LIFE CYCLE AND ENVIRONMENT OF LOCHMIOPSIS SIBIRICA Woron.

by

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(With Tab. VIII)

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STATEMENT OF THE PROBLEM.

Subject of this study is the green alga *Lochmiopsis,* belonging to the Family *Chaetophoraceae,* Subfamily *Leptosirae.* The alga was discovered by N. N. Woronichin (50)

in 1927 in several alkaline lakes of the Kulundinsteppe in the Gouvernment Tomsk, Siberia. In 1927 G. M. Smith and L. G. M. Baas Becking also observed this alga in a number of small saline ponds near Marina, California. In 1929 H. Heath received a sample from Mono Lake, California, preserved in formalin, which contained *Lochmiopsis*. E. W. Galliher collected saline muds in Ventura County in 1931 from which this plant also appeared.

The plant has a very specific flocculent appearance when submerged, and we can say that, with great probability, O. Loew, then a scientific member of Lieutenant Wheeler's Expedition (29), in 1875 already encountered *Lochmiopsis* (or an alga very much like it) in Owen's Lake, California, when the waters of this basin were still in a more dilute condition.

The following passage is taken from Loew's paper:

„Das organische Leben im alkalischen Wasser des Sees ist auf eine einzige Pflanzenart und sehr wenige Thierarte beschränkt, die niedrigen Lebensformen angehören. Erstere ist eine aus Fäden bestehende Alge, welche in runden Massen von ¼—½ Zoll Durchmesser in ziemlicher Menge herumschwimmt, theils weiss, theils schwach gelblich grün ist und voraussichtlich eine für die Wissenschaft neue Art darstellt".

It seems that the form occurs only in alkaline, saline waters of variable concentration. Not only that the concentrations of different lakes differ greatly, but the same lake may show, during the season, great changes in salinity. After the winter rains the salinity is low, while, at the end of the season the waters may be saturated with salt.

The aim of this study is to account for the developmental cycle of this alga in its relation to changes in salt-concentration, composition and temperature of its environment. The sum total of the factors delineating the possible environment may be called the "potential milieu". When

we depict this environment by an area, we may say that the "natural milieu" should be more restricted. For many salt combinations which may be successfully withstood and which may be tried in the laboratory, never occur in nature.

CHAPTER I.
NATURAL ENVIRONMENT.

a. Chemical and Physical environment.

The data for the description of the natural waters are chiefly taken from a publication of N. N. Woronichin (49) on the lakes of the Kulundin-steppe and from the unpublished work of E. W. Galliher, Mrs. D. S. Cope and L. G. M. Baas Becking on the Salina's near Marina, California.

Samples of the waters of the Siberian lakes were collected in 1927 by the Geological Committee, during a survey of the Slavgorod Region, Gouvernment Tomsk. Three lakes, in which *Lochmiopsis* occurred, deserve special attention. They are:

a. Bolschoje Petuchowskoje. Dimensions 4 by 1½—2 km. South, east and west shores covered with Phragmites, north shore covered with an organic mass, 2—3 meters thick. The sapropele in the shallow lake is 1—2 m. thick. Green parts of this mass float on the surface. The water is clear, concentration 2°—3° B.

b. Belenkoje. Dimensions 456 by 225 m. North shore barren, with salt-crust, the other sides covered by dense shrub. Bottom sapropele 1—1½ m. thick. Concentration 1°—3.6° B.

c. Maloje. Dimensions 500 by 300 m. Shore barren, covered with a salt-crust. Some small islands in the n.w. part covered with Phragmites. The water is clear, concentration 11°—25° B. The occurrence of reeds is striking as the salt-tolerance of Phragmites is certainly not marked enough to account for its presence in those highly con-

centrated brines. The ground water in the immediate environment of this lake may be fresh, a phenomenon which is repeatedly observed in desert salt lakes.

The salines of Marina consist of a series of small pools, situated immediately behind the dunes (which are about 300 meters across) at the Californian coast between Monterey and Santa Cruz. In the winter and the spring they contain 30—100 cm. water, in summer they may be dry or nearly dry, often covered by a saltcrust of 1/2 to 3 cm. thickness. The length of the ponds varies between 30—300 m. Several are situated below sea-level. The bottom consists of quartz sand and black sulphide-mud. There is no connection between the ocean and the ponds; no sea water filters through the dunes into the lakes, as the groundwater flow is directed from the coast hills towards the ocean. Wells dug around the pools show the presence of fresh ground water in the immediate neighbourhood of the brine. The chemical composition of the waters in these pools shows the peculiar character of a desert salina rather than of a seawater-concentrate. Seawater-concentrate contains always large amounts of magnesium, while this metal is usually precipitated from the desert water at comparatively low concentrations (Pyramid-lake, California: 1000 mg/l. total solids). The alkaline rocks of the coast range are leached by the ground water, which evaporates in the pools. This is apparent also from the changes in concentration with depth. Fresh water may be obtained from under the lake-floor.

The chemical composition of various lakes of the Kulundinsteppe and of the pools at Marina are shown in the following tables. As a comparison the composition of sea water (mean of 77 analyses, Challenger Expedition) is given. In the first row of every column the amount of a certain ion is given in grammes per litre; in the second row the amount of gramme-ion per litre. Salinity is given

as grammes dissolved substance per 10^6 grammes of solution The analysis of Mono Lake is taken from Clarke (10) pg. 158.

TABLE 1.

	B. Petuchowskoje		Belenkoje		Maloje		Mono		Ocean
Cl	3.5426	0.100	2.869	0.081	47.832	1.349	11.943	0.337	55.292
SO_4	0.1037	0.001	0.130	0.001	1.8916	0.020	0.658	0.007	7.692
CO_3	5.5723	0.093	8.1068	0.131	63.37	1.562	11.984	0.120	0.207
HCO_3	3.3126	0.054	4.668	0.077	16.968	0.262			
B_4O_7							0.1639	0.001	
Na	} 7.8360		} 7.566		} 88.444		19.4087	0.279	30.593
K							0.9466	0.012	1.106
Ca	—	—	—	—	—	—	0.0005	—	1.197
Mg	0.0639	0.005					0.0021	0.005	3.725
Salinity p.p.m.	20 367		23 339		218 505		51 170		30 000

The analyses of Mrs. D. S. Cope of the pools near Marina gave the variation in composition during the year as the following table shows.

TABLE 2.

	12 Dec. 1929	16 Febr. 1930	14 May 1930	11 July 1930		
				surface	1 foot	2½ foot
Cl	4.46 mol	0.589 mol	0.996 mol	3.82 mol	0.746 mol	0.138 mol
SO_4	0.014	0.044	0.006	0.064	0.002	0.002
CO_3	0.0543	0.024	0.028	0.52	0.035	0.010
Na	4.47	0.614	1.409	4.82	0.778	0.143
K	0.97	0.010	0.022	0.226	0.011	0.011
Ca	—	—	—	—	—	—
Mg	—	—	—	—	—	—
Salinity p.p.m.	266 700	37 100	62 800	291 700	47 200	9 150

A natural water may be characterized by a "simplified solution" which allows of graphical treatment, which treatment still represents its peculiar composition.

In this simplified solution we only consider the kations Na, Ca and Mg (classing K under Na) and the anions Cl (with Br, J, Fl), HCO_3 (with CO_3) and SO_4.

We will consider water chiefly as a solution of two sets of three components each and represent each set in a triangular diagram as used by Bakhuis Roozeboom [1]).

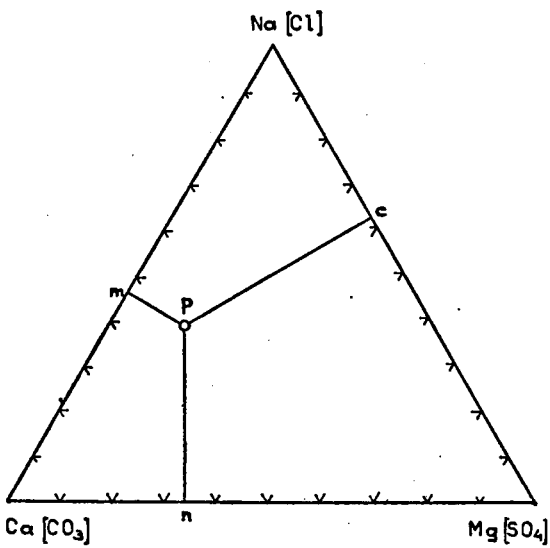

Fig. 1. Equilateral triangle, showing mode of representation of the proportions of three components.

In the equilateral triangle Ca-Na-Mg point P represents a certain solution. The molecular proportion of Na, Ca, Mg is such that $C_{Na} : C_{Ca} : C_{Mg} = Pn : Pc : Pm$.

For according to a well known property of the equilateral triangle $Pn + Pm + Pc = \text{constant}$.

[1]) The method described below is taken from unpublished work of L. G. M. Baas Becking and A. Massink. See Figure 1.

Point Ca represents, therefore, 100 % calcium.

The distance Pc = 48; Pn = 38 and Pm = 14. The proportion Na-Ca-Mg is therefore 38 : 48 : 14. Similarly by considering the corners as Cl, HCO, SO_4, P should represent Cl : HCO_3 : SO_4 = 38 : 48 : 14.

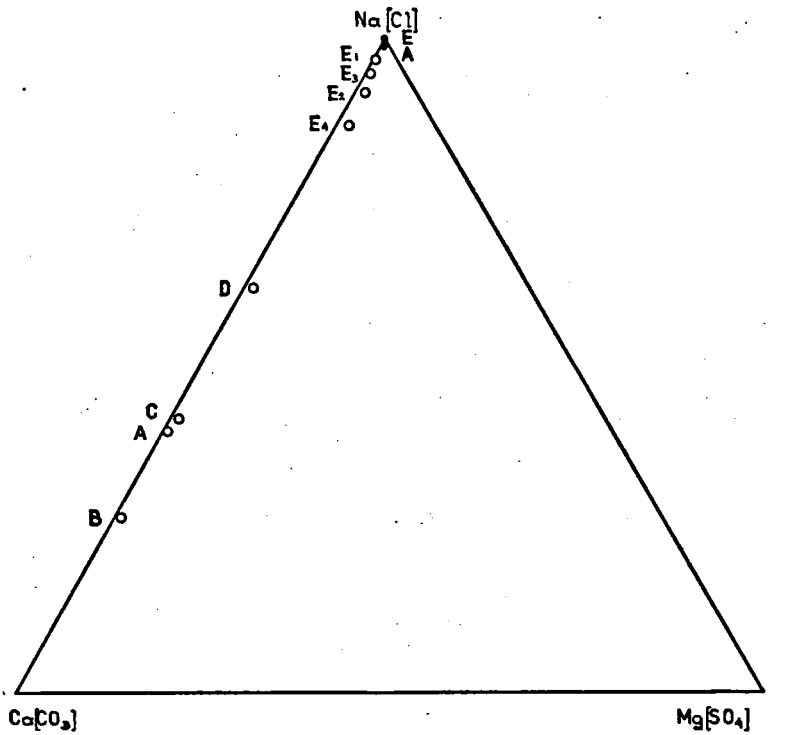

Fig. 2. Triangular diagram, depicting the natural milieu of Lochmiopsis. *A.* B. Petuchowskoje, *B.* Belenkoje, *C.* Maloje. *D.* Mono Lake, E. Marina.

The composition of a natural water may therefore be represented on a triangular diagram by two points; one point depicting the relation Na-Ca-Mg, the other Cl-HCO_3-SO_4.

In Figure 2 kations and anions of the different waters

are represented graphically, the open circles anions, the solid dots kations. The corners Na, Ca, Mg represent 100% Na, Ca, Mg or the corresponding anions respectively. The sides give the proportions of the mixtures Na/Mg, Ca/Mg or Na/Ca or the corresponding mixtures of anions. Points on the triangle represent mixtures of the three ions.

The waters belong to the chloride-carbonate type of C l a r k e (10) as the diagram shows. The proportion between the carbonate and the chloride ion varies from 73/27 to 3/97, while the sulphate content never exceeds 1.4 %. The considerable annual fluctuation in sulphate content may be caused by sulphate-reduction and by the oxidation of the sulphide-mud by photosynthetic oxygen. The waters of Marina lack magnesium and calcium, while these elements were present in the bottom mud. Small quantities of those elements, escaping even qualitative detection, should be present in the waters however, as considerable amounts of algae occurred in them. The small quantity of both magnesium and calcium may be caused by the high alkalinity of the water (pH = 10). Lake Petuchowskoje may have had a little lower pH while the other Siberian lakes are very alkaline. The waters appear, therefore, chiefly as solutions of sodium chloride,- carbonate,- bicarbonate with very little sulphate.

A second factor to be considered in studying natural environment is the salinity, the specific gravity and the temperature of the brines. The following data were available.

TABLE 3.

	Salinity	s.g. ° Beaumé	s.g.	temperature
B. Petuchowskoje	20 367	2 — 3	1.015—1.020	20.5—25
Belenkoje	23 339	2.6— 3.5	1.017—1.025	20 —24
Maloje IIA		15.2—18.5	1.125—1.149	21.1—24.1
Maloje IIB		13.3—18.5	1.100—1.149	20.5—23.3
Kupalnoje		25.0	1.205	19
Krolitschie		1.1	1.007	23.6
Mono	51 170			

The seasonal changes of Marina are presented in the table below.

TABLE 4.

	December 1929	February 1930	March 1930	May 1930	July 1930
Salinity	266 700	37 100	—	62 800	291 700
Specific gravity ..	1.195	1.022	1.023	1.040	1.224
Temperature	18°	18°	18°	18°	18°

The water temperatures around Marina do not vary much from 18° C. throughout the year. For the lakes in the Kulundinsteppe only water temperatures in the months of July and August were available, showing a range of 19°—25° C. According to Katz (20) the annual temperature-variation in the west-Siberian steppe amounts to 35°—45° C. The mean temperature during the vegetation period is 16.9° C.

Considering these data it appears that *Lochmiopsis Printzii* occurs independent of the concentration of the brine. *Lochmiopsis sibirica* is only observed in the waters with a concentration up to 3.5° Beaumé.

b. Biocoenose.

The living components of the environment in which *Lochmiopsis* occurs are for a large part influenced by the concentration of the waters. In spring, after the decrease in concentration due to the spring-rains, a great number of plants and animals develop. Subsequent increase in concentration during the summer causes a decrease of the number of living beings, a part of which awaits the next rains in a resting condition. In the concentrated brines only a few halophilic species grow and multiply, while the halotolerant forms which persist in the strong brines as cysts or resting spores, find their optimum-development

in the diluted solutions in spring. The point where, according to Walter (47), active life ceases, is situated at about 3.6 mol. NaCl [1]).

In this paper *halophilic* organisms are called those that are able to grow and develop in solutions of more than three mol. NaCl (± 17.5 %). A special mechanism enables those organisms to persist in this curious environment. The nature of this mechanism is as yet but little understood; moments of possible importance are:

a. a special type of permeability.

b. surface-charges.

c. the possible existance of "fixed" or "bound" water.

