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Contributions to our Knowledge of Freshwater Cryptomonads

By. E.G. Pringsheim, Göttingen [1)]

With Plates 148 (1) - 154 (7)

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1) Pflanzenphysiologisches Institut der Universität Göttingen (Institute of Plant Physiology of the University of Göttingen, Federal Republic of Germany)

Introduction

A microscopic examination of algal material often reveals flagellates which are conspicuous by their irregular swimming movement and are found to be Cryptomonads. Nothing is known about the circumstances under which they encounter good conditions for propagation. FRITSCH (1935, p. 652), who usually includes some ecological remarks preceding each class, omits them in the section on Cryptomonads. p.368
In most cases they are scantily represented among other algae, rarely in such quantities as to produce a peculiarly iridescent coloration. The cell structure of the Cryptomonads is imperfectly known, their physiology remains almost unexplored, except for the fact that they prefer waters rich in iron.

Many years ago (PRINGSHEIM 1944) I had, therefore, already attempted to obtain an abundant and homogeneous material by means of clonal cultures using the soil-water culture technique and limiting the investigation to freshwater forms. Although Dr. J.W.G. LUND of Windermere, England, whom we owe excellent descriptions, had assisted me in the most disinterested fashion, the planned monograph failed to take shape.

It is quite possible that it will never be written. PASCHER (1913) announced a monograph, but is has not been published. It is true, there are numerous descriptions, illustrations and names of Cryptomonads; but since no consensus could be reached on the taxonomic importance of their characteristic features, since it remained unclear whether these features were persistent and since they also appear in various combinations, it was impossible to accomplish a comprehensive treatment.

PASCHER's (1913) treatment of the Cryptomonads in "Freshwater Flora" was still based on an imperfect knowledge of forms and an inadequate understanding of the shape of these organisms. HUBER-PESTALOZZI (1950) presents all available diagnoses without reviewing them critically and without listing biographical references. Most of the descriptions are not adequate for identification purposes even where they are supported by such beautiful illustrations as the ones in GEITLER (1922, 1924) and SKUJA (1948, 1956). The number of taxa would appear to be excessive, and of these only

a few are described in a manner that would enable them to serve as stable points in the network of related forms. They are linked by intermediate stages.

SKUJA (1956, p. 347) finds (as did PASCHER 1913, p. 105 before him) "... more and more new forms upon closer examination of this polymorphous and heretofore badly neglected group which cannot be correlated with the forms that have already been described"; a situation which applies to asexual microorganisms in general (PRINGSHEIM 1962).

Great obstacles are encountered when an attempt is made to define the diagnostic characters of the Cryptomonads in a collection of mixed material: these obstacles include the usually small number of individuals, their constant movement, their instability under laboratory conditions, the difficulty in fixing them, the small size and indistinctness of the cellular constituents, etc.

I. Physiology

p.369

1. Clonal Culture

F. WETTSTEIN (1921) was the first to grow Cryptomonads under artificial conditions by means of peat agar. This was impressive but did not lead anywhere since his success was due to an accident as most of the species do neither grow on agar nor in media like his.

Useful clonal cultures in larger quantities were not obtained until the development of the soil-water culture technique in test tubes (PRINGSHEIM 1944, 1946a, 1946b). This technique permitted a certain approximation to natural conditions by using soil of varying composition, adding peat or nitrogen fertilizer and the like. Fortunately, the growth requirements of many strains proved to be not as narrow as they appear when they are cultivated axenically. The conditions in such cultures are subject to the respective life cycle and the organisms that are introduced are waiting, so to speak, until they are ready for reproduction as this would also take place in nature. Generally, individual cells introduced by means of the capillary pipette after precleaning developed within a few weeks to produce dense populations, provided care was taken to ensure the pH level was the same as that of the locality from where the cells came. Of course, this method involved a certain selection.

About 60 clones of Cyrptomonas were cultivated under water, usually with loamy soil, sometimes mixed with some peat. The living material was collected in Czechoslovakia, England, the U. S. A. and Germany. Most of the strains could be cultivated further in identical tubes. Since after the first isolation a large amount of cells could be transferred, the development proceeded much more rapidly from then on and supplied a healthy and more uniform cell material. This cell material could usually be maintained for months and was available for repeated studies and for comparison of the

various clones. If after some time the cultures appeared to deteriorate, which happened particularly when they were invaded by iron bacteria, they were cleaned again by washing.

Not all of the Cryptomonads collected produced cultures, and not all isolated strains could be perpetuated indefinitely. They were limited to 20 as diverse strains as possible, one of which is 30 years old, several others are more than 20 years old. They have not changed as will be seen from a comparison with old sketches.

Although this provided a beginning in clarifying p.370
the wealth of forms and the stability of the diagnostic characters, the publication was nonetheless delayed more than once since the taxonomic units could not satisfactorily be defined.

This was due to the fact that even with good nutrient supply as evidenced by abundant growth, the conditions in such cultures vary considerably both in terms of time and space. This is one of the peculiarities of the soil-water culture technique. When development commences, clouds of swarmers or coloured spots are revealed on the glass by means of the magnifying glass. Thus, the organisms are not evenly distributed in a homogeneous liquid but assume a certain position relative to the light, to the soil slurry surface, to the direction of gravity, or the like.

Suitable conditions must obtain at the points where growth commences and, in this regard, it is likely that phase boundaries (PRINGSHEIM 1964) are important habitats. No attempts have yet been made to study the conditions at such points, e.g. to measure the redox potential. In addition, growth does not take place at the starting points immediately but a certain period of time has to elapse first. What we are observing is not a steady state but a stage that must be passed through. In most cases, the space occupied by the cultivated organisms gradually expands and may temporarily encompass the entire culture liquid.

This technique, therefore, involves both advantages and disadvantages. Different conditions prevail at different depths of the culture vessels and these are subject to local shifting in the course of time, thereby possibly exposing the organisms to an unfavourable environment. Unless, tactically guided, they can withdraw to a more favourable habitat, they assume steady states or perish.

The differences that exist within the culture space have a particularly conspicuous effect on Cryptomonas whose members are evidently limited to narrow environmental conditions. This is evidenced by a cessation of movement, accumulation of reserve food substances and discoloration. In primary cultures arising from a single cell, only a small number of cells of a population are in a viable state and show a healthy colour. The ratio is more favourable after mass inoculation; but even then it was often barely possible to choose the "right" condition for diagnostic purposes.

2. Pure Culture and Nutrition

In order to determine the morphological and physiological characteristics it was necessary to eliminate foreign organisms and to use defined media. However, we were not quite successful in providing a homogeneous cell material. But whenever suitable conditions of growth were found, axenic cultures constituted a considerable step forward in this direction. p.371

Foreign organisms could usually only be separated by means of the washing technique. Collecting them with a capillary pipette and repeating this several times did occasionally not succeed because of the minute size and susceptibility of the cells. The main obstacle was the necessity of finding the correct pH level which was not easy since the organisms can tolerate only highly diluted and therefore poorly buffered solutions. If the pH level was unfavourable, the cells adhered to the glass or seemed to disappear completely. If enough cells could without damage be passed through five sterile baths then the game was won.

Sixteen strains have been cleaned in this manner. My Micrasterias solution, which contains potassium nitrate and ammonium phosphate (PRINGSHEIM 1946b, 1954) proved to be a suitable starting point for producing the nutrient solution. When adding trace elements, special attention should be given to iron, particularly since waters rich

in iron are preferred by Cryptomonads. This is achieved by separately dissolving ferrous sulfate with half of the EDTA and using the other half for chelating the remaining trace elements.

The species of Cryptomonas are limited to a narrow pH range. The species I cultivated may therefore be subdivided into three groups. In strains deriving from peaty locations in the Weser and Harz Mountains the optimum pH range was between 4.5 and 5.5, or 6 at the most, in those from nutrient-rich ditches near Cambridge, England and from the polytrophic pond in the botanical gardens of Göttingen the pH ranged from 6.7 - 7.2 and in those from garden basins and from pools near Göttingen the pH range was 7.5 - 8.5. They are thus adapted to acid, neutral or alkaline waters. The threshold values are somewhat affected by the composition of the nutrient solution; inoculated cells suffer from marked shifts but these are accepted as a consequence of development.

