THE INFLUENCE OF THE ENVIRONMENT ON THE DEVELOPMENT OF RETICULARIA LYCOPERDON BULL.

by

PETRONELLA S. J. SCHURE.

(With Tab. I)

(from the Botanical Institute Government University Leyden)

Table of Contents.

CHAPTER I.

A. INTRODUCTORY.

The literature on the Mycetozoa contains some data on environmental conditions for the development; the papers by Bruck (3), Constantineanu (7), Jahn (22), Pinoy (41), and Skupienski (48, 49, 50) deal with this matter.

Jahn (22) found that the spores of Reticularia Lycoperdon *) take thirty minutes to germinate at 20° C., up to ± 30° the germination time is shortened, at temperatures higher it is always longer. At 37° no germination takes place. In several experiments Jahn found another value for the optimum temperature. The experiments were made in distilled water.

He also studied the influence of the cane-sugar concentration on the spore-germination of several species of Mycetozoa. For Reticularia a solution of 4% cane-sugar prevents germination, while the germination of the spores of Didymium difforme is prevented in a 25% solution. Other species are intermediate in their behaviour.

Constantineanu (7) is of the opinion that it is not the osmotic pressure which is important for the germination, but the chemical nature of the solution. He compared the influence of isotonic solutions of cane-sugar, NaCl and K_2HPO_4 on the spores of Aethalium septicum. Cane-sugar was most favourable for germination.

As regards the influence of high temperatures on the protoplasm of the spores he found that dry spores of Reticularia Lycoperdon and Didymium sqamulosum can bear a temperature of 70° C. during one hour without damage. In a fluid the spores of some species still germinate at 40° C. For Reticularia the germi-

*) As the responsability for the scientific names cited from the literature rests with the authors, no attempt was made to complete the nomenclature cited with author's names.

nation still takes place at 35° C., but is very much retarded; 50% in 24 hours. Low temperatures (2—4° C.) are found for several species (Reticularia inclusive) to have an influence on the germination procent.

According to Pinoy (41) bacteria influence the spore germination of Didymium difforme and Didymium effusum. Germination does not take place in these species without bacteria. Especially Bacillus luteus acts favourably. The spores of other species, Didymium nigripes and Dictyostelium mucoroides are always highly infected with bacteria, which are attached like spines on the wall of the spores. For this reason he believes that the bacteria act on the wall of the spore.

In the case of Reticularia there is no bacterial action. (Wilson and Cadman, 58). The germination takes place in a very short time (from thirty minutes to two hours) and only few bacteria have developed by that time.

Bruck (3) deals with the influence of nutrient medium on the development of Didymium effusum and Didymium difforme. Transfers made of myxamoebae from a three day old culture in fresh nutrient medium give swarm-cells after division. When the myxamoebae are transferred in sterilised exhausted nutrient solution the growth was accelerated and the plasmodia were formed earlier than in the normal solution.

Constantineanu (7) also studied the influence of the environment on the formation of plasmodia and sporangia. The formation of plasmodia takes place in several species in decoctions of maize, bean stalks and bark. Most of the species form plasmodia in media made solid with agar, while Reticularia and some others only form plasmodia in solutions. The plasmodia of Reticularia were only formed in moist chambers and always encysted soon. As for the sporangia-formation he states that in Aethalium septicum the sporangia are only formed in the air. In Physarum didermoides and Didymium effusum they are also formed in solutions.

In regard to the influence of temperature on the formation of plasmodia and sporangia Constantineanu found that Didymium effusum forms plasmodia from 5° till 30° C. and Aethalium septicum from room-temperature till 35° C., the optimum being at 30° C. The sporangia are formed in the same range. Physarum didermoides forms plasmodia from 7° till 25° C.

Skupienski (48, 49, 50) studied the influence of external factors on the development of Didymium difforme and Didymium nigripes. He cultivated these forms on agar with decoctions of potatoes and carrots, with hay decoctions and with malt in mass cultures. The results of his investigations on the influence of temperature and pH we give in a table:

	Didymium difforme	Didymium nigripes
Temperature (in ° C.)	optimum (in hay decoct) 18-20°	minim. 2-4°
	„ (in decoct. of potatoes and carrots) 13-15°	optim. 8-10° (in decoct. of potatoes and carrots)
		maxim. 18-20°
pH		4.8 no development, neither bacteria nor slime-mould.
		5.5 development; takes about 30 days.
		6.5—7.8 development; takes 10 days, is normal in duration and in shape of the fructification.