The terms *halophilic* and *halotolerant* have been used by Hof and Frémy (18) in the same sense, while in other systems of classification, for instance that of Kolbe (23), the limit for „euhalobe" organisms is situated much lower, namely at 4 % salt concentration. Fauna and flora do not show the pronounced specificity which characterises the truly halophilic being.

Organisms of the brine.

In the lakes studied the Arthropods are well represented. In the first place we mention:

1. the brine-shrimp, *Artemia Salina* forma *Franciscana Semon*, which occurs in all these lakes in large quantities (Baas Becking and Boone (5), Baas Becking and Jacobi (19)).

   The decomposition products of this shrimp, chiefly chitin, form a large part of the sapropele. The eggs float on the strong brine and are beached at the lee-shore where they accumulate in large quantities.

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[1]) Calculated from Walters vapor-pressure data.

2. *Ephydra millbrae* Jones, the brine-fly, occurs in large quantities, its pupae and larvae forming floating cakes of a brown colour, the chitin of which contributes to the sapropele.

3. *Coryxa* spec., a water-boatman, pries upon the adult brine-shrimps.

4. Beetles,

5. terrestial Hemiptera, the larvae of which develop in the salty mud.

6. several Nematodes as yet undescribed.

7. colourless Flagellates and Ciliates, probably of cosmopolitan distribution.

   Several of those forms were described by Namyslovsky (31) from the subterranian salt pools of Wielicska.

   One was described from salt lakes in Siebenbürgen by Entz Sr. (12) while a new Ciliate was found by Kirby in one of the Marina pools. (21).

   Mrs. F. Wiersma-Verschaffelt has cultured most of these organisms from the Marina-material and the following information is taken from her unpublished data.

Flagellates:

a. *Tetramitus salinus Entz.*
b. *Amphimonas salinus Namysl.*
c. *Pleurostomum gracile Namysl.*

Ciliates:

a. *Rhopalophrya salina Kirby.*
b. *Lionotus sp.*
c. *Stichotriche sp.*

8. Amoeba.
   a. In the lower concentrations a form resembling *Amoeba Limax* was observed.
   b. In the high concentrations a typical *salt-amoeba* was encountered, previously described by Miss Hamburger (16) from the salines of Cagliari and later encountered in almost all solutions investigated: *Amoeba salina Hamburger.*

9. Bacteria.
   a. A large number of *purple sulphur bacteria* and also:
   b. *Colourless sulphur bacteria* live in the concentrated brines. These forms and the active sulphate-reducers are the most striking representatives of this group.

      It may be said, however, that according to the work of Miss T. Hof (as yet unpublished) a great number of bacterial species thrive in similar solutions, so that it seems out of place to give a longer list here.

10. The number of blue-green algae in the alkaline brines is very large, but the frequency and the number of species seem to be variable. In the Siberian lakes especially they seem to occur in large masses.

    In this association there occurs:
    a. *Phormidium tenue Gmt.*
    b. *Oscillatoria Tambi Woron.*
    c. *Spirulina tenuissima Ktz.*
    d. *Aphanocapsa salina Woron.*

       The first and last named form seem to contribute largely to the formation of sapropele (Petuchowskoje 1—2 m. thick; Belenkoje 1—1½ m. thick).
    e. *Aphanocapsa marina Hansg.*
    f. *Nodularia sphaerocarpa Born.* and *Flah.*

The last two forms occur in the Marina pools, develop up to the concentration of 1 mol. NaCl. They contribute largely to the formation of the algal mat on the shores of the pond when the waters are partially evaporated.

11. Diatoms occur in the lower concentrations; above 14 % salt they disappear.

12. Coloured Flagellates.

    a. the green polyblepharid *Dunaliella viridis Teod.* persists in very high concentrations together with two other forms.

    b. *Dunaliella salina Teod.* (= *Dunaliella kermesina Labbé*) and

    c. the closely related form which will be described by dr. E. Nicolai in another place: *Dunaliella Peircei.*

13. The higher Chlorophyceae are represented by the two forms of *Lochmiopsis*: *L. sibirica Woron.* and *L. Printzii Woron.*

The annual changes in composition of the association may be represented graphically by curves (Figure 3) which show the changes of salinity and specific gravity of Marina waters during the season.

We have to keep in mind that the temperature of these pools is very constant (18° C.). Boone and Baas Becking (5) found that the optimal conditions for the germination of the eggs of Artemia salina are situated at the concentration of 0.24 mol. and those for the formation of the egg-membrane at 0.48 mol. NaCl. We. therefore, expect the development of *Artemia* in the first weeks of January.

In June the development is completed, for the maximum

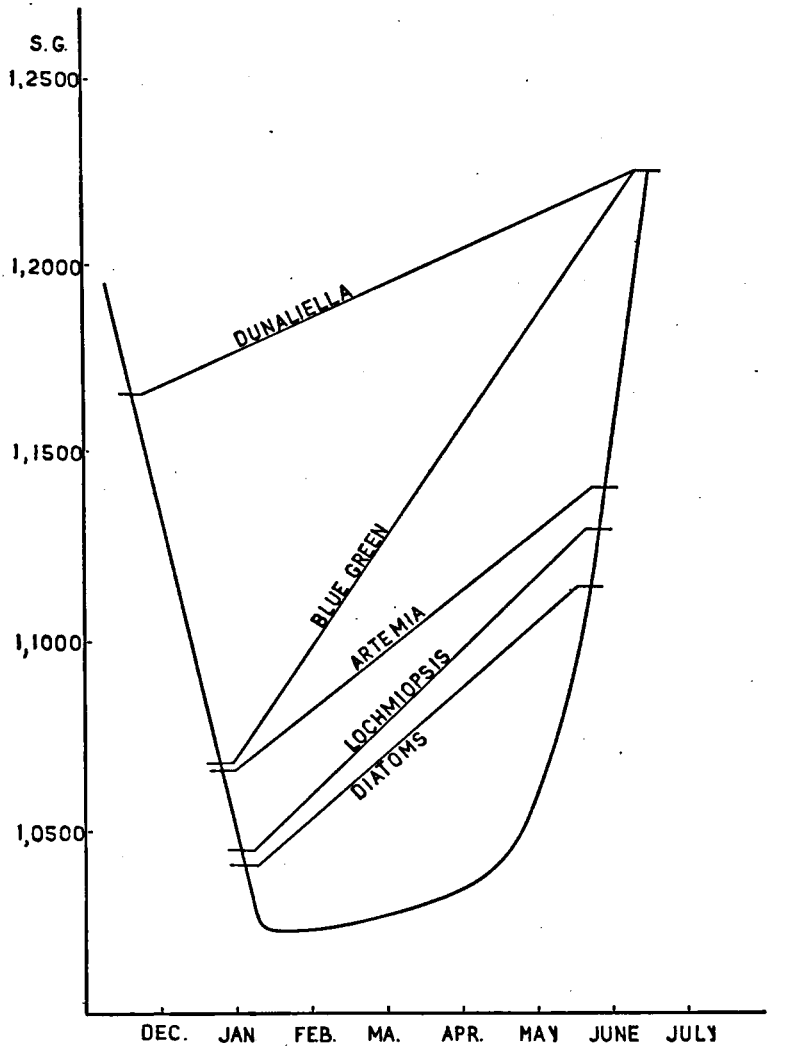

Fig. 3. Biocoenose of the ponds at Marina, California, as a function of specific gravity of the brine.

tolerance for adults seems to be situated in the neighbourhood of 3.5 mol. NaCl.

Dunaliella viridis (Baas Becking (4)) develops, from the palmella-stage, independent of concentration up to 4 mol. NaCl within two weeks. This organism will be present throughout the year.

The development of *diatoms* has been studied by H. Budde (6) in the Westfalian salines. In waters containing 44000 mg/l. chloride, corresponding to about 1.2 mol. NaCl, a copious vegetation occurred. Only a few forms, *Nitzschia frustula Grun, Nitzschia ovalis* and *Navicula longirostris* are able to develop in concentrations up to 2.4 mol. NaCl.

We might expect, for the Californian salines, a beginning of the diatom-vegetation at the end of January and a cessation of the development in May.

According to the observations of Hof (18) the blue-green algae which occurred in Marina and which were mentioned above are only halotolerant and do not show development above one mol. NaCl. My own observations show that germination and development of these forms lie between 0.5—1.0 mol. NaCl. The period of development for these blue-greens is situated between February and the end of the season. The cysts are formed when the pools are nearly dry.

The resting forms of *Lochmiopsis sibirica* (akinetes) do not show any germination in concentrations higher than 1.4 mol. NaCl. The alga shows optimal development at 0.3—0.7 mol. NaCl. The time necessary for development of reproductive stages is ten to twenty days.

Normally *Lochmiopsis* will appear in the middle of March while it dies off in May; the akinetes survive during the dry season, being the halotolerant stage of the alga.

For the Siberian lakes much higher water-temperatures were mentioned (20°—29.5° C. in July and August). The

development of the alga should be terminated by that time as these temperatures are too high for development. Earlier in the season temperatures of the Siberian lakes seem to be comparable to those of Marina (17° C. according to Katz (20)).

CHAPTER II.
MATERIAL AND METHODS.

The available material consisted of the algal mat, so-called "meteoric paper" from Marina, California, collected in the years 1929 and 1930. The akinetes seem to retain their germinating power for several years in this mat, the greater part of which consists of dead vegetative cells of *Lochmiopsis* which form a dense felt, from 2—5 mm. thick. In this felt we find, moreover, the cysts and eggs of other brine-inhabiting organisms, of which we name: eggs of *Artemia salina*, palmellae of green flagellates, cysts of *Amoebae* and colourless flagellates and ciliates, resting stages of blue-green algae, Diatom-frustules, and spores of an *Oospora*.

The resistant part of *Lochmiopsis* are the akinetes.

Between these organic components of the algal paper many crystals of NaCl may be observed. The concentration of this salt in the meteoric paper is therefore $\pm$ 5.25 mol.

The material collected in 1929 was dry, the 1930 material was moist, contained black mud and putrified rapidly. No *Lochmiopsis* plants could be raised from this material after one year. The 1929 material yielded after an incubation-time of 12 and 26 days copious cultures, respectively of *Dunaliella Peircei* and *Lochmiopsis*. It is remarkable that the palmellae of *Dunaliella Peircei* seem to be more resistant to this long period of dryness than those of *Dunaliella viridis* or *D. salina*.

From Mono Lake I received material preserved in formalin. It consisted of about 2—3 cc. of "fluff". The filaments show the sibirica-type, the cell-wall is thickened by fixation and the structure of both plastid and protoplasm, is partly destroyed by shrinkage. The contents of the akinetes are also contracted. Akinetes may be arranged in long rows sometimes with zoosporangia between them which contain 8—16 cells. Between the filaments cells of blue-green algae may be observed, and also several species of diatoms (4 species of *Navicula*, 3 species of *Synedra* and 2 of *Nitschia*).

The initial culture fluid had the following composition.

NaCl.....	3—6 %
K_2HPO_4	0.002 %
KNO_3.....	0.02 %
$MgCl_2$.....	0.01 mol.

tapwater.

Glass flasks and cuvettes were used and a small amount of the 1929 material was put into the solution which was placed near a north window. The culture methods which are often used for purple bacteria and several green algae, in which continuous illumination in a lightbox is used, proved to be unfit for these experiments. The temperature of our lightbox varied between 20°—24° C. These temperatures are already too high for germination.

In a north window where the temperature fluctuated between 18°—20° C. rapid germination could be observed within 10 days (the time being longer for older material). After three weeks the formation of akinetes could be observed at the end of the filaments and the lateral branches. These akinetes yielded the material for the further observations on germination, ionic tolerance and other environmental conditions.

Before a transfer was made a small amount of the

material was washed in tapwater and a few times in distilled water. By this treatment the living vegetative cells burst and part of the protoplasm and the plastid is extruded. Palmellae and cysts are washed away. The material was now dried with filter paper and transferred to the fresh culture-medium. Therefore, only akinetes, which are resistant, remain as infection-material. The development was observed daily by examining representative samples.

The algae were also raised in Petri-dishes on 5 % agar immersed in culture fluid. The sessile forms find a suitable substrate in the agar, while growth in a vertical direction is possible also. The free stages easily distribute themselves throughout the liquid.

The objection may be raised against both culture methods, that they do not allow of continuous observation of one and the same individual. Moreover, the chance of fungus infection is very great, while for a direct observation of the agar culture the lid of the Petri-dish had to be removed; moreover, observation under high magnification was excluded because of the intervening layer of solution.

Another well-known method, in which slides or coverslips are immersed in the culture fluid, yielded good results for the forms that developed from flagellates. On the long run, however, observation was hampered by the heavy growth; more and more individuals developed on the glass.

The hanging-drop proved to be unsuitable. Flagellates or part of the algae in a hanging drop form resting stages after a short time. They remained alive (no changes could be observed after 14 months), but no growth occurred.

The method was modified by placing the culture in a drop on the slide. Above the drop a cover was placed, which rested on a pair of parallel capillaries; pressure-damage was therefore excluded. In this way a sort of micro-aquarium was made, which allowed of microscopic inspection

also under higher magnification. The evaporation of the culture medium is checked by placing the slide on a pair of glass-rods in a Petri-dish, the bottom of which is covered with the same culture solution.

Cultures prepared in this way keep for months, while the development may be studied daily.

In the study of the influence of ions on germination and development, use was made of hard-glass tubes, containing 10 cc. of the medium. The tubes were plugged with cotton in the usual way and placed in an almost horizontal position in a north window.

As observations usually did not exceed one week, the cotton-plug seemed to check evaporation sufficiently, as it amounted in the cases measured to less than 1.5 % of total volume in one week, which amounts in our experiments to an error of 0.0015—0.015 mol. NaCl. In experiments of longer duration the tubes were stoppered with corks.

Solutions were made of salts unrecrystallized from the "pro analyse" brand from Merck or Kahlbaum.

Stock solutions were made of the various salts as follows: NaCl 2 mol.; KCl 1 mol.; $MgCl_2$ 1 mol.; $CaCl_2$ 1 mol.; Na_2SO_4 and K_2SO_4 0.25 mol.; $NaNO_3$ and KNO_3 0.5 mol.; Na_2CO_3 and $NaHCO_3$ 0.25 mol.

The desired molarities were prepared by mixing various amounts of the stock solutions with controlled distilled water.

CHAPTER III.

DESCRIPTION AND LIFE-HISTORY OF LOCHMIOPSIS SPEC.

Description.

Although Woronichin (50) has given a full description of the alga, it seems advisable to give a short account of the form here as his paper is written in Russian and two

or three phases of the life-cycle have been omitted, as perhaps they did not occur in the samples studied by him. In the following description the observations of Woronichin are printed in parantheses in as far as they differ from my observations on the material raised from the algal mat of the Marina ponds.

Woronichin distinguishes between two species: a sessile form, *Lochmiopsis sibirica* which reaches a diameter of 10 mm. and which occurs occasionally in the plankton and, moreover, a floating form, *Lochmiopsis Printzii*, which is sometimes found adhering to a solid substrate and which never reaches more than 1.5 mm. in diameter.