I conducted my first culture tests in 1928 using material of Cryptomonas from the botanical gardens in Prague. A trace of sulfuric acid had to be added to my algal agar together with inorganic nutrient salts and soil extract in order to obtain cell development. Apart from colonies of green algae, numerous loosely mucilaginous, brownish colonies developed from the material admixed to the liquid agar which responded in the same manner upon repetition. Without acidification no growth took place. Pure cultures were not obtained at that time.

The pH range for developing a culture from a few p.372
cells is usually narrower, even where the other factors are favourable, than that for the washing process since they are subjected to this action for only a short period of time. The cleaned cells must therefore be placed in a series of nutrient solutions with closely graduated pH levels in order to ascertain the best pH level. In nutrient solutions using acetate this can be achieved to some degree, but in purely mineral nutrient solutions this is left to chance. In addition, such solutions, even where a series of variations is employed, provide only scant growth and no development at all after a second inoculation.

RODHE (1948) was unsuccessful in propagating a strain designated as Cryptomonas ovata without the use of soil extract or lake mud. HUTNER and PROVASOLI (1951, p. 102) note: " In our experiments, the growth of a Cryptomonas proceeded only in the presence of soil extract, peptone and the like." This indicates vitamin requirement. In fact, cobalamine (B_{12}) appears to be generally required although this has not been conclusively determined for all strains.

The first pure culture, identified as 979-3 under the name Cryptomonas ovata var. palustris in my collection, was isolated in 1939 by means of the WETTSTEIN peat-agar plate technique. The colonies were small, dark brown and could be moved as a unit on the agar. In liquid the strain produces flocs and a non-mucilaginous rim on the meniscus when the growth rate is high. Only highly diluted solutions

permit growth. Meat infusion and sodium acetate promote growth provided no more than 0.1% is used. A weakly acid to neutral pH is best. Little growth occurs in darkness even in the presence of organic nutrients.

Other strains exhibit a similar behaviour but not all. Many strains growing in soil-water test tubes and in solutions for axenic cultures do not grow on agar. This ability is limited to those strains which form palmelloid stages and is usually associated with the secretion of a gelatinous matrix; by contrast, the continuously motile strains do not grow on agar. This also includes all species studied so far of the colourless parallel genus Chilomonas.

Apart from the adaptation to a certain pH level, we find that the secretion of mucilage is thus a further property for discriminating the various strains. However, these two properties have no systematic but merely an ecological value.

3. Colour

One of the most commonly used diagnostic characters in describing and designating the genera and species of the Cryptomonads has been the pigmentation of the chromatophores. p.373 Apart from the colourless members of the genus Chilomonas, it has been common to distinguish the blue-green forms as Chroomonas from the usually grey-olive to brownish species of the genus Cryptomonas, and the red forms as Rhodomonas. In an

earlier publication (PRINGSHEIM 1944) I rejected the pigmentation as a generic character since it was found to be unstable and since in other classes of algae, e.g. the Chrysomonads, diatoms, Dinophyceae and Rhodophyceae, colour cannot be used for discriminating purposes.

The other characters used in Rhodomonas apart from the colour, particularly the absence of a gullet, have turned out to be uncertain while in Chroomonas the cytological and physiological characteristics, although present, are only of subordinate importance.

One strain of Rhodomonas common in the Lake District and made available to me by the Freshwater Association of Windermere, England, did not correspond exactly to any of the described species of the genus. It neither differed in pigmentation nor otherwise from small delicate Cryptomonads.

As GEITLER (1924) has already noted, there are blue-green Cryptomonads which, by their cell structure, should be placed in Cryptomonas, i.e.which do not, as repeatedly stated, possess only a superficial furrow in place of a gullet (PASCHER 1913, p. 104). BURTON-ROSENBERG (1945) has confirmed this. PASCHER lists two species: the elongate Chroomonas nordstedtii and the almost spherical Ch. pulex. I have isolated five clones which had blue-green chromatophores. The gullet was not always discernible. None of the strains corresponded to PASCHER's species. They were all more rounded than the species of Cryptomonas commonly are, had a pyrenoid with few starch grains and a group

of ruby-red grains as are also illustrated in ROSENBERG.

A blue-green form which I found in Fenditton near Cambridge, England, in 1943 and 1944, has been maintained in culture for years. With increasing age it turned pale green as do the strains of Cryptomonas. Its shape was broadly ellipsoidal, its size 11-13 x 7-9 µ. I collected a narrower species (7 x 4.5 µ) from a pond near Dinas Cross in Wales in 1945, in the Flatford Mill Field Centre I found a form on green-stained mud in brackish water, together with Euglena obtusa, which was similar to that found in Fenditton but measuring only 9 x 5-6.5 µ, and in Swatham Prior Fenn, a marshland east of Cambridge, I found a Chroomonas measuring 13-16 x 5-8 µ which resembled that of Fenditton. All these blue-green Cryptomonads had unsteady but quickly swarming movements, not quite as in Cryptomonas. None of the strains could be grown as a pure culture. They are thus physiologically different from Cryptomonas just as blue-green Dinophyceae and Porphyridium aerugineum deviate from the differently coloured species. p.374

Which colour, then, is indeed characteristic of the genus Cryptomonas exhibiting such a wealth of forms?

No progress has been made since DANGEARD (1889, p.8) in answering this question. He found that Cryptomonas erosa EHRBG. may be green, yellow or violet. 60 years later, HUBER-PESTALOZZI (1950, p. 10 and 41) refers to this species as being brownish-green (olive-green), blue-green, red or yellowish, without finding anything unusual

in the obvious contradiction this statement implies. Several authors regarded environmental factors as being responsible for the variety of colours, e.g. the effect of acid bog or swamp water or the contamination by decaying matter (LEMMERMANN 1910, p. 425; PASCHER 1913, p. 106). The latter observed the preponderance of red forms in the bottom zones of lakes, particularly also in Cryptomonas. He concluded that some species were red or blue only under certain circumstances while others retained the same colour throughout. ZIMMERMANN (1924, p.5) likewise found that the colour of the chromatophores was variable with the major factor affecting colour change being illumination. He observed, however, in Rhodomonas baltica KARSTEN, that the shade appeared to be influenced by the food supply, which was as close to the truth as he could get.

FRITSCH (1935, p. 635*)) notes that the pigmentation in one cell material is often very variable, but that diverse shades of brown are most frequent, which is correct.

SKUJA's findings are, by comparison, retrogressive. Without any reservation (1939, p. 92 ff; 1956, p. 344 ff) he uses colour as a taxonomic character, such as in Cryptomonas rufescens: "Colour of the chromatophores in spring and late fall perhaps a shade more olive-brown, yet always olive-green.". C. reflexa is also described as being olive-green. Repeatedly, for example 1956, p. 344, he

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Translator's Note:

*) p. 635 is deemed to be in error and should read p. 653.

emphasizes the more or less green, brownish or reddish colour as being a species-specific character. HUBER-PESTALOZZI (1950, p. 41) has followed this example in his identification key. Further examples are found in GRAHAM (1951, p. 117) where the shades are said to change from yellow-green to brownish and red, and in HOLLANDE (1952) where the chromatophores are said to be usually olive-green but where the colour changes in the same species depending upon the pollution of the water. FOTT and ETTL (1959) provide a detailed description of C. ovata without mentioning the colour - perhaps because they have realized its unreliability.

A dense mass of healthy cells in a transparent colourless solution produces a peculiar colour impression in all strains which is difficult to describe. Depending upon the direction of light and the angle from which it is viewed one sees a differently coloured mass, almost as in certain textile fabrics which are iridescent in several colours, with reddish-brown being predominant, the same tint which the chromatophores exhibit under the microscope p.375 in transmitted light. A more orange-yellow or greenish sheen can also be observed in many cases. This is true for aggregates at the bottom of good cultures.

In cases where the cells are distributed in the liquid, the latter appears in a somewhat different colour, depending upon type and density, but always conspicuously pale and transparent. Fairly good growth may have taken

place already without this being apparent to the naked eye. This transparency of the cultures also obtains in Chilomonas (PRINGSHEIM 1963, p. 19). In the explanation to Fig. 1 it is stated: "... which is characteristic of Cryptomonadaceae."

