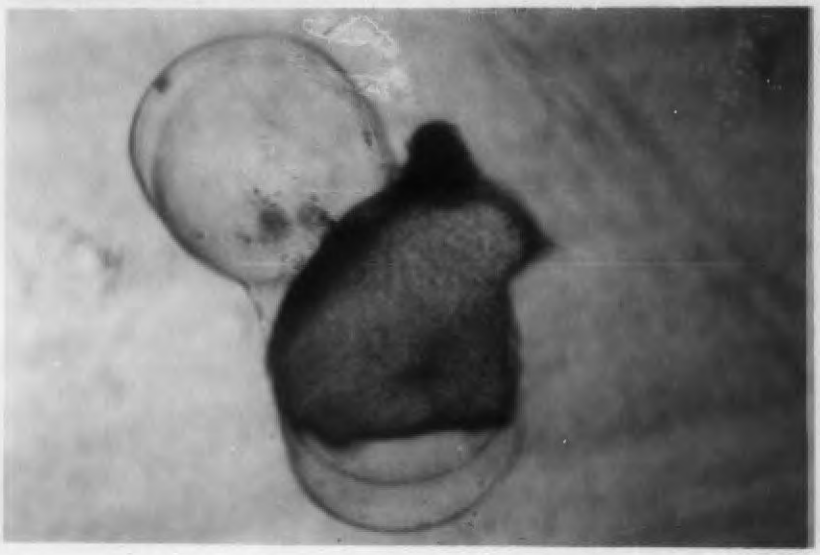
XL



XLI



XLII



XLIII

XLIV

was observed. The sphere had grown very large (out of focus in the photomicrograph) and the migration of the protoplast of one of the cells into the other was completed. The "coalesced" protoplast had become darker in colour and did not look very healthy. Five hours after the last observation (Plate XLIV) a speck of protoplasm can be seen in the empty cell and the "coalesced" protoplast had partially moved into the sphere. No further change occurred.

The described anomalous behaviour is very rare. Only three instances were observed in almost a year of culture work.

The significance of this observation is not clear. Probably the speck of protoplasm in the empty cell contained the nucleus. If the nucleus is left behind, then what is the purpose of the movement and the apparent fusion of the protoplasts? This cannot be readily guessed at.

7. DISCUSSION

DISCUSSION

Unicellular marine phytoplankton, in common with other algae, are influenced by three main factors in nature. These are light (intensity and duration of illumination), temperature and the chemical composition of the environment. Some knowledge of the effect of these factors on the growth and life history of these organisms is essential to the understanding of their ecology and relative abundance in marine environments. Field observations have indicated a variety of patterns in the composition, distribution and succession of plankton communities. The details of this succession may change annually in the same habitat. Thus an analysis of such changes also demands knowledge of the reaction of each species or group of species to the environmental conditions. In this regard the present studies have been mainly concerned with the influence of some aspects of the above mentioned factors on the growth and reproduction of *Coscinodiscus pavillardii*.

GROWTH STUDIES

1. Effect of cell size

Cell size of most centric diatoms varies in two ways. Firstly, in vegetative growth the length of the pervalvar axis increases to almost twice its value from one cell division to another. Secondly, the cell diameter usually decreases by a constant decrement at each vegetative division. Thus the

surface/volume ratio changes with vegetative multiplication.

Fogg (1965, p. 19) has pointed out that the relative growth constant measured under standard conditions does not vary to a great extent for a given species. Its value may depend on a variety of physiological and metabolic factors but size is generally most important, since it determines the surface/volume ratio and thus the relative rate of intake of nutrients for growth. For a cylindrical cell like C.pavillardii, the surface/volume ratio is given by -

$$\frac{S}{V} = \frac{2\Pi r^2 + 2\Pi rl}{\Pi r^2 l}$$

or simply -

$$\frac{2(r + l)}{rl}$$

where r is the radius of the valve, and l the length of the pervalvar axis.

Thus a change in surface/volume ratio in C.pavillardii for a reduction in cell diameter from 400 μ to 100 μ will be 0.0247 to 0.0547. Consequently the surface/volume ratio will increase with reduction in cell size or the smaller the cell the greater the amount of surface area per unit volume of cytoplasm. This means a faster rate of absorption of nutrients per unit mass by smaller cells than by larger ones.