The two species are very much alike, nevertheless they may be distinguished by some characteristic differences. In *L. sibirica* the cell-length is 20—25 × the diameter, while in *L. Printzii* the length of the cell never exceeds 3 × the width. The akinetes are often, however not always, ellipsoidal and have a thinner cellwall than those of *L. sibirica*. In *L. Printzii* the zoosporangia are similar to the vegetative cells. The floating habit of the latter form has already been mentioned. The last two characters seem to be the more important, as Woronichin mentions a case in which after diluting the brine the newly formed branches of a plant of *L. Printzii* were shaped like those of *L. sibirica*.

As we consider *L. sibirica* as the original form and *L. Printzii* as a modification *L. sibirica* will be more fully described.

L. sibirica is found in the Siberian lakes, forming a thin bushy coating on the stems of *Phragmites communis* and on sunken logs. In the Marina pools it is free floating, forming globular colonies, 10 mm. in diameter, the filaments radiating from the centre. In the sessile form the filaments rise from a basal cell-plate or a single cell which is firmly attached to the substrate.

Examined microscopically it shows filaments of the type of the *Chaetophoraceae*. The cells are cylindrical

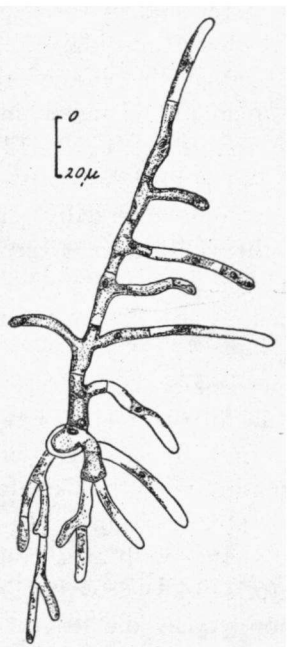

Fig. 4. Young plant of *Lochmiopsis sibirica*, Wor.

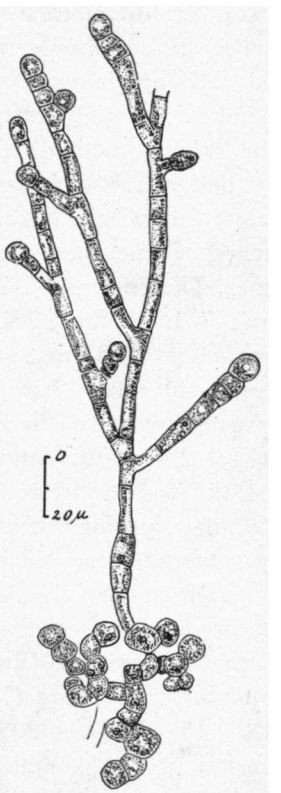

Fig. 5. Young plant of *Lochmiopsis Printzii*, Wor.

4—12 μ in diameter and 2—260 μ in length (3.5—10 μ, 10—20 times the diameter. W). The cell wall is thin. The chloroplastid consists of a parietal band, sometimes as long as the cell, mostly much shorter. 1—6 pyrenoids are embedded in the chloroplastid with 2 halfring-or cupshaped starch bodies. Products of photosynthesis are starch or fatty oil, scattered in the chromatophore. The protoplasm

forms a thin peripheral layer, surrounding one or more large vacuoles. The filaments are branched by forming a lateral prolification at the apical end of the cell. The branches are commonly not different from the main stem, occasionally much shorter by limited growth and formation of akinetes and (or) zoosporangia.

The akinetes which are formed at the end of the vegetative period are globular or somewhat ellipsoidal. They originate singly or in rows of 2—60 at the end of the branches. Sometimes the entire plant is changed into akinetes. Dimensions 8—20 μ with an average at 12—14 μ (Figure 7 a, g. Figure 18, 4) [(8,7—14.4) × (13—22) W.)]. The vacuole has decreased in volume markedly and occupies not more than 1/30 of the cell-lumen. Reserve substances are starch and protein. The wall is up to 2 μ thick (± 2.5 μ W.) and stratified. The chromatophore covers the wall entirely.

Sporangia are formed at the same time or a little earlier. They sometimes are much like an ordinary vegetative cell. They originate like the akinetes, terminally or intercalary, single or in rows, sometimes alternating with the akinetes. The size is variable (Figure 7 h. i. n. o. Figure 18, 2) Typical dimensions are (14—23) μ × 15 μ with an average of 18 × 15 μ [(9.3—20.4) μ × (6.7—14.4) μ W.]. The dimension of the lateral protrusion equals the length of the cell. According to Woronichin *L. Printzii* has oval sporangia, formed like vegetative cells and in intercalary position. The pore is placed laterally.

There are three types of zoospores: macrospores, microspores and gametes. The latter two forms are morphologically alike. They are small, pear-shaped flagellates, very plastic and without a wall. Dimensions are (4—6) × (2—3) μ. The anterior part is hyaline and has two flagella, the posterior end is blunt and shows the cup-shaped chromatophore with a pyrenoid with two half-

ring shaped starch grains. The anterior margin of the chromatophore often shows a granular appearance (crystals?). The microspores possess a red eyespot at the anterior part. The spore resembles a diminutive *Dunaliella*.

The macrospores are larger (6 × 10 μ), oval, without a wall, flagella or red eyespot. They are plastic and show ameboid movement. The hyaline pole is lacking, the plastid is cup-shaped, nearly covering the entire protoplast. These forms are probably identical with the zoospores observed by Woronichin.

Apart from the stages already described by Woronichin there occur a few other stages that are worth mentioning: the zygote and the dwarf plant. The zygote (Figure 8, 1, m) has an average dimension of 7 μ, is often surrounded by a wall (1 μ thick) and characterized by the double chromatophore. From this zygote the footplate-cells originate (Figure 8, n). They are arranged regularly in a plate, usually not larger than 100 μ. Cells are grouped in pairs, flattened against each other and show an average diameter of 8 μ. The pyrenoid is situated in the middle of the cell, in the centre of the chromatophore.

Footplates originating from parthenospores have been described by Woronichin. Here the cells are originally 6—13 μ in diameter (average 8.5 μ) which value agrees with Woronichin's observations. The cells of the footplate, especially at the margin, may elongate to more than 8 times their width.

Dwarf plants, developed from flagellates (Figure 8, f-i) consist of 1—10 cells, either elongated and placed at right angles to the substrate, with a long ribbon-shaped chromatophore as long as the cell, (6 × 30 μ,) or the dwarf plants consist of a filament of globular cells closely adhering to the substrate. The cells may change into microsporangia, the microspores formed by fragmentation of the protoplast, or they may change into akinetes.

Life-history.

A consideration of the life-cycle of *Lochmiopsis* logically begins at the germinating akinete of the "meteoric paper" which is also the beginning of the seasonal cycle in the natural environment. By the rapid dilution of the brine after the rainfall in winter and spring — a process reproduced at the laboratory by the transfer of the material in a culture fluid of a lower concentration — the akinete germinates (Figure 7, a-d). The cell-wall bursts and by the rapidly swelling protoplasm and the increasing vacuole, the margins are pushed aside and the germination-tube appears. The increase in volume of the cell, of the protoplasm as well as the vacuole, is caused probably by osmotic action. The decrease of the concentration of the environment is considerable. In the natural milieu it amounts to a difference of 4.5 mol. on the beaches of the lakes, in the brine to 1.2 mol. In the experiment these values are situated between the limits 4.5—0.5 mol. NaCl. The initial equilibrium is disturbed and will readjust itself by a decrease of concentration of the cell-contents. An argument for this opinion lies in the fact that by transference of the material in a culture-fluid in which the total concentration does not surpass 0.5 mol. NaCl germinating occurs by the extrusion of the entire contents of the cell which increases in volume by swelling into a more or less globular shape, 1.5—3 × the diameter of the akinete in width, an increase dependent of the concentration of the culture fluid (Figure 8, e). The growth occurs at the expense of the reserve-starch which will reappear at the end of the third day after germination as photosynthetic starch. The globular plant grows out normally after formation of a solid wall by forming a cylindrical cell which elongates and divides in a normal way.

The germination-tube branches once or several times immediately above the place of exit. Each of the branches

grows out in a different direction, thus giving the plant the radiating form. Secundary branching may occur in all cells by lateral prolification at the apical end. The first cell wall is formed directly above the ramification. In certain cases these branches too will grow out and attain a length similar to that of the main-stem. Sometimes the growth is limited by the formation of akinetes or zoosporangia after a few rapid successive divisions (Figure 18, 4).

Dependent upon the rhythm of division and growth, a process which is influenced by some of the salts of the culture fluid as well as the concentration and hydrogen-ion concentration (Chap. III), the plants will grow out to *Lochmiopsis sibirica* or *Lochmiopsis Printzii*. The cycle may be closed at this stage by the formation of the akinetes by rapid successive divisions of the terminal cell. The akinetes assume a globular shape; the plastid, at first a narrow band, is now lining the entire cell. The cell is filled with reserve-substances chiefly consisting of starch, but containing some protein also. In several cases, induced by the temperature, the whole plant changes into akinetes. In the diagram (Figure 9) this cycle is represented by 2—1—2.

Before the formation of the akinetes zoosporangia may be formed. Also in this case no elongation of the formed cells occurs. The cells are more or less rounded and produce, when placed intercalary, a lateral prolification in which the pore of exit of the zoospores is formed. The protoplast, lined with the plastid, shrinks from the cell wall and divides once (Figure 7, n, o). The divisions of the cells are not simultaneous, thus forming (in the case of the macrozoosporangium (Figure 7, h) 6—8 spores. In the microzoosporangium, in form and size not different from the gametangium, the cell divides 3 or 4 times, the result being 16—32 microspores (gametes). The zoospores take a more definite form, a cupshaped chloroplastid is formed at the end of the cells, the other end forming the

colourless anterior part of the zoospore. Sometimes the eyespot is formed while the spores are staying in the sporangium, the same may be said of the flagella, so that the spores may swarm away immediately after extrusion. Sometimes eyespot and flagella are formed after liberation. The spores remain motionless in the extruded mucilage in front of the pore of the sporangium.

The extrusion of the zoospores goes with considerable force. The cause of this force seems unclear. At the place of extrusion the wall certainly has another structure. In the specimens preserved in formalin the swelling caused by this preservative seems limited to the cross- and the back-walls of the sporangium, while the wall at the place of the preformed pore is still thin (Fig. 6). Microchemically, no difference could be demonstrated. In no case the formation of a bladder was observed, as described by several authors. Dodel for *Ulothrix zonata* (11), Wille for *Trentepohlia umbrina* (Kütz.) Born, Walz (48), Klebs (22) and Hirn (33) for *Oedogonium* spec. Probably the force in these cases is originated by the increase in volume of the surrounding bladder by osmotic intake of water. This explanation should not, in our case be excluded, while the formation of the bladder might have escaped attention and, moreover, a general increase in volume by swelling (osmotic?) takes place. By this increase in volume the wall may be torn at the thinnest place and the tension of the wall will be the source of the force with which the zoospores are extruded. If the pore is too small, a part of the zoospores remains inside, and sometimes leaves the sporangium by movement of the flagella.

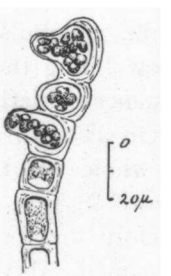

Fig. 6. Zoosporangia in material from Mono Lake, California.

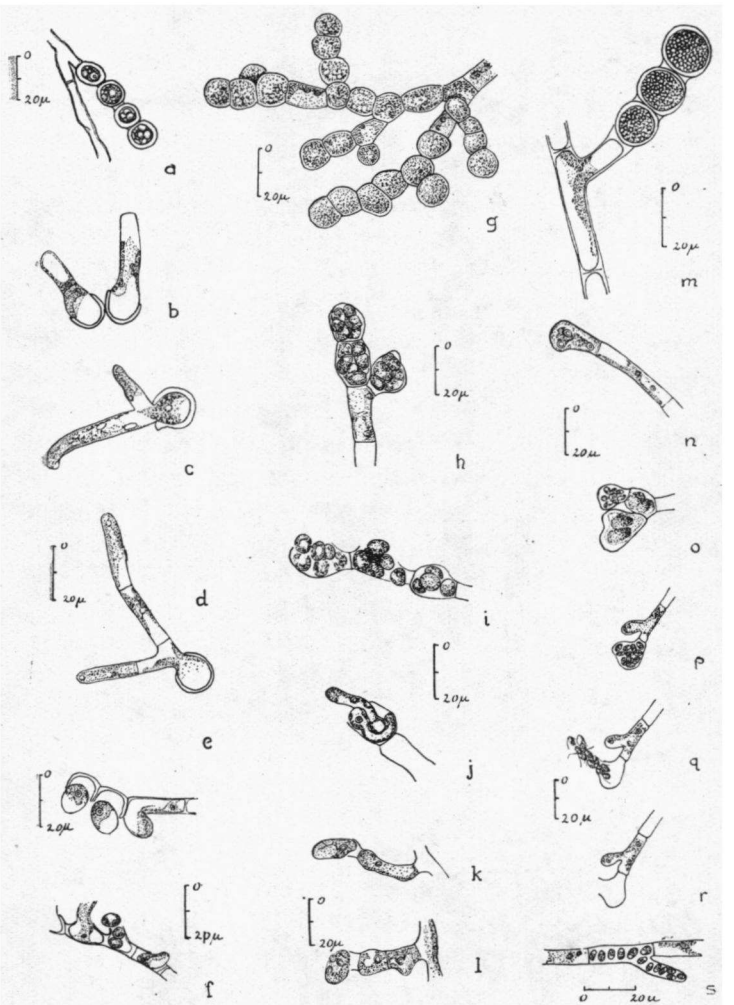

Fig. 7. Stages in the life-cycle of *Lochmiopsis*. I. *a*. akinetes from the "meteoric paper", Marina, containing starch and protein. *b-d*. germinating akinetes. *e*. germination by extrusion of cell-contents. *f*. germination by formation of macrozoospores. *g*. young akinetes. *h-i*. macrosporangia. *j*. germinating macrozoospore. *k, l*. liberation of cell-contents in an ordinary vegetative cell. *m*. hypnospore. *n, o*. formation of microzoosporangium (gametangium). *p-r*. liberation of microspores (gametes). *s*. Formation of microspores in a vegetative cell.