The intensity of the pigmentation varies in the different strains and is characteristic thereof; but when they are healthy they are all lighter or darker reddish-brown. The degree of darkness of the pigmentation is seen to vary when the chromatophores are viewed in the microscope, ranging from skin- to chocolate-colour.

Identical cells throughout cannot be obtained without mixing even in the most beautiful cultures. Since the Cryptomonads tolerate only diluted nutrient solutions, deficiency symptons occur soon followed by discoloration. It has previously been noted (PRINGSHEIM 1944) that even cultures that exhibit a bright reddish-brown will gradually discolour, turning olive-green and later ochre and straw-coloured. This sympton may be compared to the discoloration of the Cyanophyceae in old cultures which is caused by an insufficient supply of nitrogen and phosphorus compounds (PRINGSHEIM 1963, p. 109). In both cases many cells survive despite starvation and resume active life accompanied by healthy colour when fresh nutrients are again supplied.

Again, as is the case with Cyanophyceae, the intensity and composition of light is an important factor in determining the pigmentation, for example in the bottom part of lakes (GEITLER 1922, p. 688; ZIMMERMANN 1928, p. 16;

KYLIN 1935, p. 11; LINDSTEDT 1943, p. 104). The so-called chromatic adaptation in the Cyanophyceae is not, strictly speaking, caused by the colour of light but, indirectly, by the effect it has on the nutrition.

It is not easy to clarify the changes in colour that occur in pure cultures of Cryptomonads. If, from the very beginning, the concentration of nutrient elements is reduced, almost no growth will take place. It seemed more promising to allow a series of synchronized cultures to age and then, upon discoloration, to place each in a different nutrient salt hoping that reactivation would indicate which type of deficiency had produced aging. Although reactivation took place after 2-3 weeks, the results in the individual p.376
cultures showed nonetheless that without an unfeasibly extensive test program it would not be possible to obtain conclusive data. A mere change of location may be sufficient for initiating renewed swarming without thereby revealing the activating mechanism.

The coloration of the Cryptomonads is most striking in cultures on agar slant provided they grow thereon. The thick colonies are light brown to chocolate-coloured, depending upon the pigmentation of the chromatophores, and the density of the cells in the aggregates. In soil-water cultures, cells occasionally accumulate in clusters surrounded by soil on the illuminated side which appear deep reddish- to sepia-brown.

The composition of the pigment mixture is known in general terms from an examination of abundant material collected over a period of time. The examination reveals that chlorophyll a and c, phycocyanin, phycoerythrin, α-carotene, β-carotene and xanthophylls are present (BOGORAD 1962, p. 386)[2]. The biliproteins of the Cryptomonads, which resemble the phycocyanins and phycoerythrins of the Cyanophyceae and Rhodophyceae, dominate the colour impression. When the cells are killed, the biliproteins are excreted and colour the agar or solution a lilac pink, with the cell mass left behind as a greenish residue. In some strains, the colour concentration is so low that the liquid is scarcely coloured, even in good cultures, by the water-soluble pigments excreted from the killed cells.

4. Reserve Food Substances

The reserve food substances in Cryptomonads consist of starch and oil.

The cryptomonad starch appears to be the same substance as that in the Dinophyceae and Chlorophyceae as well as that of higher plants. It is also present in the

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2) GOODWIN (1964), in his treatment of the relationships between plastid pigments and systematic position of the "Protozoa" considers only the carotenoids: carotene and diatoxanthin, but disregards the chlorophylls and water-soluble pigments and morphological features. This, of course, led him to draw the wrong conclusions.

colourless Chilomonas which can manufacture it from various organic nutrients. As is common in apochlorotic parallel forms, more starch is deposited there than in the phototrophic forms, and removal of starch is scarcely possible without causing damage.

In Cryptomonads the starch usually gives the typical blue coloration with a small amount of iodine provided care is taken to ensure the proper conditions for the more physical than chemical reaction, i.e. avoiding an alkaline pH and excessive salt concentration. Occasionally the grains take on a brown-violet colour, as is also the case with other organism, but why this is so is as yet unknown. p.377

The starch grains adhere to the inner surface of the chromatophores, often so densely that they are flattened polyhedrally. Under circumstances unfavourable for growth, the cell body may be filled with large starch grains to the point of expansion, particularly so in Chilomonas (PRINGSHEIM 1963, p. 39ff.).

When cells that are rich in starch are exposed to conditions where the products of metabolism from photosynthesis or from chemical substrates are lacking but where the other environmental conditions permit growth, the starch will disappear save for some traces but will quickly reappear as soon as there is light or upon supplying suitable substrates.

The starch is not embedded in the chromatophores

but adheres to their surface, as in the Rhodophyceae, or is free in the cytoplasm. Even where pyrenoids are present, which rest on the chromatophores, the starch grains are deposited thereon only on their free surface, usually not as rounded grains but rather in the manner of a watch glass. Under these circumstances it would seem that the existence of special starch forming agents in the colourless Chilomonas was not plausible, i.e. of unpigmented organelles in the cytoplasm which the older authors believed to have identified by means of iodine. They could also not be found later with the aid of the light microscope (PRINGSHEIM 1963, p. 27, 30). However, they were later discovered by means of the electron microscope (JOYON 1963). But it is surprising that these structures should have turned out to be leucoplasts on the grounds that they contain starch grains because this is not the case in the pigmented chromatophores; unless, of course, one assumes that in this instance the plastid membrane envelops the pyrenoids and starch grains.

In the Cryptomonads, oil, apart from starch, is present in considerable amounts, i.e. it is irregularly distributed in the cell in the form of droplets. It stains with Sudan colorants and is blackened by OsO_4. It would appear that, while starch predominates in resting cells, oil is prevalent in degenerated cells.

Whenever individuals are insufficiently, but not inharmoniously, nourished, i.e. in a highly diluted nutrient solution, under weak illumination, or, in Chilomonas,

when there is a deficiency in an energy-supplying substrate, the Cryptomonads, and particularly Chilomonas, will consume p.378 their own body substance as is done, for example, in Paramaecium. When HUBER-PESTALOZZI (1950, p. 47) affirms the reserve food nature of starch in Cryptomonas by stating that it disappears "under unfavourable environmental conditions" within 24 hours, he only sees half the truth. Depending on the circumstances, various different conditions of starvation and corresponding cytological changes will ensue.

The cells of the pigmented Cryptomonads often contain one or more special structures which are located closely beneath the surface in recesses of the chromatophores. Such a granule is frequently visible at the posterior end in particular where the two chromatophore lobes are almost in contact with one another. They often occur also on the side when a chromatophore withdraws slightly from the wall. Since they are always embedded close to the periphery of the cell body, I call these structures "Randkörper" (in English: "peripheral bodies") (Fig. 8). They are highly refractive, not precisely spherical and are not stained by any of the examined stains. SKUJA seems to regard them as leucosin known only for Chrysophyta, but visually alone there is no resemblance whatsoever between them. They also do not vanish when the cells are killed.

I will list some of SKUJA's erroneous conclusions to show that I do not wish to do him an injustice. They are contained in his 1948 publication but have never been corrected.

P. 346. Cryptochrysis commutata "not uncommon". Just where a description and drawing of this dubious form would be most helpful, it is lacking. P. 351. Cyanomonas: Discoid chromatophores composed of 4-6 discs. The form is "rarely found among other Cryptomonads", which increases the doubts. It would appear that SKUJA has misinterpreted the seemingly pigmented starch grains. More detailed particulars would have been desirable all the more so since (PRINGSHEIM 1944) attention had been drawn to this error. P. 353. One species is said to include a "stock" having a shallow and another having a deep gullet. How, then, can he use this characteristic as one of the most important in the taxonomy of Cryptomonas? And this without further comment in the otherwise verbose description. P. 353. "Transformation of the pyrenoid into a viscous, satiny substance somewhat resembling leucosin, presumably volutin"! P. 354. MAUPAS' ovals, which were unknown to SKUJA, are described as highly refractive "pyrenoid-like" grains. P. 356. There are two types in Cryptomonas ovata SKUJA: one with a gullet that extends to the cell centre and one with a highly elongate gullet (see also p. 353). P. 357. Here the ovals are said to be "volutin-like" bodies!