For both species he investigated the influence of the osmotic pressure with different concentrations of malt.

Skupienski (50) also stated for Didymium difforme a progressive adaptation from the spores formed both under natural conditions and when formed in culture media with a small amount of organic material to culture media rich on food substances. When sown at once on concentrated culture media there is no development.

For both species Skupienski found that variations in temperature or in concentration of the food substances causes changes in the appearance of the sporangia and of the spores (49, 50).

As the literature on the Mycetozoa is very extensive this survey is limited further to data on *Reticularia Lycoperdon* Bull.

Life-history and Cytology. In "The Life history and Cytology of Reticularia Lycoperdon Bull." Wilson and Cadman (58) give a full description of the developmental stages when seen in living condition and when fixed and stained. It is cited here extensively and supplied with observations of my own.

Life-history. The spores (diameter of about 8 μ) are slightly turbinate in form, about two thirds of the wall is regularly rounded, the remaining part forms a blunt cone. The rounded part of the wall is thick and strongly reticulated, the blunt cone is thin, without reticulation, and provided with a few dark coloured spines. The colour is rusty-brown.

The germination was studied bij Wilson and Cadman in tap water The contents of the germinating spore swell and the wall is ruptured where it is thin. After emerging the protoplasm (which shows four to eight vacuoles) remains motionless for some moments, soon it becomes active by the formation of pseudopodia. Gradually the protoplasmic mass assumes the form of the mature swarm-cell and a flagellum is formed. The number of vacuoles is reduced to one pulsating vacuole situated at the posterior end of the swarm-cell.

The cell-division has been studied bij Wilson and Cadman in tap water and in nutrient solutions such as tap water with 0.01% lactic-acid and 2% sugar; decoctions of pine bark and wood. Divisions of the swarm cells take place in considerable number in water from four to twenty-four hours after germination. In nutrient solution the period seems to be shortened. Before division the swarm-cell is rounded off and the flagellum is withdrawn. The separation of the daughter-cells takes place bij constriction and new flagella arise from the ends of the dividing cell.

The later stages in the development; the formation of fused swarm-cells, occurred in the experiments of Wilson and Cadman in decoctions of pine bark and wood with material obtained from a stump on which aethalia were actually found growing. Fusions of swarm-cells may be observed in these solutions about sixteen hours after the suspending of the spores, mostly in pairs. Therefore the fusing swarm-cells are called gametes. (Fusion of the nuclei was also observed). Other swarm-cells, from 3 to 8, coalesce with the fused gametes. After the period of coalescence the plasmodia are spherical, some with long active flagella, sometimes six to one plasmodium. These flagella, which are much larger then those of the swarm-cells, evidently are produced in connection with refractive masses. At a somewhat later stage they have disappeared. The increase of the plasmodia now takes place by the ingestion of swarm-cells. This is quite a different process than the coalescence; the swarm-cells are attached by the anterior end, incorporated into the vacuoles and digested. The plasmodia obtain an irregular shape with active amoeboid movement. Here the cultures of Wilson and Cadman end; they could

not be kept in healthy condition longer than 3 or 4 days.

Under natural conditions the plasmodia are only seen when emerging from the wood in which they were imbedded to form sporangia. This takes place on dead wood.

The maturation of the sporangia takes according to Wilson and Cadman thirty hours. The plasmodium appears in masses of about half an inch in diameter which soon coalesce to a mass of 2—3 inches in diameter. The colour, at first, is creamy white, a silvery skin begins to form over the mass which increases in thickness. The internal mass begins to take a chocolate-brown colour, due to the formation of the spore-walls. Contractions in the sporangial wall by means of loss of water cause ruptures and the spores are set free. It was observed that the emerging of the plasmodium always takes place between 10 a.m. and noon, which may indicate a favourable influence of a rising temperature.

Cytology. (from Wilson and Cadman). The protoplasm of the resting spore consists of a course homogeneous network in which is included a number of rounded granules. The nucleus has an almost central position, its diameter is about 3 μ. The nucleolus is almost centrally placed, it contains the greater part of the chromatic material. When brought into water the protoplasm swells in a few minutes, the nucleus is more clearly defined and the nucleolus stains deeply with safranin and with gentian violet or methylene blue.