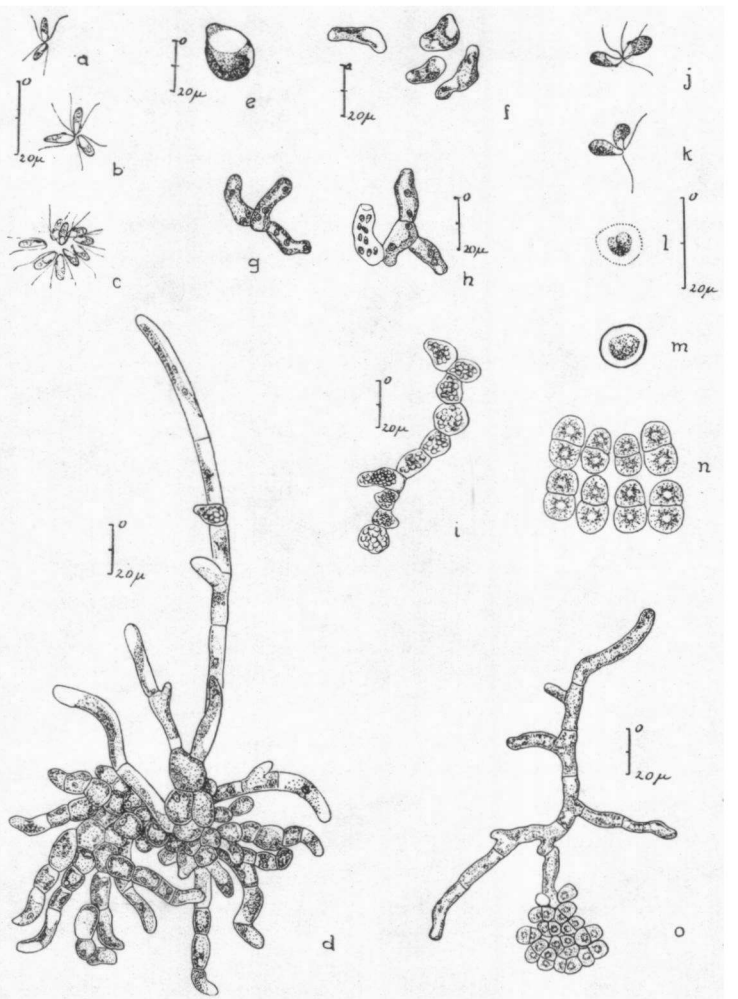

Figure 8. Stages in the life-cycle of *Lochmiopsis*. II. *a-c*. group-formation of parthenospores. *d*. part of the foot-plate originated from zoospores, showing young plant with sporangium. *e*. germination of extruded cell-contents of an akinete. *f-h*. dwarf-plants from microzoospores. *h*. dwarf-plant forming zoosporangium. *i*. dwarf-plant, changed entirely into microzoosporangia. *j-m*. pairing of gametes and formation of zygotes. *n*. formation of foot-plate by division. *o*. Young plant on foot-plate.

The macrozoospores (Diagram 13 and Figure 7, i, j) which are slightly motile and perform slow ameboid movements in the extruded mucilage, germinate on the spot where they are deposited by the sporangium or the currents. A case of ameboid zoospores is recorded by Pascher (35) for an *Aphanochaete*-like alga. Soon a solid wall is formed and the spore germinates by pushing forth a cylindrical cell which grows out to an ordinary filament by division and elongation. Commonly these spores give rise to free-floating plants. Only where they are deposited by the current on the beaches of the lakes between other sessile organisms, an apparently sessile form will originate from it. This cycle is represented in the diagram by 1—4—13—1.

The behaviour of the microzoospores differs considerably.

1°. After swarming a while the gametes pair, the colourless anterior ends coalesce first. The zygote formed in this way, swarms a while, adheres to the wall of the culture-vessel, — in natural environment the stem of Phragmites communis, logs and other firm substrate will serve that purpose — the flagella are cast off, and a solid wall is formed occasionally (Figure 8, j-m). When formed, the cell is more resistant to a rise in concentration and to drought. But this resistance seems to be considerably lower than that of the akinetes for I never saw development of zygotes out of the "meteoric paper". The zygotes, collected from the surface of the fluid in a evaporating culture germinated after transfer to a fresh culture-solution. Development occurs by division in planes perpendicular to each other, the result being a regular cell-plate (Figure 8, n). The last division is probably a tetrade division. The zygote and the cells of the foot-plate are most likely the diploid phase of the life-cycle. One or more cells of this basal plate give rise to a normal alga by a division parallel to the substrate and subsequent elongation and division

of the upper cell. (Figure 8, o). A sessile form of *Lochmiopsis* always originates from the plate.

2°. The microzoospores develop parthenogenetically. The flagellates swarm actively during 1—2 days, but as no pairing occurs, they adhere to the glass in radiating groups formed by few to several hundreds of cells. Sometimes they form freely-floating colonies. The first stages of such a colony is represented in Figure 8, a-c. The flagellates remain motile for some time, oscillating with the free end of the cells. Finally the flagella are cast off and the cells flatten against the substrate and against each other, thus forming a foot-plate as described by Woronichin and depicted in Figure 1 of his paper.

This group-formation of flagellates has been observed by Pascher (36) for *Chlamydomonas paupera*, by Hartmann (17) for *Chaetomorpha* and *Enteromorpha*, Föyn for *Cladophora* and *Ulva* (15). In all cases mentioned the flagellates paired, when + and — gametes occurred. The gametes which had not paired died off, sometimes they developed parthenogenetically into filaments. Fritsch (14) observed these radiating groups for *Stigeoclonium*, where the colonies floated freely in the water. However, the plants originating from these colonies never formed filaments more than 4 cells long. Pascher believes the formation of the colonies to be the result of a substance secreted by some of the flagellates by which the others are chemotactically attracted.

I never observed a formation of zygotes in the groups of zoospores of *Lochmiopsis*. It is probable that in the first case mentioned the flagellates are discharged electrically at the interface glass-water, as Baas-Becking (3) depicts for *Dunaliella*. A difference may be formed by the fact, that *Dunaliella* adheres with the end of the flagella, while these zoospores adhere to the glass with the base of the flagella.

The pseudoparenchymatous cell-plate formed in this

way develops into a foot-plate of 1 mm. in diameter by the irregular outgrowth of some of the cells parallel to the substrate. The filaments branch several times, thus forming a basal body with a more or less compact structure. When the cells grow out perpendicularly to the substrate a *Lochmiopsis* plant in its ordinary form occurs. A part of a the foot-plate with an outgrown filament is represented in Figure 8, d. From the free-floating colonies other free-floating colonies will result.

In this cycle (Diagram 1—4—5—8—7a—1) a close resemblance to the development of *Chaetophora* and *Stigeoclonium* is apparent as has been described by Cienkowski (9), Fritsch (14), Tilden (44), a.o.

3°. The microspores remain in the swarmer stage for a long time. In the above mentioned cases this stage lasted from a couple of hours till at most two days. Here the flagellates swarmed for two weeks. An opinion I failed to prove but which seems attractive is that this stage is identical with *Dunaliella*. The flagellates show in the cycle 10—11—12 the characteristics of ordinary vegetative propagation of *Dunaliela viridis* as depicted by Teodoresco (42, 43). However I never succeeded in raising *Lochmiopsis* from *Dunaliella viridis*.

The swarming flagellates adhere to the glass wall of the vessel, cast off the flagella, (aplanospore) form a protective membrane and soon develop into a filament. These filaments never grow out to more than a dwarf plant, as represented in Figure 8, g and h, which in this one-celled stage form microspores. Sometimes it will form a short filament which adheres to the substrate of 3—10 cells. Each of these cells may form zoospores or akinetes according to environmental conditions. This cycle is shown in the diagram by 1—4—10—11—12—9—4. The aplanospore may form a cyst. In this form it is resistant to drought and high salt-concentrations.

The hypnospore is to be considered as a transition-form of akinete and zoosporangium (Fig. 7, m). Here the cell contents appears contracted and fragmented. It germinates by extrusion of number of microzoospores which will follow in development the cycle 10—11—12—9—4

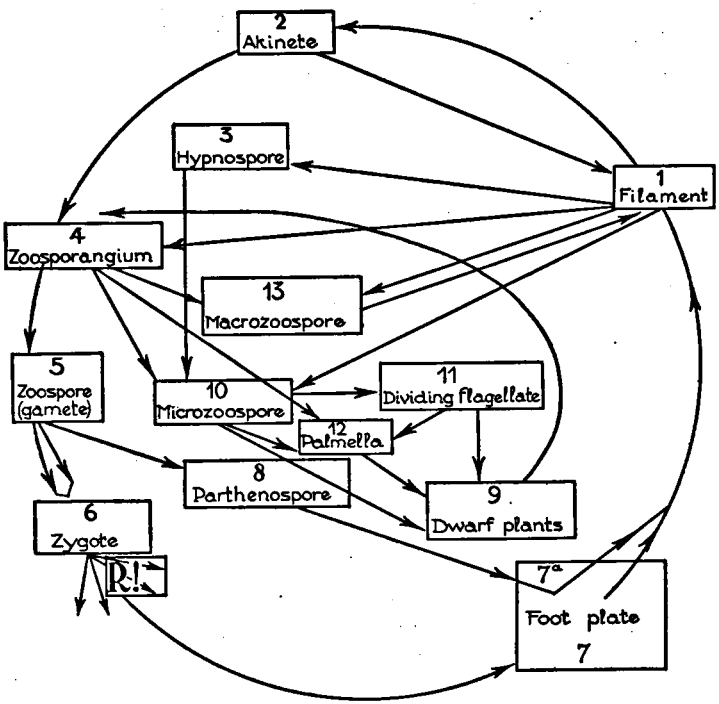

Fig. 9. Diagram of the Life-cycle of *Lochmiopsis*.

of the diagram. In the cycle described above a sharp distinction has been made between its different components. Really the distinction is not so clear as transition-stages occur. Thus akinetes and zoosporangia are to be considered as modified vegetative cells. Every vegetative cell may produce at a moment, determined by environmental conditions, one or more zoosporangia. Clearly this

was demonstrated in a culture where zoospores were formed in the cells of the basal plate as well as in specialized zoosporangia. Striking is the case pictured in Figure 7, s, in which in a common vegetative cell several zoospores had been formed. The change of the filament over akinete to zoosporangium is also gradual. This is illustrated by Figure 7, k and l, in which, after a few rapid successive divisions the whole contents of the cell left the wall by ameboid movement; by Figure 7, e and f, where germinating occurs by extrusion of the contents as a whole or in fragments. The difference with the production of zoospores, described on page 749, appears to be very slight. The hypnospores have already been mentioned as a transition-stage from vegetative cell to propagative one.

Quite apart from these cycles is the one designated as 1—4—5—6—7—1, in which pairing and reduction-division occurs and in which the difference in phase is clear.

Furthermore it is characteristic of the alga that, influenced by the conditions of environment, it is able to form resting stages which are resistant to drought and heat. In the most simple form these are represented by the akinetes which are formed at the end of every cycle, with a highly dehydrated protoplasm, a high amount of reserve-products and a thick wall. Furthermore the palmellae, aplanospores, parthenospores and zygotes are to be considered as resting stages.

The various transitions of the alga from the different stages are gathered in a diagram (Figure 9) and mentioned in the description of the life cycle by the numbers representing the stages. After what has been said some trajectories might be depicted, in which transitions have been observed. For example (7, 7a)—2, 7a—4, 9—2, 8—1, which would represent respectively the palmella-like footplate, as figured by Woronichin in Fig. 23 of his paper, the formation of the zoospores in the cells of the footplate,

the formation of akinetes out of dwarf plants, the filament arising from the germinating aplanospore. This should give an even greater complication to the diagram.

In view of the facts described, the polymorphism of the alga appears evident.

In order to study the influences by which the different stages are induced, the salts occurring in the natural environment were studied on the specific action they exert morphologically and physiologically.

For this purpose the different processes, such as germination, cell-division and elongation were observed first in simple solutions and also in mixtures of 2 and 3 salts and finally in a composite culture fluid to which the components were added in varying quantities.

Cultures in optimal salt-combinations were started to study the influence of temperature and hydrogen-ion concentration.

Finally a series of culture fluids were prepared after the analyses of some natural waters mentioned by Clarke (10).

CHAPTER IV.

THE INFLUENCE OF CONTROLLED ENVIRONMENT.

a. Influence of Electrolytes on Germination.

The first question in relation to the influence of electrolytes was to ascertain in which concentration the diverse salts allowed germination. Taking the main constituent of the natural waters, NaCl, a series of culture fluids was prepared, varying from 0.1—2.0 mol. NaCl, with an interval of 0.1 mol. The influence was measured in percent germinating akinetes with an uncertainty of $\pm 5\ \%$.

The akinete was considered to have germinated when

the cell wall had burst and the cell contents had appeared.

Living akinetes only were observed. The percentage recorded is the mean of 4 × 100 akinetes. Two samples were taken from each tube and examined at high magnification under the microscope. For every 100 the position of the slide was changed and another part of the sample was observed.

As the akinetes are formed in rows, counting was not difficult and mistakes made by counting the same cells did not occur. The result of one of these series is recorded in Figure 10.

The character of the curve is typical, having two tops respectively at the concentrations of 0.3—0.4 mol. and 0.7—0.8 mol. and three minima respectively at 0.1, 0.5 and 1.0 mol.

In concentrations lower than 0.1 mol. no germination could be observed.

In distilled water the akinetes die off within 6 days; the cause of the lower limit is, therefore, understandable.

The third minimum is also easily understood, as at this point the limit of active vegetative development is situated. Quite mysterious seems the second minimum which will appear regularly, independent of the fact whether the inoculum is taken from plants raised in lower or from plants raised in higher concentrations than 0.5 mol.

Next an explanation of this second minimum should be attempted. First of all the thought occurred that the material must be morphologically heterogeneous. Woronichin distinguished between two species of the alga and one of the characteristic differences lies in form and diameter of the akinetes. So the akinetes of *Lochmiopsis sibirica* are recorded to be globular, formed at the end of the branches and 13—22 μ in diameter.

Those of *Lochmiopsis Printzii* are globular or elliptical, formed intercalary or terminally, measuring respectively

11.6—20.3 μ and (16—31) × (12.8—20) μ. The last ones are in my opinion only differentiated vegetative cells. But sticking to the idea of the morphological heterogeneity it seemed most probable that such difference must show itself in the size of the akinetes.

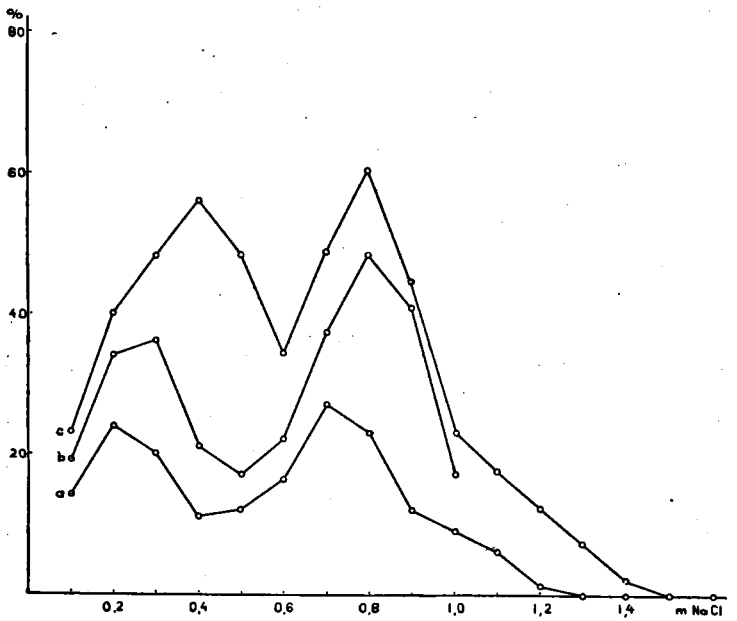

Figure 10. Percentage of germination as a function of NaCl-concentration and as a function of time. *a*. after 6 days, *b*. after 12 days, *c*. after 18 days.