FRITSCH (1935, p. 654, Fig. 216), who does not mention MAUPAS' ovals, assigns the letter n for nucleus to the granules at the posterior end, as did SENN (1900) and PASCHER (1913, Fig. 169a) before him. However, the small size, high refraction and the occurrence of more than one

of such structures in the cell render such interpretation improper. The nucleus is disposed somewhat anteriorly thereof and is considerably larger.

5. MAUPAS' Ovals

p.379

The ovoid, double refracting structures named after MAUPAS (1885) are cellular constituents which are peculiar to Cryptomonads. They had been drawn as early as 1870 by CIENKOWSKY and 1878 by STEIN but not yet named. SCHEWIAKOFF (1893) and DANGEARD (1910, p. 209) described them in more detail and found them to be puzzling, which they have remained to this day. They have often been observed; but usually the authors were not aware of the earlier findings. MAST and DOYLE (1935) and even SKUJA (1948, 1959)much later seem to believe that they have discovered something new[3]. It is, therefore, not surprising that these structures have been given a variety of names, e.g. "Exkretkörper" (excretory bodies) (SCHEWIAKOFF 1893), accessory bodies (GATENBY and SMYTH 1940), "corpuscules biréfringents" (HOLLANDE 1942), "Ellipsoide" (ellipsoids) (GALIANO 1942) and HUBER-PESTALOZZI (1950, p. 39). I call them "ovals" for the sake of brevity.

Because of the double refraction of the ovals, DEFLANDRE (1938) made reference to the crystalline excretions of the ciliates by way of comparison without elaborating thereon any further. SKUJA has regarded the structures as a kind of pyrenoid substitution even though they may occur side by side with them in the same cell.

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3) GATENBY and SMITH as well as SKUJA confuse them with pyrenoids.

MAUPAS' bodies are highly refractive, elongate-ovoid structures, appearing black before a bright background, which are usually located, in pairs, close to the anterior end of the body, with their longitudinal axes at an oblique angle to one another. They dissolve in acids and alkalis and disappear as soon as the cells are squashed. Their chemical composition is not known. They are presumably rich in phosphate.

The ovals are found in many, if not all, Cryptomonads, but only under certain conditions which have not yet been investigated. They appear in cultures that have lost their vigour but not in thriving populations. HOLLANDE's (1952, p. 292) conception that occurrence and structure of the ovals are influenced by the food concentration is based on correct observations but is not sufficient. If the ovals are excretions, they are of a type than can be reabsorbed into the metabolism because they disappear again after transfer from old cultures into a fresh nutrient solution (PRINGSHEIM 1963, p. 165, 166, p. 384 note). There is no evidence which would deny that the ovals have reserve food character.

MAUPAS' ovals have been observed in many instances. JAVORNICKY (1956) mentions them in all five of the species he describes. SKUJA draws them occasionally. They have p.380 no systematic value within the class. They are also found in Chilomonas, i.e. in all species and/or strains that have been investigated (PRINGSHEIM 1963, p.166-168, Figs. 27, 28,

29, 30; JOYON 1963a, 1963b).

Staining experiments done by various authors did not yield any conclusive results. A special sheath could be identified by means of the electron microscope. But admittedly this did not shed any light on the nature of the ovals. This can only be expected from culture experiments supported by chemical techniques.

During cell division, one of the two ovals is transferred to each of the two daughter cells. This implies that the ovals are specific organelles in the cell. Soon a second oval appears but its origin is unknown. Division and new formation account for the often considerabe variation in size of the two ovals. More than two ovals are rarely observed and must be regarded as anomalous. In certain populations I consistently found only one oval in each cell.

II. Morphology

1. Shape and Size

It is difficult to describe the shape of Cryptomonas because the body lacks symmetry so that it appears different depending from which angle it is observed.

Thus, when FRITSCH (1935, p. 652) primarily emphasizes the dorsiventral construction and the flattening, he fails to convey a satisfactory picture, all the more so since some species are not oval or elliptical in cross-section,

as he indicates, but approximately circular. FRITSCH then views the shape of the cell in side view and differentiates between a dorsal and a ventral side. But these are not the usually upper and lower and, according to him, flattened parts of the surface but instead are perpendicular thereto. Viewed in this manner the more arched (convex) part of the outline is the dorsal side, the approximately flat or somewhat withdrawn (concave) part is the ventral side.

HOLLANDE (1942) does not elaborate on the shape of the Cryptomonads in his 50 page description of these organisms. This and many additional omissions may well be due to the fact that he has used fixed material for his investigations.

To describe and illustrate the shape of Cryptomonas p.381 is not only made more difficult by the asymmetry of the body but also by the constant movement of healthy cells. Inhibiting agents and fixatives alter the shape of the delicate organisms. Osmium tetroxide, corrosive sublimate, formaldehyde, etc. will paralyse the flagella, but the form of the cell is preserved for only a very short time. The best technique so far has been to mechanically arrest the organisms, e.g. on the thin surface of small hanging drops.

One characteristic of the cryptomonad shape is its spiral torsion although it is occasionally not very pronounced. FRITSCH only mentions a shallow, oblique furrow which gradually dies out posteriorly. However, the spiral torsion is recognizable from the shape of the entire body and the orientation of the inner parts, as CONRAD (1942) describes

this for one species but may, to a certain degree, be extended to all.

PASCHER (1913, p. 96) relates his description regarding the form of the organism to the "side view" so that the outlines are determined by the dorsal and ventral side (in the sense of FRITSCH). However it is not possible to clearly define the shape of the body in this manner. The curved species of Cryptomonas are lacking in PASCHER[4].

One and the same cell may appear straight or curved depending on its orientation relative to the microscope axis. The degree of torsion proves to be variable when comparing many strains;but it is always recognizable. During rotation, the posterior end of flattened and curved cells of the Cryptomonas curvata group appears more pointed when the organisms are swimming than it really is. This is an optical illusion. The pointed end which SKUJA (1948) and SCHILLER (1954) depict for Cryptomonas marssonii and Chilomonas acuta respectively is not a sharply conical tapering but rather the side view of the flattened, narrowed and curved apical end. (PRINGSHEIM 1963, p. 168).

The degree of flattening and thus the cross-sectional form are to some extent influenced by the food supply. When the cells are filled with products of assimilation they are thicker than during vigorous growth and less twisted;

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4) According to a letter from Prof. FOTT (August 9, 1960), C. curvata is one of the most common species and is the only one which can be recognized from EHRENGERG's illustration, "apart from the fact that it is not curved at all".

under the influence of starvation they are thinner and more twisted, with the posterior tapering being more prominent. A strain may consist solely of "tailed" cells when found, whereas they are posteriorly more rounded in a flourishing culture.

The internal structure of the Cryptomonas cell is also dominated by the spiral symmetry, e.g. the manner in which the depression extends from the anterior end into the p.382
interior and which is usually identified erroneously as "Schlund" ("gullet", "pharynx", "sillon") but in contrast to most ciliates does not perform the function of ingesting food particles just as there is no ingestion of food by way of similar grooves in other flagellates.

The tubular to sac-like depression either extends almost to the centre or somewhat further posteriorly and is closed at the end. Its depth, which has been utilized as a taxonomic character since EHRENBERG, is somewhat difficult to determine; but generally it is lined with granules which are in turn arranged in a spiral fashion. Their outlines are thus easily recognizable. This depression does not extend in a straight line rearwardly but in a curved line as part of a spiral. The oblique orientation of the ovals also reflects the internal spiral tendency.

The Euglenineae also reveal spiral symmetry in contrast, for example to the Volvocineae (PRINGSHEIM 1956, p. 10). But theirs is of a different nature and cannot be regarded as an indication that these two are

related even though, in the Euglenineae, it does not only determine the external shape but also the internal structure of the cell (LEEDALE, MEEUSE and PRINGSHEIM 1965, p. 137).

The function of the glossy granules lining the depression is to secrete protective mucus. Under certain conditions, mainly chemical stimuli, they eject long slime threads and are therefore called trichocysts, but they differ from the organelles of the same name in the ciliates both in structure and function. Such mucilaginous bodies are present in all Cryptomonads but also in the colourless Chilomonads where they are not only situated in the depression but also on the surface of the body (HOLLANDE 1942).