In the present study the relative growth constant was fairly invariable and independent of cell size (Section 5.4). The growth

rate (division rate) was influenced by cell size. Therefore, if recently divided cells in the course of their development have to double their volume of cytoplasm before division can occur, smaller cells would do so faster than larger ones because of their relative surface area. This indicates that the proportion of cytoplasm to vacuole in the cells remains unchanged.

Jørgensen (1964) in his studies on the relation of cell size to photosynthesis in the centric diatom Cyclotella meneghiniana, found that the chlorophyll a content, as well as the rate of photosynthesis per unit of chlorophyll a in the cells of varying volume, are approximately constant and independent of cell volume. He suggested that this is due to a decrease in the cell vacuole with decreasing cell volume while the volume of cytoplasm is nearly unchanged. The assumption here is that a given amount of chlorophyll a is always uniformly dispersed in a definite volume of cytoplasm. Therefore if the chlorophyll a content is independent of cell volume than the proportion of cytoplasm to vacuole changes with cell size i.e. it is inversely proportional to increasing cell size. This hypothesis seems rather curious in the light of work by Paasche (1960) on a number of phytoplankton (principally diatom) species. He demonstrated that the chlorophyll content per unit of cell surface area is more nearly constant than chlorophyll per volume of cell or per cell. Furthermore Jørgensen's hypothesis does not explain the difference in division rates between smaller and larger cells.

If Jørgensen's hypothesis is tenable one can expect, as a result of continued vegetative growth, smaller cells lacking vacuoles. Paasche has pointed out that the correlation between the relative growth constant and surface/volume ratio may not hold when large cells with vacuoles are compared with cells lacking vacuoles. He felt that in the large vacuolated cells the part of the cytoplasm in which photosynthesis will occur may have just as much surface available for the absorption of nutrients as would the same volume of cytoplasm in smaller cells lacking vacuoles. Consequently both kinds of cells may have the same division rate. However, field as well as laboratory studies on the division rates of diatoms indicate that in general large diatom species tend to have a lower division rate than smaller ones. Evidence as to whether this is the case within a species is as yet wanting or unavailable, though the author's results appear to indicate this.

2. Effect of Light

Kain and Fogg (1958), using fluorescent lights, found that the saturating light intensity for Asterionella japonica was about 4,000 lux. Thomas (1966) found that it was about 6,480 lux for a tropical species of Chaetoceros. In this investigation the value for Coscinodiscus pavillardii has been found to be about 6,000 lux (Section 5.5). This value is slightly less than that for the Chaetoceros sp. How is it then that the diatoms are able to survive in the tropics where the total incident solar radiation is usually very high?

The daily average of total illumination from solar radiation during the dry season is of the order of 67,000 lux on the surface water of the estuary (Bainbridge, 1960 estimated it as 0.4 g cal./cm.^{2}/min.) Ryther (1956) found great inhibition of photosynthesis in marine diatoms at such an illumination from solar radiation but little at 25,000 to 33,000 lux (0.15 - 0.20 g cal./cm.^{2}/min.). Following Strickland (1958) Bainbridge has suggested that about 50% of the total incident solar radiation lies within the photosynthesis range of 3,800 - 7,200 Å. Such a high illumination within the photosynthetic range would greatly inhibit the growth of diatoms at the surface. Bainbridge found that the transparency of the estuary water is poor and as a result the depth of the euphotic zone is rather shallow. He also found that the high detritus content coupled with the tidal water movements complicated the picture when considering the depth of the euphotic zone in relation to the average depth of water. There was no clear cycle of turbidity between springs and neaps, as the extinction coefficients varied from 0.1 to 0.5 with a mean of 0.27. The average depth of the estuary water is about 15 to 20 m. at low water. He calculated that the maximum depth of the euphotic zone at low water is about 4 m. at springs and about 9 m. at neaps. Thus the plant material is suspended as a result of the intensive vertical mixing taking place during the tidal water movements.