So samples were taken out of three different cultures with a concentration of NaCl of 0.5 mol., 1.1 mol. and 1.5 mol. In each sample 100 akinetes were measured. The result is given in Fig. 11. Striking is the fact that in the three cultures, independent of concentration, a maximum frequency is formed in the class 12—14 μ. Morphologically no heterogeneity in the available material could be observed.

A second assumption may be made; the resulting curve is a combination of two physiological reactions, the first giving a maximum at 0.6 mol., the other with an antibate relation to concentration. Only two processes may be considered in this relation. Germination is apt to have

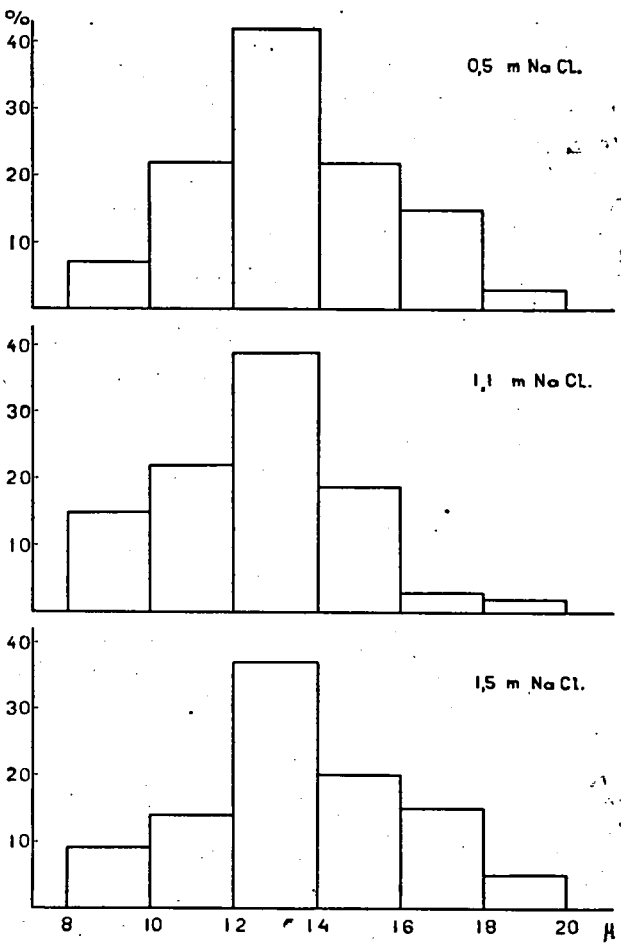

Fig. 11. Frequency diagrams of the diameter of akinetes from different salt-concentrations.

an optimum at the concentration 0.6 and the other factor may be a decrease in mortality with increasing NaCl concentration. In order to test the possibility of this assumption a series of tubes was prepared with NaCl solutions, varying in concentration from 0.1—1.0 mol.

After transferring the alga in the new culture fluid samples were taken on the second, fourth, sixth and eighth day.

4 × 100 akinetes were examined and the percentage dead akinetes, germinated as well as ungerminated, the percentage germinated akinetes (alive and dead) and the percentage germinated akinetes of the total living cells was recorded.

This last procedure was followed in all further observations.

TABLE 5.

	total % dead akinetes				% total germination				% germination of living cells			
days	2	4	6	8	2	4	6	8	2	4	6	8
0.1 mol. NaCl	17	28	52	84	4	6	7	9	4	5	10	12
0.2	35	41	49	64	8	8	12	17	8	10	15	23
0.3	19	23	38	35	12	19	15	18	12	20	18	19
0.4	13	20	15	20	8	28	23	26	7	29	22	26
0.5	13	..	18	22	8	..	21	22	7	..	22	20
0.6	14	..	22	28	5	..	16	15	4	..	19	16
0.7	25	..	23	23	9	..	22	24	11	..	23	24
0.8	34	..	35	21	11	..	14	12	14	..	18	13
0.9	23	..	27	18	4	..	9	18	9	..	7	20
1.0	14	..	18	40	4	..	11	5	3	..	7	9

From the table it appears that after two days the double top has appeared already and that this type of distribution is conserved throughout the whole experiment. (Compare this result with those depicted in Figure 10).

Furthermore it is clear that the curve showing the dead akinetes runs parallel to that showing germination, but that at the lower concentrations (0.1—0.3 mol.) a rapid increase in the number of dead akinetes occurs. It appears

that at the lower concentrations the number of surviving akinetes is small and that even in high concentrations a high number of akinetes seems to survive. A noxious influence of low concentration on germination is therefore apparent and it might very well be that the double top represents a composite action of an influence on survival and on germination. The shape of the curve which represents the influence of salt concentration on germination cannot directly be ascertained; only the resultant of the two factors is observed.

To study the effect of potassium a similar range of concentrations of KCl was prepared. This element proved to be highly toxic to the alga. Within a week the cells degenerated. The plastid, instead of lining the inner wall almost entirely, shrank to a small plate and finally disappeared altogether. In none of the tubes any germination occurred. Within six weeks all cells had died off.

Now, as this element is one of the constituents of the natural environment, next question to ask was to which amount the salt was tolerated by *Lochmiopsis*, when dosed with NaCl. In connection with the experiments of Baas Becking on salt effects on *Dunaliella* (4) a range of mixtures was prepared up to a total concentration of 1.0 mol. in which KCl and NaCl were varied. This method failed utterly, for just like in the KCl-solutions the cells degenerated within a week and showed the typical discolouring of the plastid from bright green to a brownish green. So the method was changed and the KCl was combined in varying quantities with a constant concentration of NaCl that varied from 0.1—1.0 mol. NaCl.

The concentration of the liquid was no longer constant, but varied with the added quantity of KCl.

KCl was added in concentrations: 0.005 mol., 0.010 mol., 0.020 mol., 0.040 mol., 0.060 mol. and 0.080 mol. (Figure 12).

In the lowest concentrations used (0.005 mol.) no influence

of the salt could be observed. The curve depicting germination is quite identical to that of NaCl. In raising the concentration of KCl to 0.010 mol. the minimum at 0.6 mol. NaCl disappears and becomes an optimum, considerably higher than the optima observed in 0.3 and 0.8 mol. NaCl. After adding 0.020 mol. KCl the optimum is preserved, but much lower than the former one. In 0.040 mol. the minimum reappears with still an indication of the two optima near 0.6 mol., which characteristic has fully diasappeared after adding 0.060 mol. KCl to the stock. The limit of tolerance is situated at 0.080 mol. KCl, where in none of the tubes any development could be observed. Noteworthy is the apparent occurrence of only one top in the curve marking the influence of 0.060 m. KCl. which may be explained by assuming that in the lower concentrations of NaCl (0.1—0.4 mol.) the amount of this salt is not sufficient to counteract the toxity of the KCl. For an antagonism of these two salts seems evident and is shown most clearly in Figure 12, no. 2, where the influence of the initial concentration of 0.6 mol. NaCl is fully antagonized by 0.010 mol. KCl. The proportion of NaCl/KCl which seems to be antagonistic, apparently lies between 30—80. Beyond these limits the specific action of one of the metals will become predominant, as shown in Figure 12. In the concentration of 0.005 mol. KCl (proportion 20—200) no distinct difference in character of the germination curve of NaCl could be observed. In the concentration 0.060 mol. KCl (proportion varying from 1.66—16.6) a considerable decrease in germination was observed and by adding 0.080 mol. KCl (proportion 1.25—12.5) germination had altogether stopped.

A similar behaviour was observed by adding the bivalent kations which occur in the natural environment. However, in natural environments, the quantities may be so small that they escape quantitative determination.

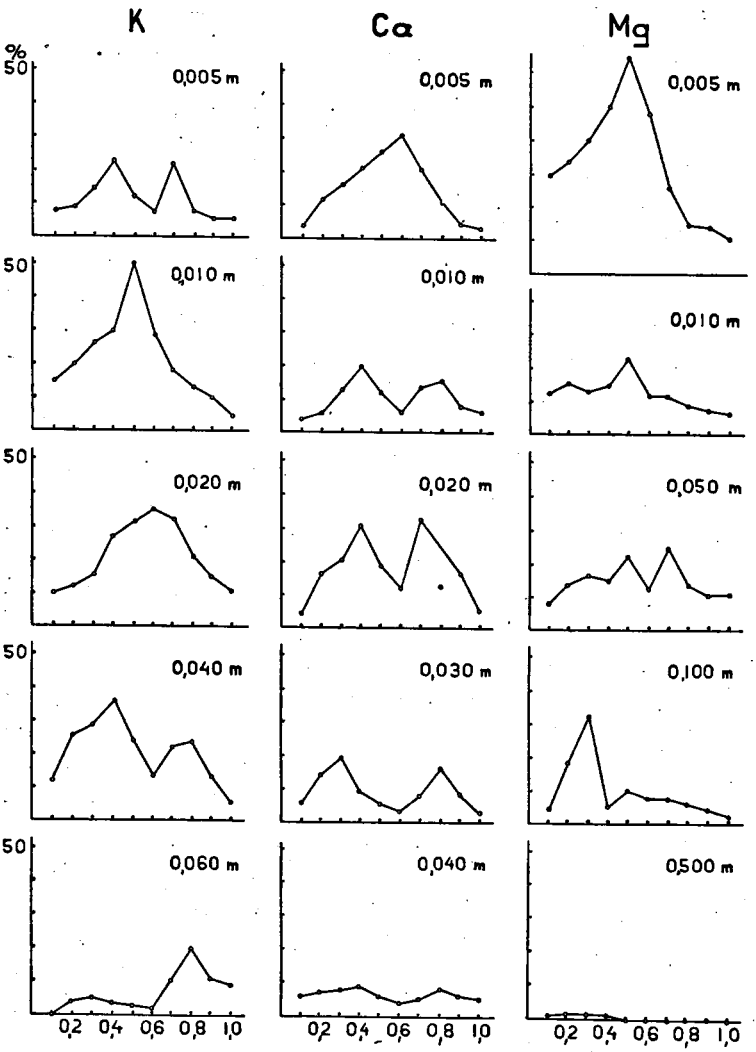

Fig. 12. The influence of K, Ca and Mg in combination with NaCl on germination.

Calcium. The calcium-ion used as a solution of $CaCl_2$, without NaCl, was tolerated in no concentration by the alga. In none of the tubes germination could be observed. The cells died off within a week.

Combined with the sodium chloride in the range 0.1—1.0 mol. tolerance was determined and also the influence of the amount of calcium on germination. As already said, the result was similar to the NaCl-KCl mixtures. Only the limits are much narrower than in the case mentioned before. The limit for calcium-tolerance lies here at 0.050 mol. $CaCl_2$. Distinct antagonism could be observed in adding 0.0005 mol. $CaCl_2$, where an optimal germination occurs in combination with 0.6 mol. NaCl. The value of antagonism is situated at 60—160 (30—80 in equivalents).

Adding $CaCl_2$ in higher concentrations shows germination up to a maximum of 70 %. However, the limit of tolerance has to be taken at 0.030 mol. because in the higher concentrations no further germination occurred, while a number of the young plants died off. The plastid decreased in volume and discoloured to a pale green.

Magnesium. In contrast with the other salts magnesium proved to be tolerated by *Lochmiopsis* in solutions of the chloride without addition of other salts, up to a concentration of one mol. Little or no germination could be observed, but the greater part of the akinetes stayed alive in a concentration not exceeding 0.5 mol. In more concentrated solutions a number of the akinetes died off. Typical is the bright green colour of the plastids. Transferred to a normal culture fluid the spores, after a stay of three months in $MgCl_2$, germinated and formed normal *Lochmiopsis* plants. In combination with NaCl the results are not so clear as in the experiments mentioned above.

Added in a concentration of 0.005 mol. it antagonized the effect of 0.6 mol. NaCl. The one-topped curve changes in a double-topped curve when the $MgCl_2$ is administered

in a concentration higher than 0.050 mol. Maximal dosis is 0.5 mol. $MgCl_2$. Above this concentration no germination occurs. This limit is not only to be considered as a limit in toxicity, but it is quite probable that in this case the total concentration surpasses the limits of germination. In the same way the divergent behaviour of the curve (NaCl + 0.1 mol. $MgCl_2$) may be explained.

The proportion for antagonism of $NaCl/MgCl_2$, is 60—160 (30—80 in equivalents), just like the sodium-calcium antagonism.

In order to see in how far the process of germination is influenced by the valence of the ions, two more salts were used in the culture fluid.

Lithium, as a monovalent ion, proved to be highly toxic and was never tolerated in pure solution. The cells died off within a week. Added to the NaCl solutions it had the same influence on germination as the NaCl-KCl mixtures. Only the cells died off immediately after germination.

It therefore appears that the properties described in the lyotropic series K-Na-Li, etc. apparently have little to do with the germination of the akinetes.

The same may be said of the trivalent *hexamine-cobalti-chloride*. Striking is the result after adding 0.0008 mol. of the salt. Here along the range 0.1—1.0 mol. NaCl the percentage of germination is 15, 25, 30, 33, 35, 27, 22, 16, 10, 6 %.

The effect is apparently influenced by, but not proportional to, valence as appears from the following summary.

Amount of equivalent additional kation-chloride necessary to cause optimal germination in NaCl solutions, expressed as Na/kation.

K.....	30— 80
Ca	30— 80
Mg	30— 80
Hexaminecobalt	120—320

In studying the influence of the anions, choice was made from the salts occurring in natural waters, i.c. Na_2CO_3, $NaNO_3$ and Na_2SO_4.

The salts were combined with NaCl, as in simple solutions they proved to be injurious to the cells. In these mixtures the same behaviour of the akinetes could be observed as in the chloride combinations. At first the curve representing germination was double-topped, then, by raising the molarity of the second salt, it passed over to a single-topped curve to become double-topped once more by further raising this quantity.

The typical concentrations of the salts and the resulting percentages germination are recorded in the following table.

TABLE 6.

NaCl in mol.	$NaNO_3$		Na_2SO_4	
	(single top) 0.002 mol.	(double top) 0.01 mol.	(single top) 0.010 mol.	(double top) 0.050 mol.
0.1	0	8	0	0
0.2	6	20	8	0
0.3	28	34	10	4
0.4	48	15	13	12
0.5	66	7	15	13
0.6	36	2	16	4
0.7	9	9	15	10
0.8	6	16	9	6
0.9	4	9	6	4
1.0	3	4	4	2

The results of the NaCl-Na_2CO_3 mixtures are not to be compared with the other combinations mentioned, because in this case two effects are exerting their influence. For, by adding the soda, the alkalinity of the fluid is greatly raised. In the cases of the neutral salts the solutions are almost neutral, while in the NaCl-Na_2CO_3 mixtures the pH varies

from 9—10. As will be demonstrated later, the germinating power increases with increasing alkalinity.