In the protoplasm of the swarm-cell granules may be observed like in the resting spore. They have a marked Brownian movement in the living cell. From their reactions it appears that they consist of lipoid material (are stained red with Sudan III, reddish-brown with iodine solution, and are ether soluble). The length of the mature swarm-cell is 14—16 μ, of the flagella 14—22 μ. The nucleus is situated at the anterior part, its diameter is 3 μ, it is only surrounded by a thin layer of cytoplasm. At the sharply pointed anterior end the blepharoplast is situated, between the blepharoplast and the nucleus there appears a cone shaped structure, it appears to consist of fibrils. It is achromatic and without granules. In the central part of the cone is seen a more conspicious fibril, which connects the blepharoplast with a granule on the nuclear membrane. The mature swarm-cell possesses a contractile vacuole towards the posterior end. In the protoplasm of the resting spore no vacuoles are present but they appear soon after germination in a number from three to eight. During elongation and maturation of the swarm-cell their number decreases, the last ones coalesce to one contractile vacuole.

Before division the swarm-cell rounds up. In the dividing swarm-cell the division of the blepharoplast may be first observed, the two parts of it stay connected by a strand, the centro-desmose, later on the two parts of the blepharoplast function as centrosomes. The centrodesmose forms the spindle. The division is karyokinetic, four chromosomes are present, the new flagella arise from the centrosomes.

In the fusing swarm-cells the blepharoplasts come in contact with the nuclei, then the gametes shorten by the formation of an oval fusion-cell (Zygote). Then the nuclei move and when contact is established they fuse. This process is followed by a fusion of the nucleoli. The diameter of the zygote nucleus is 5—6×3 μ, while the zygote has a diameter of 9—10 μ.

From the coalescing swarm-cells the nuclei mostly divide once and are digested.

The nuclei of the young plasmodia contain eight chromosomes. The division of these nuclei takes place without centrosomes.

The plasmodium, when emerged from the wood, consists of protoplasm with

a large number of nuclei. During formation of the sporangia large parts of the protoplasm and of the nuclei degenerate and form walls, hypothallus and strands. In the nuclei of the remaining protoplasm appear two nuclear divisions, the first division is heterotypic, the second division is homoiotypic. The protoplasm is regularly cleaved into uninucleate portions which form the spores.

Our own observations on the several developmental processes are for a great deal in accordance with the descriptions given by Wilson and Cadman. All observations were made on living material. Several of them, of the germination of the spore and of the formation of plasmodia were reproduced cinematografically. The film was taken by Mr. J. C. Mol, Director of the N.V. Multifilm, Haarlem, whom I kindly thank for this work and also for the photo's which he ceded to me for the illustration of this paper.

The germination of the spore was studied in distilled water, the spore-suspension was placed between slide and coverglass which were closed with paraffin-oil. As the process was similar to that observed by Wilson and Cadman, I omit the description here. (figure 1, 2, 3)

The division of the swarm-cell was studied in decoctions of maize, sometimes it was also observed in distilled water or in salt solutions. Our observations were also in accordance with those of Wilson and Cadman (description is omitted).

As regards the formation of plasmodia our observations are in many respects in accordance with the descriptions and figures given by Wilson and Cadman. Their interpretation however does not agree with ours.

The formation of plasmodia was studied in salt solutions, which according to the experiments on the influence of the salts proved to be most suitable, namely solutions of $MgSO_4$ and KCl 100 to 2 milli aequivalents. For the further development they were also put afterwards in maize decoction. In most cases they were put between slide and cover glass, the edges of which were closed with paraffin-oil. From the process of plasmodia-formation we have observed the following stages:

1°. Fusions of swarm-cells in pairs (figure 4); they are present in large number in cultures of about sixteen hours. The swarm-cells become attached with their posterior ends, they remain moving with their flagella, connected with a strand of protoplasm. Gradually their protoplasm is fusing and also the contractile vacuoles.

2°. At the same time are present collections of swarm-cells (figure 6). In some cultures the collections are very large, of about 40 swarm-cells, also smaller ones occur. In the small ones it is

evident that the swarm-cells are arranged round a pair of fused swarm-cells. In the larger collections fusions are soon seen. The contact between swarm-cells and protoplasmic mass always seems to take place with the posterior end of the swarm-cell.

3°. From those collections of swarm-cells arise large rounded off masses of protoplasm (figure 7). The protoplasmic mass mostly consists of homogeneous granular plasm, in which are a few large more refractive granules. In few cases when the masses were very large the protoplasmic mass was not homogeneous but the separate swarm-cells were seen in it actually moving (figure 8). This case I first saw on the film. Later on it was also observed a few times in the cultures. Gradually the protoplasm becomes homogeneous. The rounded off masses sometimes are provided with long flagella.