Strickland (1965) has defined the depth of the euphotic zone as the depth at which 1% of the surface visible light energy remains. Thus the incident daily radiation is greatly attenuated within the euphotic layer and the amount of light available to the diatoms in the estuary must be greatly reduced. Bainbridge also found that throughout the whole year concentrations of holoplanktonic forms within the estuary showed no appreciable differences between the surface and 10 m. levels, either at spring or neap tides. Therefore it is not surprising that the saturating light intensity for C.pavillardii is about 6,000 lux, and that it can suffer little or no inhibition of growth even at 18,000 lux.

3. Effect of Temperature

The optimum temperature range for exponential growth appeared to be 25 - 29°C for the cultural conditions employed (Section 5.5). Temperatures below or above this range either reduced the growth rate or inhibited its growth. A change in temperature of 10°C, i.e. from 25 to 15°C, resulted in erratic growth. This effect may be due to the previous history of the cells as the stock cultures were usually kept in a temperature range of 24 - 28°C.

Some studies have been made on the combined effect of temperature and light on cell division rates (Spencer, 1954; Kain and Fogg, 1958; Sorokin and Krauss, 1959; Jitts et al., 1964; Jørgensen and SteemannNielsen, 1965). These studies have shown a wide temperature and light tolerance especially if the

cells are allowed to adapt themselves on transfer from one culture condition to another. This was found to be important when cells are changed from a higher to a lower temperature, or from a higher to a lower light intensity. For the adverse effects of low temperature are exaggerated at low light intensities, and the damaging effects of high light intensities are made worse at high temperatures.

In this study no attempt has been made to study the combined effect of temperature and light on the growth rate of the diatom. Thus the diatom's temperature and light tolerance range and its ecological implications cannot be assessed. This will form a basis for further study.

4. Effect of Salinity

The slower growth rate at the 32.9°/oo S was probably a consequence of the larger diameter of the cells. Since size has been shown to affect the growth rate (Section 5.4) it can be assumed that the rate would not have changed had the cell size been the same as those at the other salinities. Thus the growth rate can be considered as the same in the salinity range 13.3°/oo to 32.9°/oo.

Spencer (1954) has shown that small inocula sometimes produced a short lag when cells are subcultured into fresh medium during the exponential phase. He found that such a lag did not affect the growth rate during the exponential phase. Probably

the observed lag at the 10.1°/oo S can be explained as such for the same size of inoculum was used at the 13.3°/oo S. However it is felt that the slower growth rate of the diatom at the 10.1°/oo S is a direct effect of the difference in salinity. Therefore it is probable that the growth rate slows down appreciably at salinities below 13.3°/oo.

During the dry season (November to April) when the diatom can be found as part of the standing crop of phytoplankton the minimum and maximum monthly salinity recorded were 12.2°/oo and 34.4°/oo respectively (Watts, 1960). Within this range of salinity the diatom has been found to grow well. This, therefore, must be an adaptation to the salinity changes in the estuary. For to survive in estuarine conditions an organism must adapt itself to a wider range of fluctuations in environmental factors than in the sea or fresh water. It is, therefore, not surprising that Coscinodiscus pavillardii can tolerate such a wide range of salinity changes as borne out by the experimental results.

5. Effect of Vitamins

About 70% of the marine phytoplankton organisms thus far examined have been found to be auxotrophic and require cobalamin (B_{12}), thiamine (B_1) and biotin, either singly or in combination. Coscinodiscus pavillardii appears to require vitamin B_{12} only (Section 5.8).

The CM and CM - BIOTIN media contain other vitamins than the three mentioned above whereas the PLAIN + B_{12} lack them. The faster growth rate in the CM and CM - BIOTIN media, as compared with that determined in the PLAIN + B_{12} medium, probably indicates that the other vitamins (apart from B_{12}, B_1 and biotin) are complementary in enhancing the growth of the diatom. The difference in growth rate of the diatom in CM and CM - BIOTIN media also probably indicates that biotin, although not required by the diatom, has a retarding effect on the growth rate.

The moderate growth of the diatom in the PLAIN, CM - B_{12} - BIOTIN, PLAIN + BIOTIN, and PLAIN + THIAMINE media, on the first transfer, may be due to the carry-over of vitamins in the inoculum or the effect of the quantity already absorbed by the cells. The failure of the cells to divide from the third transfer onwards and their consequent death indicate that the specific action of vitamin B_{12} is on cell division or that vitamin B_{12} is involved at some stage in the processes of nuclear synthesis which affect cell division.