The slime threads, among other things, form a protective sheath against injurious influences in a similar manner as in the species of the Euglena sanguinea group (PRINGSHEIM 1956, p. 91). Presumably they are also responsible for the peculiar recoil action during discharge characteristic in Cryptomonads which takes place without the action of the flagella, as was evidenced by observations under conditions of darkness (cf. HOLLANDE 1942, p. 35ff.). When the slime threads are ejected it is no longer possible to recognize the glossy granules in the depression which makes it outline indistinguishable. In the intermediate stage, the otherwise regularly arranged mucilaginous granules break up leaving gaps therebetween, which would support the above interpretation.

Older authors, e.g. PASCHER (1913), maintain that there is no depression proper in certain Cryptomonads but only a superficial furrow lined with mucilaginous bodies. They regard this type of structure as primitive and attribute taxonomic significance to it. This conclusion p.383 is based on the examination of delicate, poorly fixed cells (Sennia, Hillea). Sometimes it is difficult to tell where exactly the boundary of the groove runs. But in most cases the depression extends into the interior. Its blind end can be adjusted in the microscope together with the nucleus. In Chroomonas, which was said to possess no "gullet", ROSENBERG (1944) has found one and I can confirm this. It is very likely that we will find a gullet everywhere once better techniques are available.

In the most recent statement on Rhodomonas, JAVORNICKY (1967) cites newer authorities for the lack of a gullet in the Cryptomonadaceae. He lists the features which are characteristic of Rhodomonas, among these pyrenoids surrounded by a shell, but which are not illustrated in his 11 drawings. None of these constitutes a difference relative to Cryptomonas, certainly not the statement: "... in some specimens at least a reddish colour."

It has been affirmed that Cryptochrysis does not possess a gullet but no convincing observations are provided. Cryptochrysis pochmannii HUBER-PESTALOZZI is said to differ from Cryptochrysis commutata PASCHER only by the

presence of oval bodies, which he also uses elsewhere for discriminating the various species[5].

An attempt to utilize the depth of the depression, the flattening and curvature of the body or the like for discriminating the various species groups has had little success since such features occur in changing combinations.

In Cryptomonas, the dimensions are not as important as in other flagellates since there are wide variations within the individual clones which are due to unequal cell division. If, for example, a range of 18 - 25 µ is specified as the length of one species, this may well be true for a clone, whereas such a wide range would indicate varieties as regards size in Oscillatoria or Euglena. Although each taxon of Cryptomonas has a specific size, the range of variation is nonetheless conspicuously wide, particularly under unfavourable environmental conditions, following a general biological principle.

The dissimilarity of the sister cells is associated with cytological characteristics as a result of which one daughter cell receives the entire flagella apparatus while the other produces it anew. Prior to cell division, a second blepharoplast is formed which produces two new, somewhat unequal flagella (HOLLANDE 1942, p. 34, Plate III, Fig. 9, 10, 11). Thus, the blepharoplast does not undergo division,

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5) According to SKUJA (1948, p. 350), PASCHER's observations on the absence of the gullet in Chroomonas and Rhodomonas are incorrect.

as, for instance, in Euglena, in order to produce two new and identical flagella from each half which would result in two unequal pairs. It is assumed that the new formation taking place in one of the sister cells originates from the nucleus. There are several indications that the sister cells are not identical in Cryptomonas.

The dissimilarity of the daughter cells is apparently responsible for the difference in size of the individuals in a clone. The Cryptomonads differ in this regard from other organisms. In the diatoms, the difference in size within a clone is due to the insertion of the new cell wall into the old, in the Chlamydomonadaceae it is due to the formation of several daughter cells in a parent cell, in Euglena only the transverse diameter changes during division while the length remains the same and this is so because there is a strict division into two halves which affects the flagella apparatus as well (PRINGSHEIM and HOVASSE 1950). p.384

2. Cell Structure

The internal structure of the Cryptomonas cell is greatly influenced by the previously mentioned invagination of the anterior end. Situated anteriorly of this opening is a contractile vacuole which is divided into two halves during cell division.

The most conspicuous organs are the chromatophores. Most of the authors assume that there are one or two. HOLLANDE (1942) suggests that one chromatophore only is

present consisting of two parts linked by a ridge. JOYON (1963) follows this view which may well obtain in most cases.

The chromatophores are not always easy to recognize. They cover the major part of the surface closely beneath the outer membrane, are often of irregular outline shape and leave a free triangular space at the anterior end and, at the opposiste end, mostly a strip as well. The chromatophore lobes, which are of unequal size, often leave only a narrow colourless strip therebetween which resembles a wavy band. The margins are then approximately parallel to one another, giving the impression as if the chromatophores avoided contact with each other, similar to the chloroplasts of other plants which are lenticular when there is sufficient space but polyhedral otherwise, although they do not touch one another.

Where pyrenoids are present, each adheres to one half. Their number need not correspond to that of the chromatophores as in the Euglenineae. But apparently the ridge may be withdrawn, thereby indeed providing two chromatophores. This phase may however be indicative of an impending cell division. After division, it would seem, only one two-lobed chromatophore is present in each instance. Whether or not various species may be discriminated in this manner will have to be investigated further.

The unequally long chromatophore lobes usually cover the ventral and dorsal side, with the dividing line extending approximately lengthwise over the flank. In a p.385 Cryptomonas anomala described by FRITSCH (1914), the "two"

chromatophores are apposed to the relatively flat flanks. It would thus appear that number and position of the chromatophores have no particular significance.

The pigmentation of the chromatophores varies in intensity in the various species but in healthy populations (cf. p.374-376 German text; p. 13-17 English translation) it is always a reddish-brown shade, ranging from almost skin-coloured to chocolate or sepia-brown. When lying flat, the two flanks usually appear darker than the rest since the light must penetrate a thicker pigment layer than on top and at the bottom. The shade is however not the same in all species. Some are more brown, others almost a pure coral-red. But this cannot be used as a generic character which would distinguish Rhodomonas from Cryptomonas since there are intergradations and since other characters are not available for discrimination purposes taking into account that there is still uncertainty as to the apparent absence of a depression proper in Rhodomonas - unless this question will be resolved in the future[6].

The cryptomonad nucleus is a relatively large transparent vesicle in the posterior one-third of the body. It shrinks upon fixation which makes it more distinct and a granule, invisible in the living cell, appears in its centre. It is not known whether this is a nucleolus which dissolves

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6) LUND (1962) notes with respect to Rhodomonas:

"... it may be that a gullet is present as in other Cryptomonads but it is not clear here because the cells are so small. In general this distinction between the Cryptochrysidaceae and Cryptomonadaceae is open to doubt."

(cf. p. 383)

during cell division as in other organisms, or a centrosome, as in Euglena (LEEDALE 1958, 1959), which remains present and exercises a support function. Its invisibility in the living cell would support the view that it is analogous to Euglena.

According to HOLLANDE (1942, p. 63) the nucleus may be indented anteriorly by the depression of the cell. This would be one of the cases where a plasmic organ, by external forces, is forced to assume a form that is different from its own shape; but perhaps this is merely the result of fixation.

SENN (1900, Fig. 169a), PASCHER (1913, p. 96) and FRITSCH (1935, p. 654) have identified a rounded structure at the posterior end as the nucleus. In fact it is smaller and more highly refractive than the nucleus which is situated anteriorly thereof and which they overlooked, and it belongs to the structures which I called "Randkörper" (peripheral bodies) (p. 378 German text - p. 21 English translation). SKUJA (e.g. 1948, p. 12), presumably on the basis of fixed material, shows the two organelles at the proper position.

After DANGEARD (1910) and BELAR (1916) it was p.386
mainly HOLLANDE (1942) who examined the nucleus and its division in Cryptomonas and Chilomonas and who presented his views in numerous drawings. He emphasized the behaviour of the Cryptomonads which differs from that of other organisms and therefore renders an understanding more difficult. His results are somewhat unsatisfactory but this may only

partly be due to the susceptibility and poor fixability of the organisms. The figures, although making it appear cruder, reveal this. HOLLANDE seems to have missed the phase of karyokinesis proper during fixation and thus, by accident, observed delayed and disturbed figures of cell division. He does not indicate when he fixed his material. Phototrophic flagellates usually divide after nightfall.