4°. Gradually the protoplasmic mass becomes more amoeboid, large pseudopodia are put out in all directions and it becomes attached to the surface, on which it spreads, most times branched, in some cases very thin and frond-like (figure 9, 10). The granular contents are moving in a rhythmic flow backwards and forwards. In this stage fusions between plasmodium and swarm-cells were still observed. It was actually observed twice after the addition of fresh swarm-cells, in other cases it could not be seen. It was observed furthermore that rounded off swarm-cells, which were granulated were encircled by pseudopodia and taken in within a vacuole in the plasmodium. Also ungerminated spores and spore-walls were taken in.

From our observations we interpret the course of the process as follows:

After germinating the swarm-cells fuse in pairs; like W i l s o n and C a d m a n we call a pair of fused swarm-cells a zygote. The zygote attracts other swarm-cells, they become attached to the zygote and the protoplasm of the swarm-cells mixes up with that of the zygote to one protoplasmic mass, called the plasmodium. When the process goes very fast the mixing up is very incomplete and intact swarm-cells lay in the protoplasmic mass.

The plasmodium starts moving by the projecting and withdrawing of pseudopodia and by the circulation of the granular endoplasmic mass. The protoplasm stretches on the surface and the movement of the endoplasmic mass becomes more marked, and is a rhytmic movement in backward and forward direction. For its further development the plasmodium takes in young swarm-cells by fusion, and old degenerate swarm-cells by engulfment.

We are in difference with the opinion of W i l s o n and C a d m a n on the duration of the coalescence period. According to

these authors the coalescence period takes a very short time and is bound to the haploid phase of the plasmodium. However their figure 80 shows coalescence between a diploid plasmodium and a swarm-cell. At the same time a swarm-cell is ingested. They describe this condition as quite unusual. From our experiments we have concluded that the coalescence depends from external factors, temperature and salts (Chapter II and III). And it seems probable to us that it also depends on the age or condition of the swarm-cells. Whether there exists a correlation between the nuclear condition of the plasmodium and the external factors we have not investigated further.

The ingestion of normal swarm-cells observed by Wilson and Cadman on stained preparations we never saw in our cultures, only the ingestion of rounded off, degenerate swarm-cells.

Data on environmental conditions of the species. For germination of the spore the temperature and concentration conditions were investigated bij Jahn and Constantineanu (Cf. p. 2).

Wilson and Cadman state that when the spores are present in small number in drop cultures the germination is not succesful. Either it does not take place or if it does, the swarm-cells round off directly, without the production of flagella.

As to the cell-division Wilson and Cadman (58) found that when the spores are sown in nutrient medium the cell-divisions occur more abundantly but the period in which they do occur seems to be shortened in comparison to swarm-cells in water.

According to Wilson and Cadman (58) very special conditions are required to obtain a large number of fusions followed by coalescence. These conditions may be satisfied by the use of wood which has been dead for just the right length of time. In decoctions of pine bark and wood from material obtained from a stump on which aethalia were actually found, the plasmodia-formation went on readily. When wood from stumps which did not bear sporangia was used the plasmodia-formation was not so satisfactory, neither when the first solution was some days old or when it was contaminated with bacteria.

Geographical distribution. The species is known from several places in the world. A survey of the lists shows that the distribution of the species is chiefly extra-tropic. It has been observed in:

non-tropic.
Edinburgh (Wilson and Cadman, 58)
The Netherlands (Scholte, 46)
Poland, Polish Carpathians (Jarocki, 24)
Bialowietz Forest (Jarocki, 23)
Roumania (Brandza, 2)
Russia, Ukraine (Zelle, 59)
Switzerland, Jura (Meylan, 34)
Algeria (Maire, Patouillard, Pinoy, 33)
Tunis (Maire, Patouillard, Pinoy, 33)
Japan (Minakata, 35)
S. Australia (Cleland, 5)

N.S. Wales (Cleland, 5)
New Zealand (Lister, 30)
E. Massachusetts (Gilbert, 12)
Lord Cornell Reservation (Muenscher and Wann, 36)
Michigan Rock River (Kauffman, 25)
Western Washington (Greene, 15)

tropic. Hispaniola (Raunkiaer, 43)

Gilbert (12) who described the species for E. Massachusetts calls it fairly common, but not in great quantity where it does occur.