6. Effect of Spore formation

If cells of cultures A (Table X) were grown outside the salinity range for the induction of spore formation their growth rate would have been 21.6 hours (calculated from Fig. 4c). Because of spore formation the growth rate was slower (27.3 hours).

In cultures B and C the growth rate should have been the same at the different salinities for the given diameter range of cells. Where the initial percentage spore formation was the same the growth rate was similar irrespective of the salinity. Where the initial percentage spore formation was greater the growth rate was slower. Therefore the amount of spore formation determined the extent to which growth rate would be affected.

At any time during exponential growth the "vegetative" cells would consist of three kinds - presumptive male gamete and auxospore mother cells as well as the normal vegetative cells. The presumptive spore mother cells could not be easily determined as there are no apparent morphological differences between them and the normal vegetative cells. Thus the percentage of "vegetative" cells may have been over-estimated, and that for spore formation under-estimated. Nevertheless the percentage drop has been considered real and significant. If the growth rate of the diatom had not been affected by spore formation the percentage "vegetative" cells would have been higher than observed. Furthermore it was noted that spore formation had its greatest effect on the growth rate during early exponential growth, for it was then that a high proportion of the cells were induced to spore formation. It was also considered probable that induction of male gamete and auxospore formation took place simultaneously during the period of exponential growth.

REPRODUCTION STUDIES (SPORE FORMATION)

With regard to the problem of the factors promoting spore formation in diatom, one must first consider how far spore formation is a normal part of their life history. The continual reduction of cell size is a consequence of the method of vegetative division. When a very small size is achieved death ensues. This limit is reached at about 70 μ in the C.pavillardii clone. Regeneration of the maximum size is only achieved by auxospore formation, so that spore formation may be regarded as essential for the continued existence of the clone. On the other hand, in those diatoms which do not decrease in size, spore formation does not apparently occur (von Denffer, 1949). Consequently spore formation does not appear to be an invariable requirement for every diatom. It is theoretically possible to envisage continued growth of a diatom species without spore formation. Starting from a cell of maximum diameter, the epivalve will always remain the same size, and will always form part of the frustule of one of the daughter cells resulting from vegetative growth. If such daughter cells remain immortal then a population, which would die out because a majority of the cells had become too small, can be maintained by such cells. Under natural conditions this is extremely unlikely as it is reasonably probable that such daughter cells will be ingested by a grazing animal or suffer some other calamities.

Spore formation can then be considered to be an essential part of the life history of those diatoms undergoing continued decrease in cell size. Hence, if such species growing in culture does not produce spores, it can be said that it is not growing naturally i.e. not under satisfactory environmental conditions. One may therefore ask what are the optimum conditions which bring about the formation of spores? They seem to be a combination of an internal condition governed by cell size, and of external environmental factors.

1. Internal Condition

It has been shown by Geitler (1932) that sexuality in diatoms is intimately connected with a progressive decrease of average cell size. It only occurs in cells of a definite size range which is characteristic for each taxon, and is correlated with a specific internal physiological condition. In the Centrales this critical size is within the range of 10 - 50% of the maximum valve length or diameter. Geitler (1932) and von Stosch (1954) have also shown that in the upper limit of the critical size range, more female cells were produced than males; in the lower limit more male cells than female; and in between about the same proportions of male and female cells. The present observations on C.pavillardii are in agreement with the findings of Geitler and von Stosch.

The formation of male gametes and auxospores in Centric diatoms has been reported principally by von Stosch (1956) and Holmes (1967). Both von Stosch and Holmes found that they were only produced in smaller diameter cells. However, the author's observations indicate that male gamete and auxospore formation occurred throughout the sexual induction size range. There was a tendency for the relatively small diameter cells to become gamete mother cells, but this was not restricted to them alone.