The chromosomes are numerous and minute. An arrangement in two parallel plates has been seen by several authors. THAKUR (1965) suggests that HOLLANDE may have misinterpreted the metaphase. It is also not clear whether the nucleolus and the nuclear membrane disappear during division. A spindle has not been seen.

The most recent investigations (GODWARD 1966) report only on the number of chromosomes. Thus, for example, they number 209± 7 in strain 37 of my collection, whereas Cyanophora, also from my collection, and only tentatively placed in the Cryptomonads, has only 10 chromosomes. Because of the large and highly varying number of chromosomes depending on the species it is assumed that the chromosomes are polyploid and anomalies are suspected to be due to the long period of culturing the cells artificially, an assumption with which I cannot concur without new investigations. Cryptomonas ovata var. palustris, having been grown in pure culture for 25 years, possesses 86 ± 6 chromosomes which indeed is much less than Cryptomonas 37 cultured for 20 years

in soil-water tubes, i.e. under more natural conditions. Chroomonas mesostigmatica with 24 ± 2 chromosomes may well be diploid.

These investigations, likewise, fail to elucidate the behaviour of the nucleus during division, i.e. nuclear membrane, nucleolus and spindle, just as they do not reveal anything about the process of cell division and duplication of the various organelles.

3. Motility

The Cryptomonads are not always motile but, in many species tend to adopt the palmelloid stage or form aggregations embedded in mucilage from which they quickly revert to the motile state under appropriate conditions. Others swim constantly during their entire life. Chilomonas p.387 is practically always in motion. However it is assumed that species of this genus also form cysts, under certain circumstances, about which nothing is known as yet, since they can be cultured from dry material.

The tendency to form palmella varies with each strain, as does the tendency to form mucilage aggregates. But both depend on nutrition and water supply. Some taxa live in gelatinous aggregates for a long time and multiply in this stage but revert quickly to flagellated motility as soon as they are flooded with water. The cells are capable of performing circular movements in the mucilaginous envelope. They are often arranged in groups of four.

These phenomena have been described and illustrated by GEITLER (1951) and compared to those in the palmelloid stage of Euglena viridis. In Volvocineae and others, the palmella formation is again somewhat different corresponding to their different cell structure and symmetry.

Flagella arising in pairs from one point serve as swim organs. Their internal structure corresponds to that of other flagella. It is not known why two flagella of somewhat unequal length are required for the rotating forward movement. A division of labour may be detected in that, occasionally, one flagellum, i.e. always the same, beats freely while the other rests.

The two flagella of Cryptomonas and Chilomonas are stated to have lateral appendages (cilia) according to PITELKA and SCHOOLEY (1957). This finding could not be confirmed by JOYON (1963). It would seem that what they recorded were mucilage deposits which they interpreted as appendages (MIGNOT 1967). In older publications, including HUBER-PESTALOZZI (1950), the flagella are described as being band-shaped from the image appearing in the light microscope. However, this would appear to be contrary to the true structure as well. According to older descriptions (PETERSEN 1925), the longer flagellum has appendages on one side, the shorter none.

The kind of swimming movement is very characteristic in Cryptomonads.They never swim in a straight line even when stimulated by phototaxis, but change direction frequently

swimming unsteady, with a characteristic swaying. They can also swim backwards, but whether this is accomplished by means of the flagella I do not know. In any case, it is possible to recognize Cryptomonads by their characteristic swimming even at a low magnification.

Chemical stimuli cause the cells of Cryptomonas to recoil a short distance, only to resume their forward movement in another direction. This recoil or backlash, often several times the length of the cell, is caused by the discharge of slime threads from the so-called trichocysts, i.e. a kind of rocket reaction, and may well constitute a useful escape reaction. p.388

Several authors (cf. PRINGSHEIM 1963, p. 77ff.) have observed phototaxis in Cryptomonads but this need not be associated with the presence of eyespots, as in most flagellates and algal swarmers, which makes their role very unclear when present since the absorption of light by the chromatophores would seem to be sufficient. In Chilomonas, which does not possess pigmented cell organelles, phototaxis is in response to light of short-wave length, including ultraviolet (LUNTZ 1931, p. 90).

Chemotaxis is also known in Chilomonas but investigations in this regard are as yet incomplete (GARREY 1900).

The behaviour of motile Cryptomonads in cultures reveals sensitive reactions to various stimuli. However, appropriate studies have not been undertaken for many decades.

III. Description of Some Strains

In order to better illustrate the visible properties of Cryptomonas I will give a short description of some strains which exhibit general or specific characters preceding the plates with the drawings. The dimensions refer to the mean length and width of clonal populations.

When I attempt, by way of drawings, to illustrate the diversity within a conspicuous uniformity in the various structural forms of Cryptomonas, because the written description cannot convey the immediate impression, I am aware that what I present is nothing more than a small selection within a bewildering variety[7].

(A). Kummerteich,*) Hirschberg in Bohemia, June 1939: The strain prefers a slightly acid pH, approximately 6.0-6.5. 18-24 x 9-12 µ. It was named Cryptomonas ovata var. palustris at a time when it was still considered useful to name the taxa. Cleaning by means of smear on agar plates. The cells are elongate-ellipsoidal, with broadly elliptical cross-section. The gullet may almost extend to the nucleus. The chromatophores almost cover the entire inner surface and are separated only by a narrow strip. Two large pyrenoids adhering to the chromatophores with their somewhat flattened sides and covered wholly or in parts by starch grains. p.389

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7) These descriptions are not uniform because they are based on notes extending over a long period of time. I have used the term "Schlund" (gullet), although it is technically incorrect, because I have not found any other short term and because so far it has been universally used.

Translator's Note:

*)Kummerteich: Teich = pond

In addition there are usually lenticular starch grains on the inner surface of the chromatophores. Colour reddish-brown, flagella shorter than the cell. The strain produces little mucilage, but tends to adopt the palmelloid stage.

(B) Cherry-Hinton near Cambridge, England, ditch, 1940: The strain prefers a neutral pH. The cells are approximately 20 μ long, somewhat angular-ellipsoidal with cross-section showing furrow on one side. The gullet extends almost to the centre, the flagella are as long as the body, the chromatophores a light reddish-brown. Mucilaginous aggregates are formed on agar and along the edge of the liquid; the cells are grouped in fours in the aggregates.

(C) Wray Castle, Windermere, England, 1943: Grows in slightly acid pH. Mean length 26 μ, highly curved with thick body envelope, anteriorly snouted, posteriorly laterally curved in various ways. The gullet does not extend all the way to the centre but has sac-like dilation. Body circular in cross-section. Flagella as long as body, peripheral bodies situated at various locations.

(D) Jesus Green, Cambridge, England, 1944: Usually 32-35 μ long. Elongate-ellipsoidal with almost parallel flanks and snout-like protrusion adjacent the depression carrying the flagella. I saw only one oval body and occasionally a peripheral body. Gullet very short. Flagella 3/4 of body length. A red spot was always present

but its interpretation as an eyespot remains doubtful[8].

(E) From sphagnum pools east of Clausthal in the Harz Mountains, 1957: The strain prefers a pH of 4-5 corresponding to its habitat. It is phototrophic(*) and requires vitamin B_1 and B_{12}; but peat extract proved to be best. The smallest additions of organic nutrient solutions promoted growth. A slimy, dark brown rim is formed on the edge of the liquid, on agar a sepia-coloured gelatinous mass is formed.

(F) From swampy ditches with pH 6.1 near Buntenbock in the Harz Mountains, 1957: Growth at pH 5.5-6.5 but not without organic nutrients at low concentration, e.g. amino acids. Our Euglena gracilis solution at 1/4 of the usual concentration was very effective, above and below such concentration the effect was reduced. A gelatinous mass is formed both on the meniscus of a good nutrient solution and on agar. But the colour is not brown but brownish-orange. It would appear that B_1 is necessary p.390 apart from B_{12}. The cells are ellipsoidal and measure 13-15 x 9-11 µ. The gullet is deep. Pyrenoids are present.