The percentage of germinations after addition of Na_2CO_3 is raised from 10—20 %, independent of NaCl concentration over the simple NaCl solution.

TABLE 7.

NaCl	Mg	0.005 mol.		0.010 mol.		0.100 mol.		
	Ca	Mg/Ca/Na %	Mg/Na %	Mg/Ca/Na %	Mg/Na %	Mg/Ca/Na %	Mg/Na %	Ca/Na %
0.3	0.005	31	40	89	5	90	33	10
	0.010			84		51		14
	0.050			56		36		6
	0.100					62		—
0.5	0.005	32	65	72	16	65	9	26
	0.010			56		55		12
	0.050			34		35		—
	0.100					33		
0.7	0.005	4	27	70	8	59	8	21
	0.010			63		51		14
	0.050			35		12		—
	0.100					6		—
mol.	mol.							

After the mixtures of two salts, three salts were combined. As usual NaCl-solution formed the foundation, to which KCl, $MgCl_2$ and $CaCl_2$ were added in varying concentrations.

Three proportions of the salts were used; the first one corresponding to the single-topped curve, the second to the double-topped curve, while the third solution represents a limit for development in the two-salt-combinations.

For $CaCl_2$ these values were: 0.005, 0.010 and 0.050 mol.; for KCl: 0.010, 0.040 and 0.080 mol. and for $MgCl_2$: 0.005, 0.010 and 0.100 mol. (see Figure 12).

No antagonism could be observed for K/Ca or K/Mg. Ca combined with Mg, on the contrary, showed distinct antagonism. Some of the results are collected in the table above.

Considering these results it seemed quite probable that by adding the salts of Mg and Ca in an adequate proportion the limits for germination should be much widened and that germination should occur at much higher concentrations of the NaCl. This supposition proved to be inexact as germination did not occur in $MgCl_2$-$CaCl_2$ mixtures and the upper limit was shifted from 1.5—1.6 mol. NaCl only.

The limits for Ca-tolerance in germination as well as in development are widened, being shifted after addition of 0.100 mol. $MgCl_2$ from 0.030—0.050 mol. $CaCl_2$. (See tab. 7).

In all cases observed, potassium proved to be toxic in concentrations above 0.060 mol. KCl.

The following three-salt combinations proved, therefore, to be most suitable for germination:

$MgCl_2$	0.01 —0.100	mol.
$CaCl_2$	0.005—0.010	mol.
NaCl	0.3 —0.500	mol.

b. Specific Salt Influence.

After the observations on the influence of various salts on the germination of the akinetes, the next step was to establish the influence of several ions, present in the natural environment, on growth and further development of the alga. Various culture media which differed only in the kations, showed that germination is possible in combinations of chlorides, but that growth is excluded. This phenomenon holds for pure NaCl-solutions as well as for combinations of NaCl with the chlorides of K, Ca and Mg.

The results obtained with these combinations are given above. It seemed logical to add other anions to the medium, and we chose those anions which are commonly assumed be be indispensable for green plants; NO_3^-, $SO_4^=$, $CO^=_3$ and $HPO_4^=$, added as $NaNO_3$ and KNO_3, Na_2SO_4 and K_2SO_4, Na_2CO_3, $NaHCO_3$ and Na_2HPO_4. Mg and Ca were added as chlorides.

An empirical solution which was previously found suitable, was used as a starting point:

$NaCl$	0.5—1.0	mol.
Na_2HPO_4	2/15000	mol.
KNO_3	0.002	mol.
K_2SO_4	0.001	mol.
$MgCl_2$	0.01	mol.
$CaCl_2$	0.005	mol.
Distilled water.		

NaCl varied in concentrations 0.3, 0.5, 0.7 and 1.0 mol., representing the important points in the germination-diagram. The other salts were added in quantities varying from zero to the point of maximal development, varying one component at the time. The influence was expressed in % germination of 400 akinetes, the length of the plants formed, length and width of the cells, size and character of plastids, and the stages of the life cycle which occurred.

It soon became apparent that different optima occur for various stages of development. Akinetes germinate even in solutions in which further development is impossible.

As an example the data of one experimental series are given in the following table. The first six vertical columns show the variation in the culture medium. The vertical lines indicate that the concentration remained the same as indicated by the number printed above. In the last three columns germination is given in %, length of plants and length of cells in μ. In culture 12, for instance, we have

0.7 mol. NaCl, 0.00015 mol. Na_2HPO_4, 0.002 mol. KNO_3, 0.001 mol. K_2SO_4, no $CaCl_2$, 0.01 mol. $MgCl_2$.

The detailed report on all experimental series is not included in this paper. The results will be reported on page 772 a.f.

TABLE 8.

Number of cultures	NaCl	Na_2HPO_4	KNO_3	K_2SO_4	$CaCl_2$	$MgCl_2$	Germ.	Length plants after 8 days	Cell-length after 8 days
1.	0.7	0.00015	—	0.001	0.005	0.01	95%	280	30
2.			0.001				70	140	24
3.			0.002				45	160	48
4.			0.004				40	130	40
5.			0.016	0.001			25	16	8
6.			0.002	—			95	220	30
7.				0.0005			30	32	26
8.				0.0025			95	200	36
9.				0.005			45	140	68
10.				0.010			30	40	32
11.				0.100	0.005		80	88	22
12.				0.001	—		50	420	76
13.					0.005		80	64	30
14.					0.010		90	44	20
15.					0.020	0.010	85	16	16
16.					0.005	—	80	160	26
17.						0.005	90	144	18
18.						0.100	40	680	60
19.		0.00015				0.500	80	germinated akinetes	dead.
20.		—					10	60	22
21.		0.00075					80	200	50
22.	0.7	0.00150	0.002	0.001	0.005	0.010	95	68	40

Sodium. Na as the only kation is able to sustain the cycle from akinete to filament and vice versa. The plants show poor growth and soon form akinetes, especially in concentrations higher than 0.4 mol. NaCl. The akinetes are small (diameter 10 μ) and often show the characters of hypnospores,

by their contracted protoplast, in which a large amount of starch is formed.

These „hypnospores" do not germinate and die at the second or third transfer. Akinetes germinate, transferred to a new culture fluid, in a normal way or by extrusion of the contents. They show the specific behaviour as to concentration mentioned in the previous chapter. The colour of the chromatophore becomes pale green and the plants usually die after the second transfer immediately after germination.

The plants all showed a large accumulation of starch in the cells.

Potassium. K is noxious for germination even in small quantities. In the pure chloride some germination only occurred in concentrations 0.0004—0.03 mol., but the plants died immediately after germination. In higher concentrations there is no germination. The plastid decreases in size and becomes brownish green. The protoplasm disintegrates into a large number of small granula. Starch appears. The wall seems swollen.

In complex culture fluids potassium seems to play a role in the formation of the zoosporangia, probably in combination with sulphate. The zoosporangia only seemed to occur in solutions containing 0.0005 mol. or less K_2SO_4.

Magnesium. Mg stimulates growth and division in concentrations of 0.010—0.100 mol. The chromatophore is large, and a bright green. In the 0.050—0.100 mol. concentrations in combination with 0.3 and 0.5 mol. NaCl it seems to depress germination about 50 %; in higher NaCl-concentrations it also promotes germination, however. The high NaCl-concentration may antagonize the magnesium.

If the surplus of magnesium is too large, germination is depressed again. In this case the total salt-concentration apparently became too high.

Pearsall (37) who based his conclusions on the work of Loeb (28) claims that, in freshwater, the ionic quotient

$\frac{Na\ (+K)}{Ca + Mg}$ cannot exceed a definite value without changing the character of the vegetation by causing great damage to the development of certain plants. Apparently a similar phenomenon was observed here. Solutions of 0.1—0.4 mol. $MgCl_2$ are tolerated in a latent condition. The germinated material is partly destroyed (50 %) in these solutions. The surviving material stayed green. The upper limit seems to be situated at 0.5 mol. $MgCl_2$.

Calcium. The presence of measurable quantities of calcium seems unnecessary for development in culture fluids of low total concentration. In higher concentrations the action of Ca seems similar to that of magnesium. Highest tolerance 0.020 mol. $CaCl_2$ in combination with $MgCl_2$ and 0.7 mol. NaCl or higher. 0.010 mol. $CaCl_2$ seems to be optimal. In combination with nitrate it shows a curious effect upon the cells. Their mutual adhesion is lessened and they break apart, forming individual small plants. In cultures without calcium germination occurs by extrusion of the cell-contents. It seems therefore, that, contrary to the observations of Vischer (46), calcium may promote the liquefaction of the cell-wall.

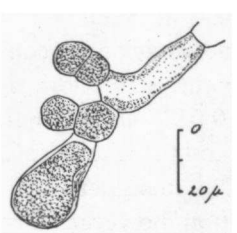

Fig. 13. Giant cell.

$SO^{=}_4$. Sulphate seems to regulate growth and to influence the formation of chlorophyll. When this ion is present in very low concentration (0—0.0002 mol.) giant-cells occur, which cells originate by irregular protrusions of the wall. (Figure 13). The dimensions vary, the width may increase to 28 μ; the lateral protrusions may be 80—90 μ long. Sometimes only the bases of the vegetative cels swell to a globular structure. The wall is irregular in texture with local thickening. The formation of cross-walls seems to be defective and is

sometimes checked in the "diaphragm" stage. The resulting filament presents itself as a long tubular coeloblast. The chromatophore also shows abnormal growth in various directions. It does not cover more than $^1/_4$ of the wall, and often seems torn in various irregular fragments. We may look upon this phenomenon as one of exhaustion of the nutrient medium. It often occurs in older cultures of high or low concentrations.

In concentrations of 0.3 — 0.5 mol. NaCl the phenomenon seems limited to a globular swelling of the cell or of its base. In higher concentrations of NaCl irregular formations of akinetes and giant-cells occur. (Budde, 6).

Zoospores are formed in a higher concentration when potassium is present (0.0005 mol.) The decrease in sulphate-concentrations in natural waters, which seems to be necessary for this phenomenon, may be observed repeatedly. Intake of sulphate by plants and sulphate reduction by bacteria are a few of its biological causes. If the conditions in a lake are sufficiently anaerobic, the sulphate may disappear almost entirely and the fact that we accidentally found both empty sporangia and giant-cells together shows that a variation of SO_4 does occur.

0.0005 mol. appears at higher NaCl-concentrations to be limiting for the formation of akinetes. Germination and development go through an optimum with increasing SO_4 concentration, dependent upon the concentration of NaCl. The sensitivity to $SO^{=}_4$ increases with increasing NaCl-concentrations.

The maximum tolerated dose is 0.1 mol. Na_2SO_4. It limits germination at 0.3 mol. and 0.5 mol. NaCl to a low percentage, germinated cells either die directly, or elongate very rapidly. In 7 days a 1—2 mm. long plant is formed. These plants consist of long strands of cells poor in protoplasm, with small, granular chromatophores.

NO^{-}_3. Cultures to which $NaNO_3$ or KNO_3 are added

show a bright green colour. Growth seems to be hampered; the plants consist of short, round or elliptical cells. The adhesion between the cross-walls is decreased, the slightest pressure will separate the cells. This phenomenon is enhanced by the addition of calcium. The optimal quantities seem to be, independent of the NaCl-concentration, 0.01—0.02 mol.

$HPO^{=}_{4}$. Apart from the regulatory influence on the pH, no specific effect could be observed. Optimal concentration is 0.00075—0.00150 mol.

We are now enabled to make a culture medium in which most ions are present in optimal quantity.

The fundamental solution is:

NaCl in concentration of 0.3 mol. to 1.4 mol. Further add:		
$NaNO_3$ or KNO_3	0.010—0.020	mol.
Na_2SO_4 or K_2SO_4	0.001—0.005	mol. (varying with conc. of NaCl)
Na_2HPO_4	0.0015	mol.
$CaCl_2$	0.005	mol.
$MgCl_2$	0.010	mol.
Iron	trace.	

Instead of Na_2HPO_4 Na_2CO_3 may be used in concentrations 0.010—0.020 mol.

A second result of this method is the determination of the boundary of the potential milieu for germination and development, in as far as this milieu is determined by the ionic proportions (Figure 14). The second factor is, of course, total concentration, which factor cannot be shown in the diagram. It appears from Figure 14 that the limits of germination are much wider than those for development. For the preparation of culture fluids only the latter area may be used.

The influence of organic components and of the so-called minimum-factors [1]) have not been studied in this paper.

Practical reasons limit the amount of cultures and there-

[1]) Such as Bo, Si, Mn, Cu, Mb.

fore it seemed advisable to restrict our study to the simplified inorganic environment. It may very well be that the organic material which may be exceedingly abundant in natural environment, as well as the minimum factors

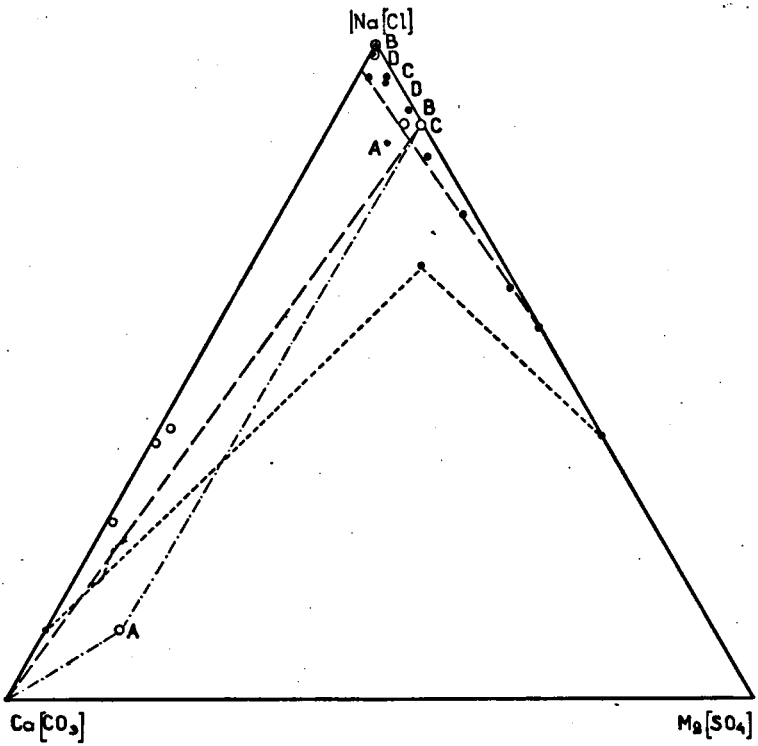

Fig. 14. Triangular diagram, showing potential milieu (— — — — —), and limits of germination (-.-.-) for anions, (- - - - -) for kations. A. Natron Lake, B. Dead Sea, D. Kisil Kul.

exert a marked influence on the development of the forms studied. Inasmuch as the results obtained with our simplified environment actually surpass the potentialities of the natural environment, no very important factor that favoured development could have been omitted.

c. The Influence of Temperature.