Not all cells of a culture were induced to form spores. Some continued vegetative growth resulting progressively in smaller and smaller diameter cells - a characteristic feature associated with vegetative multiplication. Whilst this reduction in cell size was in progress small diameter cells became large diameter cells with respect to their products. Then the occurrence of spore formation in the relatively smaller diameter cells changed progressively. In effect both large and small cells produced spores as was observed in the experiments using cells of the same average diameter. However, the relatively smaller cells did so more readily than the larger ones in the same culture. Therefore it is felt that there is not a definite restriction of spore formation to smaller diameter cells only. Moreover, it would appear that von Stosch and Holmes arrived at their conclusion from an unselected group of cells of widely varying sizes and

not from observations on a uniform size of cells within the sexual induction size range.

The relative growth constant, as measured by the volume of cell material produced, did not change over the range of cell size. Consequently the induction of sexuality cannot be related to a change in the relative growth constant. This is also apparent in the experiments, as spore formation only occurred during the exponential phase when growth is unrestricted. The invariability of the relative growth constant of cells of different diameters is interesting, as the surface/volume ratio increased with cell size. The rate of absorption of nutrients depends, amongst other factors, upon the surface area of the cell. Therefore the absorption of nutrients is not limiting the growth of the diatom under the experimental conditions. This will be the case unless absorption is limited to certain areas of the cell surface which remain constant despite changes in cell size or unless the proportion of cytoplasm to vacuole changes as the cells decrease in size.

2. External Factors

(a) Salinity

It is clear that salinity plays an important role. Although cells remaining in medium of 32.9°/oo S and cells growing in 26.7°/oo S and transferred to 32.9°/oo S produced male gametes in small quantity, cells grown in 19.9°/oo S and 16.5°/oo S do not

complete the process of male gamete formation (Table XI). The absolute value for the change in salinity does not appear to be as important as the level to which the cells are transferred. Thus an increase in 13.3°/oo S (from 16.5 to 29.8) gives normal development of male gametes, but an increase of 13.0 to 16.4°/oo S (from 16.5 or 19.9 to 32.9) gives male gametes which are not released. The former transfer also gives auxospores which the latter transfer does not. Moreover, in contrast to the findings of Schreiber (1931) with Melosira nummuloides, there does not appear to be much difference in the rate of production of auxospores in transfers from low to high salinity or vice versa. The results with Coscinodiscus pavillardii suggest that there is an optimum salinity range for spore formation to take place. This could possibly be an adaptation to the estuarine habitat of this clone. For very large forms of the diatom, ranging from 360 to 460 µ, are usually found in the estuary towards the end of November, when the average salinity is 21.5°/oo, until January when the average salinity is 28.1°/oo (Watts, 1960). Nevertheless there is a clear interaction between the salinity difference and the levels of transfer. This most probably operates through an effect on the internal ionic concentrations. Moreover, it is possible that there is a significant difference between the ionic concentrations at which male gametes and

auxospores are formed, and at which they are liberated from the parent cell. For when cells undergoing spore formation were transferred to an unfavourable salinity gametogenesis was not inhibited.

(b) Temperature

Temperature appears to have an important effect. Presumably the effect is concerned with the rate of enzymatic processes in the diatom cell (Jørgensen and Steemann-Nielsen, 1965). Experiments with the diatom at a single light intensity range have shown an optimum temperature range for the promotion of spore formation. Bruckmayer-Berkenbusch (1955) has suggested that one of the factors responsible for spore formation is a preferential synthesis of protein. It is likely therefore that the way in which temperature acts is by regulating the protein synthesis necessary for spore formation.

It is considered that temperature and light interact in the production of spore formation as shown by Holmes (1966). No attempt was made in this study to investigate their combined effects on spore formation.

3. Light

Light has been considered to play a significant role in sex determination (von Stosch, 1954). However further study of its effect would appear to be desirable. In the present study a higher light intensity has been shown to inhibit spore formation in competent cells at the optimum salinity. At lower light intensities,

under the same experimental conditions, spore formation is promoted. This finding lends support to the hypothesis that light intensity determines the nature of sexuality in the diatom cells. A normal vegetative cell within the sexual induction range is considered to have both potentials for maleness or femaleness. The expression of either potential is dependent on the state of metabolism of the cells as well as on light intensity. Under high light intensity the metabolism of the cell is such that no expression of sexuality is favoured. This may be due to the production of organic material at a faster rate, and perhaps in greater quantity than is required for growth. Under low light intensity this does not occur and hence spore formation is promoted. Moreover, Sorokin and Krauss (1962) observed that in non-synchronous cultures, cells approaching and beginning cell division may have a low rate of metabolic activity and growth. Consequently the metabolism of some cells would be such that light can influence the initiation or non-initiation of gametogenesis. Under low light intensity some cells will be able to express their inherent potential for sexuality, resulting in the production of potential male and female cells. The foregoing are tentative suggestions and verification would require the use of synchronous cultures. It was however observed that in the experiments with alternating low light intensity, when cells had been induced to form gametes the process went on to completion whether or not they remained in the