Meylan (34) mentions Reticularia in the Jura. He distinguishes two species, *R. Lycoperdon* Bull. and *R. jurana* Meyl. R. jurana occurs as first of all slime-moulds in the spring. R. Lycoperdon occurs only in the autumn. They inhabit in the same places, 1500 M. altitude, in full sunshine and in partial shade, but never on very shaded places. They differ from each other in size of the sporangium, in colour of the surface-walls and in the reticulation of the spore-walls. According to G. Lister (31) R. jurana Meyl. which also occurs in England is only a form of R. Lycoperdon Bull, derived from small plasmodia, which have fructified under sheltered situations.

According to Wilson and Cadman the species occurs frequently in the vicinity of Edinburgh on stumps and trunks of different trees, on Pinus sylvestris, Fagus sylvatica and Acer pseudoplatanus. The sporangia were only found on dead trees, the wood of which was comparatively fresh, with no definite sign of decay.

Schinz (45) calls the species cosmopolitic.

It appears from the lists that the occurrence in the tropics is very rare, it was only found in Hispaniola (Raunkiaer, 43) and in Ceylon, known from a specimen in the British Museum (Lister, 31).

The species does not occur on the lists from:

non-tropic. S. Mandchuria (Emoto, 10)
New Zealand (Mac Bride, 32)
Suva (Mac Bride, 32)
Long Island (Hagelstein, 17)
N. E. Colorado (Smith, 51)

tropic. Buitenzorg (Penzig, 40)
N.E.I. (van Overeem—de Haas, 39)
Java and Krakatau (Emoto, 9)
Sumatra (Boedijn, 1)
Malacca (Emoto, 8)
Santo Domingo (Toro, 53)
Porto Rico (Hagelstein, 16)
Surinam and British Guyana (Gilbert, 13)
St. Croix, St. Thomas, St. Juan (Raunkiaer, 43)

So from 22 lists of extra-tropic stations Reticularia occurred on 17, while from 10 lists from the tropics it occurred only on one.

The results of our experiments on the influence of the temperature on the development are in agreement with this distribution.

Chemical nature. Iwanoff (20) investigated the protein of the spores of Reticularia. It closely resembles that of the higher Fungi. He also found that the spores contained about 2% trehalose (the enzym trehalase, however, was not found).

Data on the chemical composition of the plasmodium of Reticularia have

been given by Kiesel (26). The material used consisted of plasmodia which had just emerged from the wood before sporangia-formation. The colour was still white, a little cuticle had already been formed. They were placed in alcohol 96%. Kiesel succeeded to collect as much as 89 Grammes dry weight. The reaction of the plasmodium was a slightly acid one, calcium salts are absent, formic acid has been demonstrated. While on the contrary the plasmodium of Aethalium septicum gives an alcalic reaction and contains much calcium. We reproduce here Kiesel's results in tabellar form.

oil	17.85
lecithin	4.67
cholesterin	0.58
reducing carbohydrates	2.74
not reducing, soluble carbohydrates (except glycogen)	5.32
glycogen	15.24
polysaccharide (hydrolizable with difficulty)	1.78
nitrogenous extractive substances	12.00
protein (a part in nucleoproteid)	20.65
plastin	8.42
nucleic acid	3.68
oil of the lecithoproteids	1.20
unknown substances	5.87
	100.00

The compositions of the wall-substances like peridium and capillitium of Reticularia have been studied by Kiesel (27) and Wettstein (57), both by microchemical methods. Kiesel found polysaccharids and protein in these substances, whereas Wettstein found cellulose and keratin, the latter substance preponderating.

B. STATEMENT OF THE PROBLEM.

An investigation of the potential milieu of one single species of Mycetozoa has, as yet, not been undertaken. In particular of the acalcareous, wood inhabiting species very little is known.

The meaning of this thesis is to determine the influence of some external factors — temperature, salts, pH, osmotic pressure — on the development of an acalcareous species.

Meanwhile attempts have been made to culture this form.

C. MATERIAL AND METHODS.

All experiments were at first made with spores of one sporangium (called *Reticularia 1)*. It was collected in August 1930 from a willow stump in the vicinity of Leyden. Later on duplicate experiments on the influence of temperature, osmotic pressure and cultural conditions were made with spores of a sporangium gathered in August 1933 *(Reticularia 2)* from a felled tree (Spec. undet.) in the vicinity of Leyden.

A retardation in the germination was quite obvious after three years' preservation.

Some experiments on the action of salt on the swarm-cells and experiments on condition of culture were made also with other species. The action of salt was also studied on:

Stemonitis fusca Roth.
Stemonitis splendens Rost. var. *flaccida* List.
Fuligo septica (L.) Gmel.