A so-called "Light box" which was used in the studies on purple bacteria by Van Niel (32) and for green algae by several other authors was used at first to grow *Lochmiopsis*. This light box consisted of a wooden compartment, lighted by a row of centrally placed lamps. No development occurred in this box after 14 days; the temperature in the box fluctuated between 20° and 24° C.

One half of the flasks were removed from the light box and placed in a north window. After three days development in concentrations 0.5 and 1.0 mol. NaCl could be observed, while the controls in the box still showed no development. The temperature in the window varied during those days from 16°—18.5° C. This observation lead to a further study of the influence of temperature on the alga.

In order to be able to study the effect of a wide range of temperatures, the following incubator was constructed (Figure 15), which is an application of the well-known "temperature-organ". A metal plate which is cooled at one end and heated at the other end will show a temperature-gradient which may be varied by varying the temperature at the terminals.

A copper plate was used, 3 mm. thick. The plate was bent in the way indicated in the figure. One end was forced around a tube (K) through which flowed ice water from the ice box. An electric heater (V) with a regulator (T. R) regulate the temperature of the first culture-chamber. A similar heater and regulator kept the last culture-chamber at a constant temperature. The apparatus was connected with the city current, with a carbon-lamp as primary resistance. The low-voltage current necessary for the interruption of the city current was provided by two batteries of 2 Volt each. Figure 16 shows the wiring of the instrument. The outer mantle of the apparatus consists of multiplex wood, while the sides are closed by double plate-glass windows

which provide sufficient isolation. Individual compartments are isolated above by pieces of multiplex, at the sides by

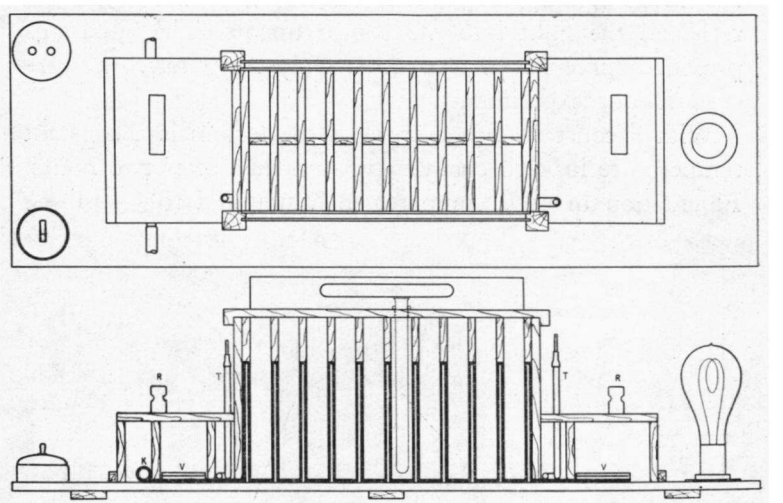

Fig. 15. Diagram of incubator.

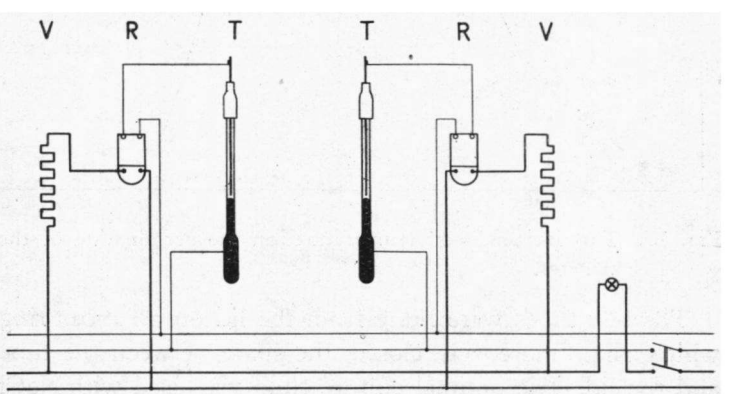

Fig. 16. Wiring of incubator.

rubber which is pressed by the glass against the copper. By a longitudinal division the apparatus is divided into two

series of compartments, enabling a dark control. Light was provided by a show-case lamp, placed horizontally above the longitudinal axis of the thermostat. Two mirrors reflected the light into the compartments. The apparatus provided space for 40 test tubes. Only the outer rows were used in our experiments.

With a constant temperature at the terminals a constant temperature in each chamber was reached after two hours. Fluctuation in each compartment amounted to $\pm$ 0.1° C.

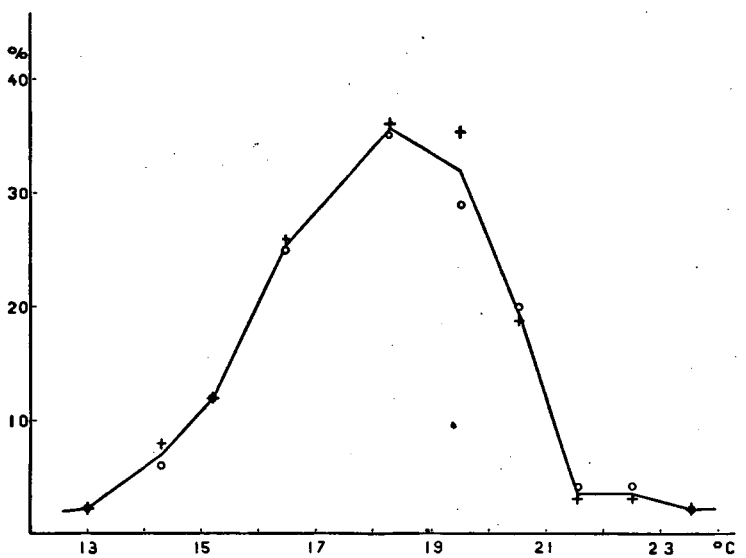

Fig. 17. The influence of temperature on the germination of the akinetes.

The test tubes were hung from the lid on a rubber ring which ring, moreover, closed the space between the tube and the lid. The optimal culture fluid was used with NaCl in concentrations 0.3 mol., 0.5 mol. and 0.7 mol. Germination % of akinetes was determined after one week. The result of one of the experimental series is shown in Figure 17.

The temperature-range in which akinetes show rapid

and intense germination is very narrow. The lowest limit lies at 14° C. At this temperature germination is reduced to a few percent, while growth has almost stopped. An optimum was found at 18° C. Here germination occurred after a few days, the young plants were almost 0.5 mm. long. Growth seems still vigorous between 18°—21° C., but here germination decreases markedly.

Optimal for germination seems the range between 16°—18° C. and the upper limit is situated at 22° C. where the germination has almost stopped.

The limits for development are a little wider, between 16°—24° C. Higher temperatures are tolerated (Woronichin) but growth has ceased.

Filaments grown at various temperatures often show when placed in the incubator at 24.5° C., a repeated division of the cells. A large number of short cells are formed which swell to a globular shape, giving rise to an abnormal formation of akinetes.

d. Influence of Hydrogen-ion Concentration.

In the natural environment of *Lochmiopsis* the alkalinity of the brine is high (pH = 10) which is due to the large amount of $CO_3^{=}$ and HCO_3^{-}. The total lack of calcium and the small amount of magnesium may find an explanation in this fact.

So the question was raised in which degree the development of the alga may be accelerated or depressed by the hydrogen-ion concentration of the culture fluid. The influence of Na_2CO_3 on germination already gave an indication in this direction. Both germination and growth were stimulated by the addition of Na_2CO_3.

A set of buffer-solutions were prepared which were added to the culture fluid. The buffers were chosen amongst the phosphate-NaOH and soda-borax mixtures in a range, varying from pH 6—pH 12. These salts were chosen as

they occur in the ordinary culture fluids, while borax is one of the constituents of the brine in Mono Lake. A difficulty in the use of these buffers proved to be the great change in pH in adding them to the culture medium which contains a considerable amount of NaCl and other salts. Moreover, some of the components seem to be assimilated much more rapidly than the others, so a change in alkalinity during development could not be prevented.

The first difficulty was met by adjusting the initial pH-value by titration with NaOH in the phosphate-mixtures and with Na_2CO_3 in the borax-buffers. The pH value was determined colorimetrically during development. The pH value of the buffers were taken overlapping each other intentionally to exclude specific action.

The buffers were added to a culture-fluid of the following composition.

NaCl	0.5	1.0	mol.
$NaNO_3$	0.01	0.01	mol.
Na_2SO_4	0.001	0.001	mol.

Ca and Mg were omitted as these elements are precipitated in higher alkalinities and so the composition of the diverse culture fluids would not be comparable any longer. A piece of iron-wire was added to provide the cultures with iron.

After a week the cultures were examined. The percentage of germination and the length of the plants were recorded.

As the table below shows, germination in the lower concentration is more than in the higher concentration. In both cases optimal conditions lie at pH 9—11. Cell length as well as the length of the filaments seems to be a function of H^+-concentration. Increased longitudinal growth also showed by the tendency to branch. Below pH = 8 abnormal forms occur. The influence is clearly illustrated by Figure 18.

After a week alkalinity in the range pH 11—12 decreased to a value between pH 9 and pH 10, independent of the nature

TABLE 9.

0.5 mol NaCl

pH	% germination	length in μ	Remarks
6	± 40	—20	Just started growing, many dead akinetes.
7	± 60	12—30	Growth irregular, tending to form giant-cells. Division retarded.
8	± 80	23—60	Plastids well-developed, 2—3 pyrenoids.
9	100	30—95	Plastids well-developed, 1—6 pyrenoids, unbranched.
10	100	60—100	Id.
11	100	60—100	Id. Branched.
12	100	60—200	Id. Id.

1.0 mol. NaCl

pH	% germination	length cell	Remarks.
6	—	—	Many dead akinetes.
7	30	—18	Just started growing, tending to form giant-cells.
8	40	28—70	Unbranched, plastids well-developed.
9	80	30—100	Branched.
10	100	60—250	Id.
11	100	60—250	Id.
12	100	60—250	Id.

of the buffer used. Hence the great similarity in the last three cases. In the further course of development the advance acquired in the first week was preserved. The plants in the culture fluid of pH 9—10 showed copious growth and branching. After addition of 0.01 mol. KOH

in 10 days the alga formed zoosporangia and spores, which, after germination, covered the glass with a heavy coating. In the lower range of pH growth was slow in 1 mol. NaCl, the plants soon formed akinetes and the vegetative cells died off. In 0.5 mol. little growth was observed; in the cultures with KOH a few zoospores were formed which formed resting stages in a couple of days.

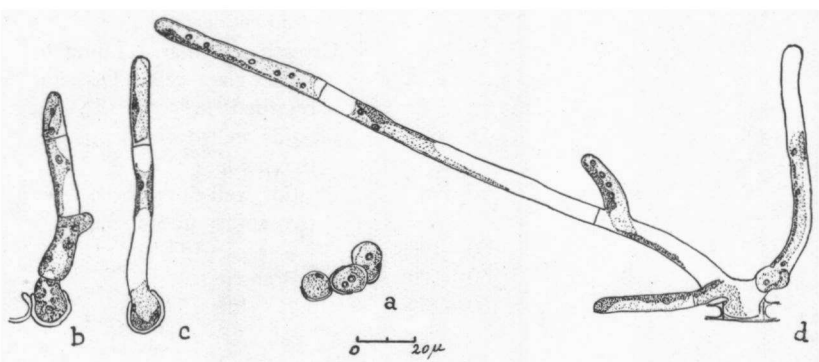

Fig. 18. Influence of pH on development *a*. pH = 7; *b*. pH = 8; *c*. pH = 9 *d*. pH = 10.

e. Natural waters. A number of cultures were started in solutions constructed after the analyses of some natural waters, mentioned in Clarke (10). In the summary on the waters of closed basins Clarke gives a classification of the lakes in a few well characterized groups. Each of the groups are represented by at least one solution, used as a culture medium for *Lochmiopsis*, in order to see wether the alga may occur in other waters than are recorded as its habitat.

The first type mentioned is the chloride water characterized by sodium-chloride. Of this group Lake Tekir-Ghiol (Roumania) and the sea water (mean of 77 analyses by Dittmar's) were taken as types.

From the second group the natural bitterns, in which Mg-salts are concentrated by evaporation, the surface-water of the Dead Sea at the north end of the lake (Clarke, l. c., p. 164) was selected as a representative of the sulphato-chlorid bittern.

As a transition of the chloride- to the sulphate-type Lake Kisil Kul (Siberia) and Schunett Lake (Siberia) were chosen as examples of the chloro-sulphate and of the sulphato-chloride waters respectively.

TABLE 10.

Type	Chloride		Bittern	Chloro-sulphate	Sul-phato-chloride	Chloro-carbonate		Triple waters	
	A.	B.	C.	D.	E.	F.	G.	H.	I.
Cl	60.53	55.29	65.81	48.28	38.89	26.00	43.47	36.51	36.67
Br	0.18	0.18	2.37	trace	—	—	—	—	—
SO_4	0.67	7.69	0.31	14.55	32.19	—	0.03	10.36	12.33
CO_3	trace	0.21	trace	0.22	0.31	32.90	15.17	13.78	10.42
B_4O_7.....	—	—	—	—	—	—	—	0.25	—
Na	34.78	30.59	11.65	34.01	16.12	31.05	41.33	36.63	33.46
K	1.68	1.11	1.85	0.35	0.33	—	—	2.01	7.09
Ca.....	0.28	1.20	4.73	0.69	0.44	3.57	—	—	trace
Mg	1.84	3.73	13.28	1.86	11.67	3.62	—	0.22	trace
Salinity p.p.m.	70877	35000	19215	1085	1519	4407	211400	113700	310000

A. Tekir Ghiol ..... Clarke, p. 168
B. Ocean ..... p. 123
C. Dead Sea ..... p. 164
D. Kisil Kul ..... p. 167
E. Schunett ..... p. 167
F. Natron Lake ..... p. 169
G. Salt Lake near Pretoria ..... p. 169
H. Soda Lake ..... p. 157
I. Katwee Salt Lake ..... p. 169

In the group of carbonate waters the Siberian lakes Petuchowskoje, Belenkoje and Maloje could be classed easily, as the CO_3 radical is predominating here.

To the following type, the carbonate-chloride waters, belong Marina and also Mono (California); the latter classed, however, amongst the "triple waters" by Clarke. Here chloride as well as carbonate are present in quantities of equal importance. As further representatives of this type Natron Lake (Egypt) and the Salt Lake near Pretoria (S. Africa) were chosen.

The "triple waters" in which chlorides, sulphates and carbonates are all present in important quantities, were represented by Soda Lake (Nevada) and Katwee Salt Lake (S. Africa).

In the following table the analyses of the various waters are given. (See tab. 10).

For the analyses of Petuchowskoje, Maloje, Marina and Mono see table on p. 729.

The culture fluids were carefully prepared by mixing measured amounts of stock solutions of NaCl, Na_2SO_4, K_2SO_4, $MgCl_2$, $MgSO_4$, $CaCl_2$, $Ca(OH)_2$, Na_2CO_3, $NaHCO_3$ and borax and adjusted to the natural salinity. In cases where the natural salinity was higher than 42 000 p. p. m. the solutions were adjusted to this salt-concentration.