light. Cells in the induction size range when kept in the dark, produced no gametes, but became bleached and died after a few days. It will be recalled that spore formation occurred earlier under continuous light than in the alternating light/dark cycle, and also the growth rate was faster under continuous light than in the alternating light/dark cycle. It is probable that under continuous light the cells were producing organic matter at a faster rate than was necessary for the optimum production of spores. Warburg (1958) has pointed out that continuous light is unnatural with respect to the evolutionary history of all cells. For "cells are forced to produce organic material continuously, and more material than they need for their own synthesis. Consequently the energy yield of the cells is reduced to a small fraction of the optimum yield." Thus the light/dark treatment favoured an increase in the energy yield of the cells which in turn enhanced the attainment of the necessary physiological state for sexual induction in many of the cells. This may also account for the observed increase in male gamete and auxospore formation under the alternating light/dark treatment.

A number of workers have reported a reduction of algal growth rates with shortened daylength (Talling, 1955; Tamiya, 1957; Huling, 1960; Jones et al., 1963, Castenholz, 1964). Talling suggested that the growth rate may be proportional to the duration of the effective light period. Castenholz has pointed out that if such

a proportionality exists then it may not hold through a great range of possible daylengths in many species. Whether such a proportionality exists for Coscinodiscus pavillardii has not been determined as only two light periods were investigated. But it did seem significant that there was proportionality between the number of cells at the two light periods during the exponential phase of growth. This proportionality was also evident in the percentage spore formation at the two light treatments.

Whilst a 12 hour low light/12 hour dark cycle did not affect the time of induction of male gametes and auxospores, it did increase the number produced compared with that in continuous low light. This is in conformity with the results of Holmes (1966). Although the 12 hour light/12 hour dark regime also reduced the growth rate, yet this is a result of the proportion of cells involved in spore formation. If it were possible to determine the truly vegetative cells and follow their multiplication then perhaps their growth rate would be found to be the same as for those in salinities outside the range for the promotion of spore formation.

The observations of Holmes (1966) and those of the author are consistent with the working hypothesis that spore formation in diatoms is a result of a short-day quantitative photoperiodic response. It is also suggested that this response is complicated by an ionic effect dependent on temperature. This suggestion will be used as a basis for further experimentation.

3. Initiation of gametogenesis and liberation of spores

The initiation of male gamete formation always precedes auxospore formation. It is very difficult to observe the early stages of auxospore formation (i.e. meiotic division) in the living cell. Both male gamete and auxospore formation may be initiated simultaneously, but auxospore formation only becomes obvious after meiosis has occurred and probably after penetration of the egg cell by a male gamete. If both are initiated simultaneously, synchrony may be achieved by the mitotic divisions of the male gamete mother cell being initiated by the factor which initiates meiosis in the auxospore mother cell. Although not impossible, it seems more likely that meiosis in both is induced by the same factor. If light is considered as the factor which determines the sexuality of the cells, then it must influence vegetative cells in some significant way such that some become male, some female and the rest remain vegetative. It is suggested therefore that in male cells light stimulates the build-up of some metabolite which causes them to divide to form spermatogonia. During the production of the spermatogonia it is also suggested that a hormone is produced which causes them to undergo meiosis. This hormone diffuses out and causes the female cells, whose metabolism differs in some significant way, to undergo meiosis directly. The delay which has been observed between the formation of male gametes and the formation of auxospores can be largely

explained by the time lag required to build up an effective concentration of the hormone in the region of auxospore mother cells. It will be recalled that meiosis in the male gamete mother cells occurs in some four hours, whereas auxospore formation is delayed for a further one to two days.