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8) Prof. FOTT (in a letter) expressed doubts about the occurrence of eyespots in Cryptomonas; according to HUBER-PESTALOZZI (1950, p. 39) it has not been proved. SKUJA (1948, p. 350) finds a stigma in Chroomonas coerulea GEITLER.

Translator's Note:

*) The German word used is "photoautotroph" which I have rendered in English as "phototrophic" both in the present instance and whenever the term appears in the following. (See definition in Penguin Dictionary of Biology: "An autotrophic organism may be phototrophic or chemotrophic as regards its supply of energy", but not "photoautotrophic".)

(G) This strain comes from wet sphagnum cushions near Clausthal in the Harz Mountains, 1964: It grows only at an acid pH between 5.0 and 5.8, at any rate below pH 6. It is phototrophic like (E) and, like (E), growth is promoted rather than inhibited by very small amounts of organic nutrients, e.g. acetate, meat infusion and peptone. Diluted peat extract is particularly good. Soil extract is harmful, even after acidification. The cells are motile for a long time, nonmotile only in old cultures, but they do not form palmella or mucilage and do not grow on agar. In a mineral nutrient solution, e.g. my Micrasterias solution, they remain healthy for a long time. They are weakly pigmented. Cells elongate-ellipsoidal, average length 22 µ.

(H) The strain was isolated in the polytrophic pond located in the botanical gardens in Göttingen in 1955: Growth at a neutral pH (pH 6.5 - 7.5), but this does not mean much since propagation was poor in pure culture, as it was also in a strain isolated from the same pond 10 years later. Good growth in soil-water test tube, particularly with some NH_4MgPO_4 at the bottom, which ensures a good supply of nitrogen and phosphate. The strain does not form palmella . The cells swim all their life. They measure 18-25 x 10-12 µ . The spiral tendency is particularly prominent in the gullet which extends to the centre and is somewhat curved. The chromatophores are light brown to reddish-brown and carry pyrenoids. The flagella are as long as the body.

(I) The strain was cultivated from a ditch near Bloomington, close to Indiana University, U. S. A., in 1964: Its optimum pH is 7.0 - 7.5. It can also be phototrophic, but growth is promoted by soil extract and even more by organic nutrients, such as acetate, peptone, yeast extract which is highly diluted, yet it does not grown in darkness. As usual, nitrate is a better source of nitrogen than ammonium salts. No growth on agar, only between agar and glass. Average size 20 x 8 µ. The body is somewhat curved, the gullet extends posteriorly beyond the centre. The chromatophores are a bright reddish-brown.

IV. Explanation to the Drawings

The following outline drawings are intended to illustrate only what can be gleaned from cells in immersion that have been immobilized shortly before. Of course, they cannot compete with SKUJA's masterpieces because, after all, the present drawings are not intended to show all the details but merely indicate the features characteristic of individual forms. p.391

The chromatophores have not been shaded in order not to obscure the shape. But all drawings were drawn to the same scale, 1:4000 and then uniformly reduced for reproduction in order to facilitate comparison.

The selection of the drawings does not correspond to that of the strains described hereinabove. In many

cases no sketches had been made at the time. This omission is partly rectified by presenting the most conspicuous features.

These are: the shape of the body in plan view, as it is commonly depicted; the gullet; the outlines of the chromatophores as far as they are discernible in the preparations; location and size of the ovals and lateral bodies*); pyrenoids and starch grains; red pigment spots (eyespots?). The nucleolus has been depicted in order to identify the nucleus even though it is not visible in the living cell.

Remarks relative to some strains compared to older illustrations which also appear in HUBER-PESTALOZZI:

No. 20 from Wray Castle may well be Cryptomonas reflexa (MARSSON SKUJA 1948**) were it not for the gullet. C. curvata PÉNARD may be closer in this regard but still seems to differ somewhat. C. rostrata TROITZKAJA corresponds to my form as far as the shape of the gullet is concerned but the sketch is too rough to permit a proper judgment. C. rostratiformis SKUJA would be very close, even as regards the shape of the gullet, were it not for the fact that the flagella, as depicted, are shorter.

No. 151, top, from Cambridge, might be identified as C. platyuris SKUJA 1948; yet I never have seen cells that are that flat. No. 32 and No. 37 are also similar but possess red spots.

Translator's Notes:

*) The author suddenly refers to "Seitenkörper" (lateral bodies) whereas previously he talked about "Randkörper" (peripheral bodies).

**) sic.

These taxa are among those which are most clearly differentiated, the others usually resemble one another much more closely. These few examples will illustrate that there is indeed not much sense in assigning a species name to each individual strain that may deviate somehow from the next.

V. Discussion

The present publication should be regarded merely as a preliminary study. But in view of the fact that our present knowledge is as yet imperfect it is nevertheless hoped that it will provide a useful contribution in the sense of a supplement, as it were, to HUBER-PESTALOZZI's overview (1950, p. 13 etc.)

HUBER-PESTALOZZI has already realized that not all p.392
named forms can be maintained as species, that forms of highly variable size should not be placed in one species but also that conspicuous differences in dimensions can be observed even within a natural population, and that various former species apparently comprise a number of elementary species. All this could be confirmed by means of clonal and pure cultures.

A number of erroneous statements are found repeatedly in the literature and these required correction.

The colour of healthy Cryptomonads is not yellow-green or olive green but reddish-brown or blue-green, as has been noted in detail above.

The Cryptomonadineae with "primitive organization" (e.g. PASCHER 1913) should be deleted. The minuteness, the poor state of preservation or inadequate optical equipment are responsible for the misleading impression that the cells are simple.

Species with numerous chromatophores, such as Cryptochrysis polychrysis, Cyanomonas americana, do not exist. The error is due to an optical illusion. Cryptochrysis is not reliably different from Cryptomonas, Rhodomonas should also be combined with Cryptomonas. The apparent absence of the gullet is due to the fact that it is difficult to recognize.

Chroomonas differs from Cryptomonas by scarcely more than the preponderance of the blue phycobilin, which has also not been recognized as a generic character in other algae, e.g. Porphyridium and Phormidium.

Nucleus, pyrenoids and oval bodies have often been confused. Their role and their appearance required clarification. LEMMERMANN (1910, p. 477) depicted Cryptomonas nordstedtii with a nucleus at the posterior end which is stated to be surrounded by two "crescent-shaped" (watch glass like?) starch grains. PASCHER (1913) finds a basal, "highly refractive nucleus" in Cyrptochrysis. But nuclei never exhibit such an optical behaviour. FRITSCH (1935, p. 645*) Fig. 216) identifies peripheral bodies with the letter n as nuclei although they look completely different. Such errors have been perpetuated indefinitely.

Translator's Note:

*) p. 645 is deemed to be in error and should read p. 654.

Leucosin, which both SKUJA and HUBER-PESTALOZZI have indicated as being present in the Cryptomonads, is found only in the Chrysophyceae and completely different in appearance.

The differentiation between Eucryptomonadineae and Phaeocapsineae (FRITSCH 1935) should not be maintained since many species of Cryptomonas can grow in the palmelloid stage for a long time. Tetragonidium (PASCHER 1914), which is said to form angular cysts, has been observed only once and requires a more thorough study since there is no conclusive evidence that the illustrated forms are indeed allied. p.393

A number of questions that have scarcely been addressed so far, although they are of general biological importance, become more accessible by means of clonal and pure culture techniques. They are also important from an ecological point of view. Thus, for example, we do not yet know whether the bleaching of the chromatophores is caused by the deficiency of each essential nutrient and the associated interruption of growth. Perhaps it is only the lack of certain trace elements, nitrate or phosphate, that is responsible therefor. In order to resolve these questions, more elaborate tests are required.

These might also reveal what causes a shift in the pigmentation, to what extent it can be cured, and whether it is ecologically important, perhaps even useful. What about the photosynthesis of discolored chromatophores and the effect of the spectral zones? Even healthy material is

conspicuously weakly pigmented. But we do not know anything about the amount of chlorophyll present which would suggest the question as to what is the quantitative ratio between the amount of chlorophyll present and photosynthesis.