The objection could be made that in none of the analyses iron as one of the most important elements occurred and in the natural waters undoubtedly a small amount of iron must be present. So some iron filings were added.

In all solutions the pH proved to be between 9 and 10.

After a week the cultures were examined.

Tekir Ghiol. Germination up to 30 %. The length of the plants varied from 30—200 μ, the width from 6—10 μ. Some giant-cells were formed. The plastids were bright green. Starch occurred in many places of the plastid.

Development ceased after some time.

Ocean. No development could be observed.

Dead Sea. Germination up to 7 %. All cells had increased their volume by swelling of the protoplasm and increase of the vacuoles. The vegetative cells were much divided and elongated. The plastids were bright green, with one or two pyrenoids and well-developed starch-bodies. No "scattered starch" occurred.

Kisil Kul and Schunett Lake showed little germination. The cells had swollen but seemed to tolerate this medium. In Schunett Lake many dead akinetes were found.

Belenkoje. Germination up to 90 %. Algae 200 μ in length, all of the sibirica type. The plastid is bright green, packed with starch. The plants are unbranched.

Petuchowskoje. Like *Belenkoje,* but germination only up to 60 %. A further difference are the broad cells at the base of the plant, reaching a diameter of 11 μ. At the top and in the ramifications the diameter of the cells never exceeds 7 μ (Characteristic of *L. Printzii.*)

In this connection it seems worth while to state that Woronichin mentions *L. sibirica* from Belenkoje Lake, while both forms occurred in Petuchowskoje.

Of *Marina* three culture-media had been prepared, respectively of a concentration 0.74, 0.59 and 1,0 mol. NaCl. The first culture showed 80 % germination. Plants had grown out to a length of 150 μ, cells 8—60 μ. The plastid was bright green, but granulated. The second culture was of the same appearance, only all akinetes had germinated and some of the plants were branched. The last mentioned fluid did not yield any germinating akinetes.

Mono Lake. Germination 10 %. The rest of the akinetes swollen by increase of the vacuoles and the protoplasm. The plastid was bright green.

Natron Lake. Germination 100%. The cells were up to 30 μ in length. The plastid was bright green, packed with starch.

Salt Lake near Pretoria. Many of the akinetes were dead. No germination occurred.

Soda Lake and *Katwee Salt Lake.* Within a week all cells, vegetative as well as akinetes had died off.

The difficulty in reconstructing a natural water for culture purposes is the uncertainty of the date and the month in which the waters have been collected. It is, therefore, quite unknown in how far biotic influences have changed the initial composition. Striking are the data obtained from the three culture fluids, constructed after the analyses of Marina. The first two being the analyses made at the beginning of a vegetation-period and of a deeper pond, the last analysis, taken in a shallow pool at the end of the vegetative period. It is plausible, therefore, that in the last fluid the akinetes remain in their more latent form and do not germinate.

However, from the above record it may be ascertained which types of waters are to be excluded as habitats of *Lochmiopsis.* In the first place the chloride waters must be excluded in as far as the amount of Ca and SO_4 is too high, as it proved to be in seawater.

In the second place the chloro-sulphate and sulphato-chloride waters do not represent possible environments when the sulphate predominates over the other negative ions. Speaking generally, developement of the alga will not occur in such basins where, in the beginning of a vegetation period, the sulfate contents surpasses 1 % of the total amount of anions. The same may be said of the "triple" waters in which the three negative radicals are present in notable quantities.

The rest, the carbonate and the carbonate-chloride waters proved to be the medium in which copious growth may be expected, a fact which might have been predicted after examination of the analyses of the natural habitat.

It seems possible, therefore, that *Lochmiopsis* will be

found in many more lakes of these types, in which the total salinity does not surpass 42 000 p. p. m. at the beginning of the growing season.

CHAPTER V.

DISCUSSION AND SUMMARY.

The object of this study, *Lochmiopsis spec.*, is by its occurrence in highly differentiated waters, quite an uncommon object.

As already has been mentioned the natural environment is exposed to considerable variations in physical as well as in chemical properties. In the first chapters data have been given of the chemical composition of the brine, salinity, temperature and biocoenose. Of one of the habitats a more extensive description could be given of the seasonal changes in milieu, but the Marina ponds represent a more exceptional case, as the Californian coastal climate is temperate by so-called summer "high fogs" and the brine is not exposed to great fluctuations in temperature as will be the case in the west-Siberian lakes and Mono Lake. Here extreme cold and heat may occur, thus varying the environmental conditions to a greater extent than has been recorded for these Californian ponds. Furthermore there remains to be considered that at the same time in one and the same lake extreme conditions in salinity do occur. Considering the data for the Belenkoje Lake where the north beach is barren and covered with a salt-crust and the salinity of the brine does not surpass 3 % of salt, a difference in concentration of 96 % may be observed within a distance of 100—250 meters.

By currents or by wind the plants may be removed from a place of extreme "physiological drought" to more moderate environmental conditions and vice versa.

Moreover, the period of vegetation is short, a fact caused

in the shallower pools (some of the Marina ponds, also Maloje, Krolitschie) by rapid evaporation, thus reducing the vegetative period to a couple of months. However, in the cases considered it never exceeds 6 months.

Summarizing these facts it seems evident that the organisms living in this milieu have to be particularly adapted to the sudden changes which may occur. Moreover, it is most probable that in a milieu, variable in composition to such extent (due to the activity of the sulphate-reducing bacteria, colourless and purple sulphur bacteria, the active denitrification, etc.) an alga may present itself under different forms. Chodat (8), Ono (34), Livingston (26, 27), Famintzin (13), Klebs (22), Piercy (39), Vischer (46), Uspenskaja (45) and so many other investigators have shown how trivial changes in the medium exert a prominent change in the morphological characters. The numerous forms of *Lochmiopsis* have extensively been described in Chapter III. The extreme complication of the cycle as illustrated by the diagram Figure 9, however, is more apparent than real, as only a few basic types of cells occur: filament-cells, akinetes and zoosporangia and their products. We shall summarize here the components of the cycle in relation to the physical and chemical environment.

The shortest possible cycle is from *akinete* to *filament* and vice versa. This cycle occurs whenever the medium consists of sodium-salts only in a concentration below 1.5 mol. The formation of akinetes is favoured by an increase in concentration and a decrease of nitrate and sulphate content, while germination is favoured by a decrease. "Exhausted" cultures may be revived by addition of nitrate. The addition of magnesium promotes the development markedly and the formation of the chlorophyll and the colour of the protoplast are favoured also. Moreover, the longitudinal growth of the cells is more

active, so that forms of the type of *Lochmiopsis sibirica* will appear. The form L. *Printzii* seems to develop when Mg is not or scarcely available and probably when, moreover, the temperature is supra optimal. (20° C.). *Woronichin's* samples were taken when the temperature of the lakes amounted to 20.5°—29.5° C. The occurrence of the "sibirica" type could be accounted for when we consider the fact that average summer temperatures of Siberian lakes seem to be situated in the neighbourhood of 17° C.

This is in accordance with the observations that the akinetes germinate in a narrow temperature range with a marked optimum at 18° C.

Addition of the other ions restricts the possibility of germination in a complicated way which is described in Chapter IV of this paper. It appears that antagonisms of various types come into play, but that they do not follow the rules laid down by Loeb (28) inasmuch as they are dependent upon concentration. The valency rule seems to hold, except for trivalent ions, while the lyotropic series — at least as far as the kations are concerned, does not seem to hold.

Zoosporangia are formed whenever the concentration of the sulphate in combination with the potassium, passes below a certain minimum (0.0005 mol.). In the analyses of natural waters where *Lochmiopsis* occurred the sulphate always surpassed the amount of 0.0005 mol. This does not invalidate our result, however, inasmuch as the sulphate contents of a water may be variable in the extreme. In certain older cultures in which the solution might have been exhausted, foot-plates as well as filaments developed zoosporangia. The foot-plate cells also showed zoosporangia which might have been developed because of the decrease of the sulphate and potassium in the solution.

It seems that the same solutions which promote the

formation of the zoosporangium also enable gametes and zygotes to develop.

A process resembling the formation of macrozoospores, which consists in the extrusion of the protoplasm (without previous division, however) occurs in solutions containing calcium and NaCl up to a concentration of 0.5 mol. In these cultures, moreover, the adhesion of the cells seem to be disturbed, so that very loose colonies are formed.

Increase in longitudinal growth was observed also in solutions of high alkalinity. Many factors, therefore, seem to contribute to this phenomenon. In this case the prolification and branching of the filament was also promoted markedly. At pH = 7 conditions become very unfavourable to development.

Natural environment. Baas Becking in his inaugural adress (2) defines two kinds of environment. *a.* The potential environment which is the sum total of the factors, chemical as well as physical, under which an organism may occur and *b.* the natural or terrestrial environment which is the sum total of the factors under which an organism does occur naturally.

The second type of environment has to be, as a rule, more restricted than the potential milieu, for the waters occurring on earth only show a rather restricted range of variation in their composition and in the physical conditions which influence them.

Objections may be raised to this mode of thinking. For, if active adaptation did not occur, materials collected from a certain natural environment might show, in their potentialities the characteristics of that environment; while when they adapted themselve in the laboratory to a set of new conditions, this change may equally well occur in nature. In this connection the work of Baars (1) on the sulphate-reducing bacteria has to be mentioned. While Baars was able to change the salt-tolerance of

Vibrio desulfuricans in the laboratory he never surpassed the natural potentialities of this organism. If, on the other hand, we are able to show that the forms studied do surpass these potentialities, then we have a good case for the contention that in our case adaptation did not play a role.

This case seems to hold for *Lochmiopsis*. The ionic ratio's which were tolerated in the laboratory are graphically represented in Figure 14 of this paper, giving a range of potentialities. It may be seen from this figure that the points representing the composition of the natural environment of this alga, are situated well within the limits of the potential milieu (compare with Figure 2).

Comparison with the work of Jacobi and Baas Becking (19) on *Artemia salina*, of Hof and Frémy (18) on *blue-green algae*, of E. Petter (38) on red bacteria, of the unpublished work of J. Reuter on *Oospora* and of the data available for *Dunaliella* (25, 3, 4) shows that the potential milieu of all these forms is different and also differs from that of *Lochmiopsis*. The biocoenose, the natural environment, in which these forms occur together, gives evidence of the fact that the natural milieu may be considered as a common denominator of many potential environments.

In relation to environment one more remark seems in place. Woronichin describes two species of *Lochmiopsis* which he names *L. sibirica* and *L. Printzii*. From the data presented in this paper no evidence for the validity of this distinction appeared. On the contrary, we are inclined to believe that those two forms are but modifications of a highly polymorphous alga, which modifications are caused by changes in physical and chemical environment.

SUMMARY.

1. *Lochmiopsis*, a green alga belonging to the family Chaetophoraceae was described by Woronichin in 1927 from lakes in the Kulundin-steppe, west-Siberia. This alga was probably already seen by Loew in 1877 in Owen's Lake, California and was rediscovered by G. M. Smith and L. G. M. Baas Becking in Marina, California in 1927 and in Mono Lake and other places in California in 1929.

2. The alga is polymorphous which accounts for the contention of Woronichin that two forms, *Lochmiopsis sibirica* an *L. Printzii* do occur.

3. The alga is halophilic in concentrations of NaCl lower than 1.5 mol.; the resting stages are halotolerant up to a saturated salt-solution.

4. pH, temperature and salt-influence were studied. They show that the alga is adapted to carbonate and chloro-carbonate-waters of the temperate zone.

   Occurrence of the alga may be predicted in Europe, South-Africa and South-Australia from the data presented in this paper.

5. The natural environment lies well within the potential milieu.

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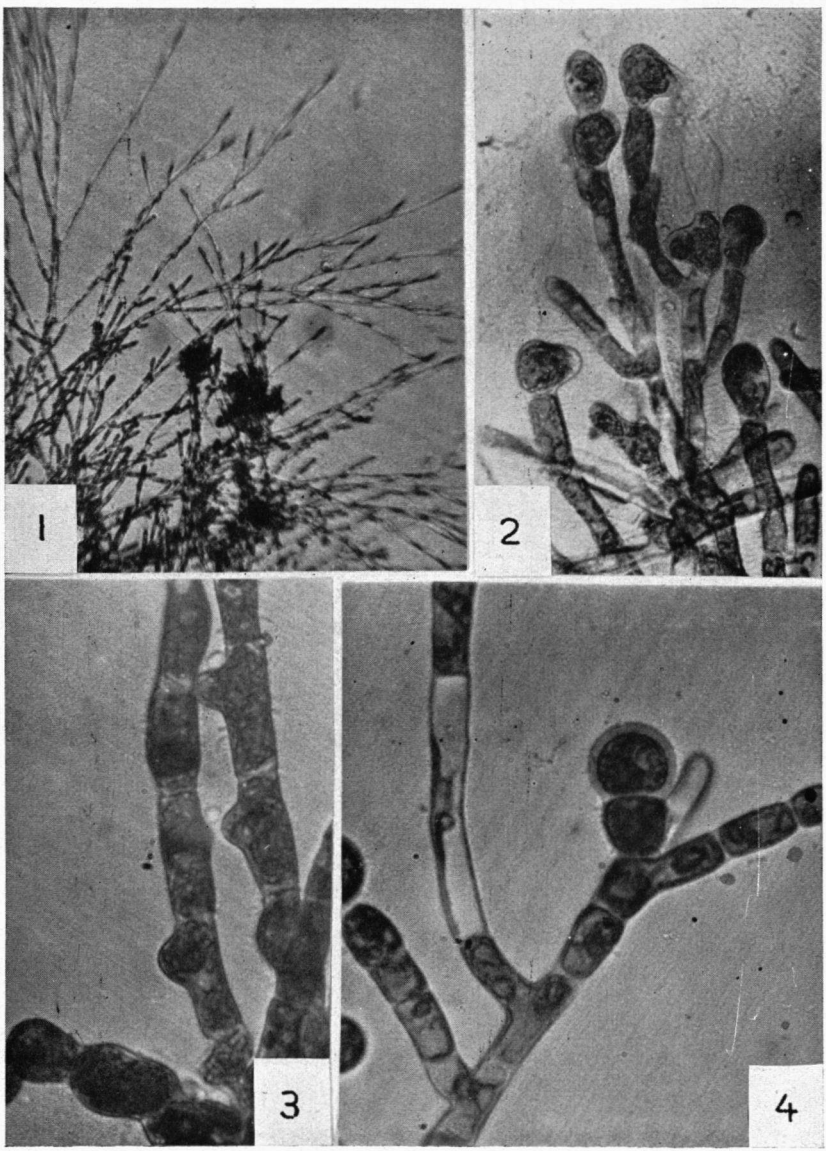

Fig. 19. 1. Lochmiopsis sibirica, thallus.
2. Lochmiopsis sibirica, zoosporangia. 3. Lochmiopsis Printzii, thallus.
4. L. sibirica, akinetes and vegetative cells. For magnification compare text-figures.

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