The experiments on the transfer of cells from one salinity to another provide some evidence for the above proposal. When cells were transferred from 19.9°/oo S or 26.7°/oo S to 32.9°/oo male gamete formation took place without the formation of auxospores. It may be objected that meiosis might have taken place in the auxospore mother cells but it seemed significant that in the former transference, male gamete formation stopped before the stage of meiosis. Moreover, in both transfers less than 1% of the cells were seen to be undergoing male gamete formation. Probably in the 26.7°/oo S, where male gametes were released, the concentration of the hormone produced in the relatively few male gamete mother cells was insufficient to stimulate meiosis in the female cells.

Among oogamous algae suggestions of chemotaxis between unfertilized egg cells and male gametes are quite numerous (Cook and Elvidge, 1951; Hoffman, 1960). In this observation of auxospore formation in C.pavillardii the male gametes, after their liberation, were seen to move towards certain cells whilst bypassing others. If the cells they approach are the egg cells then there may be some chemotactic substance secreted by the egg cells which

aid the male gametes in locating them. This suggestion needs further investigation.

What factor(s) initiates the enlargement of the protoplasm of an egg cell and the liberation of the male gametes from their mother cell? Both von Stosch (1954) and Holmes (1967) have suggested that an increased internal pressure apparently causes complete separation of the valves of a spore mother cell. It is felt that this increased pressure can be considered in terms of an osmotic effect caused by an increase in the solute content of gametes and the resultant stress on the girdle bands (points of weakness) of the diatom frustule.

However, Holmes (1967) has described slight separation (as opposed to complete separation) of the two girdle bands of C.concinnus which he felt was the result of the male gamete flagella activity and penetration. He suggested that this is quite another method of release of gametes. This method was also observed in the release of male gametes of C.pavillardii. In this case although two kinds of male gametes were observed no one kind was particularly active at the girdle region. There was clustering at the girdle region but it seemed to be a result of their being trapped by others immediately surrounding them. The significance of the "sheath" towards the base of the flagellum of one of the kinds of male gametes can only be guessed at. Probably it is a strengthening device against flagellar damage from activity at the

girdle region. For this flagellum is used in a spear-like action with only the tip region performing a sinuous motion.

It is not clear what factors are responsible for the time lag between the liberation of the male gametes and germination of the auxospores. Holmes (1966) has observed a time lag of up to 76 hours in Coscinodiscus concinnus. In the case of C.pavillardii the time lag was about 48 hours. Possibly this lag may be due to the time spent by the male gametes in penetrating the egg cell, coming into contact with it and fusion of their nuclei.

Von Stosch (1954) reported, in the case of Biddulphia mobiliensis, that the valves of the egg mother cell opened after the second meiotic division of the egg nucleus, thus making it available for fertilization by the male gametes. He observed the union of the egg and male gamete in vivo in two instances and later confirmed it cytologically. After fertilization some nine hours elapsed before germination of the auxospore began. In general fertilization is only possible when the egg is mature. If it is conceded that chemotaxis is involved in the location of egg cells by the male gametes, then the production of the chemotactic substance is most likely to take place during maturation of the egg. It is possible that the production of the chemotactic substance triggers off the enlargement of the egg protoplasm. It is felt that the production of the chemotactic substance and the

build-up of osmotic pressure within the frustule (it took some 4 hours to build up and effect the separation of the valves of the male gamete mother cell) must also contribute to the time lag between the liberation of the male gametes and the appearance of germinating auxospores.

4. General Considerations

Lewin and Guillard (1963) expressed the view that "some conditions promoting the early onset of gamete production may do so merely by promoting more rapid cell division, while others may have a more direct effect by inducing gametogenesis in relatively larger cells." This may account for the observed vegetative multiplication in some, and spore formation in others, of the relatively larger diameter cells of a culture. In the induction experiments some cells underwent cell division whilst others commenced gametogenesis. Moreover this occurred during the exponential phase of growth when, although the growth rate was constant, the relatively larger cells were giving rise to cells of their own size as well as to cells slightly smaller than themselves. However not all the cells in the exponential phase of growth would be in the same state of metabolism at the same point in time in a non-synchronous culture. Thus under the conditions of culture, depending on the state of metabolism of the cells, external factors may have a threshold effect on some cells and not on others. As a result some would multiply

vegetatively whilst the others would be induced to spore formation. Such an effect would increase the number of relatively smaller cells which are readily induced to form spores. Presumably this accounts for the large proportion of relatively smaller cells of a culture observed undergoing spore formation.