A healthy and homogeneous cell material might be obtained by means of improved culture techniques, e.g. optimum illumination and temperature, diffusing of carbon dioxide-containing air, replenishing consumed nutrients and regulating the pH level. Since the Cryptomonads can only tolerate diluted solutions, which means that an adequate concentration of nutrient salts or, as a matter of fact, of all necessary substances, cannot be maintained for any length of time, it would be necessary to conduct detailed preliminary tests as the present techniques are not capable of maintaining optimum conditions over any length of time with regard to healthy pigment and reserve food content. The conditions necessary for the development and enlargement of MAUPAS' ovals should also be investigated in this manner.

The difficulty of obtaining uniformly favourable conditions for growth is contrasted by the longevity of the cell material in old cultures. This must also be extremely important in nature for the propagation of the Cryptomonads. It is assisted by the development of mucilaginous envelopes, storage of energy-rich reserve food substances and the resistance to dehydration, presumably also the shift in pigment content.

There must be habitats, for example, above the mud bottom of lakes and ponds, where the conditions favouring propagation do not change as quickly as along the water edge or in laboratory cultures. In the phase boundary above the mud bottom, good conditions favouring propagation are p.394
created for longer periods of time due to the supply of nutrients and regulation of the pH level on the part of the colloidal masses at the bottom. For the ferrophilic Cryptomonads, the presence of iron in the form of water-soluble suboxides may well be particularly important. Occasionally this produces a brown coloration but it would appear that this has not been observed frequently.

VI. Explanation of the Plates

Plate 148, top: Hirschberg in Bohemia, 1939.

Rounded-ellipsoidal, gullet short, 2 pyrenoids with starch grains or starch sheaths. The chromatophores almost cover the entire surface. Flagella 2/3 the length of the body. More details see above.

Plate 148, centre: Cherry Hinton near Cambridge, England, 1940.

Shape broadly ellipsoidal, somewhat angular. Gullet extends to the centre. The cells are somewhat flat with a furrow. Flagella reach the same length as body. The strain has a tendency to form mucilaginous aggregates in which the cells are grouped in fours.

Plate 148, bottom: Pond in the botanical gardens in Cambridge, England, 1942.

The shape of the body is elongate-ovoid, anteriorly truncate. The gullet extends to the centre. The chromatophores have wavy margins. Oval bodies present. Flagella 3/4 of the body length.

Plate 149, top: Pond in the botanical gardens in Cambridge, England, 1943.

The cell outline is approximately foot-shaped when the cell lies on its flattened side. A protrusion appears close to the base of the flagella, the gullet extends to the centre of the cell. The outer sheath is thick as is common in large species. In one cell, a lateral body can be seen in a recess of the chromatophore. The flagella reach 3/4 of the body length.

Plate 149, bottom: Wray Castle, Windermere, English Lake District, 1943.

The body is highly curved with variously shaped posterior end frequently including a protrusion. Close to the base of the flagella is a snout-like protuberance. The gullet is short and wide, the cellular cross-section approximately circular. Outer sheath thick, flagella almost as long as the body.

Plate 149, bottom left: Barton near Cambridge, roadside ditch, 1943. p.395

The body is ellipsoidal to ovoid, as in the majority of the strains. The flagella are approximately the same length as the body. The cells contained 2 rounded oval bodies as well as lateral bodies posteriorly and on the flank. The gullet extends somewhat beyond the centre.

Plate 150, top: Fenditton near Cambridge, England, ditch, 1943.

The cells are large and had a somewhat irregular outline, which may well be due to degeneration. A prominent protrusion was located close to the base of the flagella. The body sheath was thick. In the interior, starch and fat in the form of droplets and irregular clusters. The flagella were 2/3 the length of the body. A peripheral body was observed in one cell between the chromatophore lobes.

Plate 150, bottom: Cambridge, England, ditch running between the college gardens, 1943.

Usual shape, gullet scarcely extending to the centre. The pyrenoids are covered with many small starch grains which occupy almost the entire surface. It is not known what will influence the form and size of the starch grains of the sheath; in any event form and size thereof are not alone influenced by the properties of the taxon. Lateral bodies can be discerned posteriorly and on the flank but the conditions necessary for their development have

likewise not been investigated. This material contained only one oval body in each cell. The flagella were approximately the same length as the body.

Plate 151, top: Fenditton, in the marshland near Cambridge in England, 1944.

Material from a roadside ditch. The form in question is small with short gullet. The pyrenoids are provided with starch caps. Lateral bodies and ovals present. The flagella are relatively short.

Plate 151, bottom: Cambridge, England, ditch on the Jesus Green, Jesus College, 1944.

The form in question is large with short gullet. The chromatophores almost cover the entire surface and leave only enough space for lateral bodies. A red pigment spot (stigma?) is present in all individuals.

Plate 152, top: Same locality, somewhat later, 1944.

The form is relatively slender but not flattened. The gullet extends to the centre. Ovals were present in all individuals. The flagella were approximately the same length as the body. One specimen is illustrated in plan view, one in optical cross-section. It contains much starch and is thicker than starch-deficient cells.

Plate 152, bottom: Fenditton near Cambridge, ditch, 1944. p.396

Large form with chromatophores which almost cover the entire surface. Lateral bodies and starch including irregularly shaped, highly refractive masses (oil?).

Plate 153, top: Cambridge, ditch on the grounds of Trinity College, 1945.

Medium large, slender, somewhat irregularly shaped, with snout-like protrusion close to the base of the flagella. Gullet short and wide. The chromatophores almost cover the entire surface. One rounded oval body each. Outer sheath thick. Flagella 3/4 of length of body.

Plate 153, bottom: Cambridge, Jesus Green, as 10 and 11, 1945.

Medium large, somewhat rounded form with thick body sheath. The chromatophores leave a very small area. An elongate, red spot (stigma?). Flagella 3/4 the length of the body.

Plate 154, top: Oxford, England, ditch in low lying area near the River Thames, 1945:

Small elongate-ovoid form. Gullet short. Lateral bodies present. The inner surface of the chromatophores was covered with starch grains. The flagella were 3/4 the length of the body.

Plate 154, bottom: Three strains of Chroomonas are illustrated. All are rounded and short, with flagella the length of the body.

a) Fenditton near Cambridge, 1945.

A relatively large form with groups of ruby-red granules (eyespot) and pyrenoids. Flagella the length of the body.

b) Dinas, Wales, 1945.

The shape is the same as in the most common form of the genus.

c) Flatford near Colchester, England, 1947.

The body is more rounded. A stage during division is illustrated.

VII. Summary

Freshwater Cryptomonads are generelly found singly between other Algae and Flagellates. They may, by their unsteady movements, already be recognized at low power magnification. Because of their motility and their sensibility to fixing agents they are difficult to investigate.

Cell-sizes vary much even in clones and are therefore of limited value in differentiating species. Quite useless in this respect are the colour of chromatophores, and the quantity and nature of reserve substances as well as the presence and appearance of MALPAS' ovals because all these depend on conditions.

All Cryptomonads except the blue-green species (Chroomonas) are in a healthy state more or less deeply tinged reddish brown. When the hue shifts to yellowish or brownish green, that is due to deficiency in nutrition, an experience explaining statements in the literature as to diversity in colour.

Characteristic of all Cryptomonads is a more or less pronounced screw-like structure, strongest in forms with a crooked and pointed back end. The width of the cells is to some extent influenced by the state of nutrition. Certain features used for differentiating species, such as depth of gullet and degree of curving, are of little value in practical use, even when constant in subsequent generations, because they occur in various combinations, so that habitual names can only be considered as descriptive of groups.

The chlorophyllous Cryptomonads are phototrophic. No satisfactory growth in darkness was achieved, although some strains are favoured by acetate, peptone, etc. As far as tested they all require vitamin B_{12}, while the colourless species *(Chilomonas)* need organic acids and vitamin B_1.

In most instances an adaptation to a restricted pH-range is pronounced. Another, likewise ecologically consequential difference between the strains is exhibited by either continuous motility during life-time, or a tendency toward formation of palmella, often with mucilagenous envelopes. Only the latter group may be grown on the agar-surface and can withstand loss of water.

There is an immense number of taxa differing morphologically and physiologically. They cannot all be designated as species. Those given names have only by chance been more thoroughly investigated.

The gaps in our knowledge of Cryptomonads are pointed out. They rendered unfeasible a comprehensive monograph of the genus and family.

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