Since spore formation occurs during the exponential phase of growth then there would seem to be no antogonism between the conditions favouring spore formation and those favouring vegetative growth. This accords with the view of Kreiger (1927) and Geitler (1930). Thus the factors favouring vegetative growth and spore formation appear to be the same, but the extent to which either activity may occur in a cell is dependent on its state of metabolism. If spore formation can be induced more readily in relatively smaller cells then this quality must be considered to be associated with some factor which, as reduction in cell size occurs, alters their metabolism to that required for spore formation. It is suggested that such a factor must be an expression of the genetic apparatus of the cell.

Srb and Owen (1958, p. 296) maintained that although some plants, as well as animals, regularly rely on environmental influences to direct the course of sex differentiation, this cannot imply an absence of a genetic basis for sexuality. Rather it illustrates that sex, like all other characteristics of developing organisms, is subject to threshold effects. Moreover,

von Stosch (1965) has pointed out that despite the importance of relative cell size as an internal factor in diatoms, their physiological qualities are also dependent on the genotype and on environmental conditions as in other organisms. However, cytological investigations on diatoms have only been concerned with elucidating the method of mitotic and meiotic nuclear divisions. Such investigations have revealed that the chromosomes of diatoms are very small and it is not known whether sex chromosomes occur as in other organisms. When such evidence is available it may be possible to explain the relationships among the internal physiological conditions, critical cell size, the influence of external factors and sexuality in diatoms.

8. SUMMARY

SUMMARY

1. Unialgal but non-axenic cultures were used in laboratory studies on the marine diatom Coscinodiscus pavillardii, Forti isolated from samples taken from the Sierra Leone River estuary.

2. The morphological characteristics of the clone and the taxonomic problems involved in its identification are discussed.

3. Growth rate was unchanged over the salinity range of 13 - 33°/oo and the diatom survived at a salinity of 10°/oo but not at 6.7°/oo.

The smaller cells (73 - 164 μ) grew faster than the larger cells (327 - 345 μ) but the relative growth constant remained unchanged for both categories of cells. The saturating light intensity was found to be about 6,000 lux and no inhibition was evident at nearly 15,000 lux.

4. Cells of 20 - 60% of the mean maximum cell diameter (464 ± 44 μ) could be induced to become sexual using an illumination of 5,500 - 8,000 lux in continuous light or in an alternating twelve hour light/dark treatment; at temperatures of 23° - 28°C; and at salinities of 19 - 33°/oo. A light intensity of 11,000 - 12,000 lux inhibited spore formation. The alternating light/dark treatment was more

favourable to spore formation than continuous light.

Cobalamin appeared to favour spore formation in as much as vegetative growth was maintained. Biotin influenced the quantity of spore formation. Thiamine and biotin media inhibited spore formation in as much as they inhibited vegetative growth.

5. Spore formation depressed the growth rate, the degree of depression being dependent on the initial number of cells involved in the activity. Induction of spore formation occurred during the early part of the exponential phase of growth.

6. Light, temperature and salinity are shown to influence the initiation of gametogenesis. Light plays the most important role in the determination of the sexuality of the cells. Moreover, it is considered that spore formation in the diatom is a result of a short-day quantitative photo-periodic response complicated by an additional ionic effect.

7. Temperatures above 28°C and below 23°C, as well as salinities of 16.5°/oo and below, inhibited spore formation.

8. Male gamete and zygote formation in the living material are described and illustrated by photomicrographs.

9. The occurrence of two cells in fusion is also described and illustrated by photomicrographs. This was observed in cultures of the diatom containing cells within the sexual induction size range.

9. APPENDIX

APPENDIX

Diatoms isolated and established in culture

The following diatoms were isolated and established in unialgal cultures:-

(i) *Hemidiscus cuneiformis* Wallich

(ii) *Eucampia zodiacus* Ehrenberg

(iii) *Streptotheca thamesis* Shrubsole

(iv) *Skeletonema costatum* (Greville) Cleve

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