Culture experiments were also undertaken with:

Stemonitis splendens Rost. var. *flaccida* List.
Didymium effusum Link.

The methods used in this work are fully described in the corresponding chapters. Only some general remarks will be given here.

The distilled water was obtained by double distillation; the first time in a tinned boiler, the second time in a pyrex distillation apparatus over potassium permanganate.

The buffer solutions used in the experiments were prepared according to C l a r k (6) and diluted to one fifth of the normal concentration and afterwards controlled potentiometrically or colorimetrically.

The salt solutions used in the experiments were prepared from ordinary and from "purissima" salts of the N.V. Koninkl. Pharmac. Fabrieken. Except the NaCl used as "purissimum" salt, all salts were purified by recrystallisation, the "purissima" salts twice, the ordinary salts three times. From these salts stock solutions were made. After adjusting the concentration with the refractometer the different molarities were obtained by mixing the stock solutions with controlled quantities of distilled water.

The cane sugar solutions used in the experiments on the influence of the osmotic pressure were made from saccharose Kristal 100% of the N.V. Koninkl. Pharmac. Fabrieken. The concentrations were obtained by mixing a stock solution with distilled water. They were controlled with the refractometer.

The experiments on the germination in relation to temperature, H^+ion concentration, salts, osmotic pressure were made in tubes of so-called French pyrex and of Jena glass. The first experiments were carried out with pyrex and the duplicates with Jena glass. The typ of glass did not seem to make any difference as the experiments take only a very short time, about 4 hours.

In the first experiments the tubes were cleaned with Na_2CO_3 and with potassium-bichromate + H_2SO_4 and rinsed with tap water and afterwards with distilled water. In the duplicate experiments fluoric acid 1% was used instead of the ordinary cleaning solution. This method proved to be superior. In the experiments

with single-cell cultures the cover-glasses on which the drop-cultures were placed were also treated for the cleaning with boiling Na_2CO_3 and after rinsing with acid they were treated with fluoric acid 1% during 5 minutes. Then they were rinsed carefully with distilled water and dried on filterpaper.

CHAPTER II.

THE INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT.

The influence of the temperature was established on several processes of the life-cycle:

a. On the germination of the spores.
b. On the division of the swarm-cells.
c. On the plasmodia-formation.

A. Experimental methods and sources of error.

The spore-germination and temperature. Two series of experiments were performed. In the first series the spores were suspended in distilled water. The suspensions were kept in pyrex tubes in a layer of about 1 cm. thickness.

The temperatures varied from 0°—35° C., with intervals of 5°. The temperatures from 20—35° were obtained in thermostats, 10° and 15° in Dewar vessels, 0° and 2—5° in the ice-box.

The experiments from 10°—35° took no more than 4 hours, at lower temperatures more than 24 hours.

In the second set of experiments a solution of KCl 2 milli aequivalent was used as suspension fluid, as it proved to be the most favourable medium for germination. The several temperatures form 7°—35° were obtained in a so-called temperature-organ. For the description of this incubator cf. J. Ruinen (44).

The influence of the temperature on the germination was measured by determination of the germination percentage after one half-, one-, two- and four hours or longer if necessary. By "percentage" is meant the mean percentage of germination of 300 spores. After thorough shaking samples were taken, from each tube thrice the contents of a wire loop. The samples were coloured with carbol-fuchsin and examined with the microscope. In the ungerminated spores the contents are coloured, while from the germinated spores the empty spore-walls remain uncoloured.

Division of the swarm-cells and temperature. The spores were suspended in a decoction of maize (5 gram maize in 100 cc distil-

led water) and the suspensions kept in tubes in layers of 1 cm. The range of temperature was from 0°—30° C. The temperatures from 7—30° were obtained in the temperature-organ. The experiments were run twice. In the first set only the presence of cell-divisions was ascertained. The later stages of division are quite distinct in living condition when seen under high magnification. The observations were made 17, 22 and 41 hours after the preparation of the spore-suspension.

In the second set of experiments observations were also made earlier, after 2, 4, 8 hours etc. and the frequency of cell-divisions was ascertained after each period. From each tube samples were taken and in these samples were counted the number of division-stages, occurring in thirty fields. In one field about ten swarm-cells were present. Also the total multiplication of the swarm-cells was counted.

The plasmodia-formation and temperature. The plasmodia-formation was investigated in the same temperature-range as used for the swarm-cells. A solution of about 50 milli aequivalent $MgSO_4$ was choosen as a medium. To a dense suspension of germinated spores enough $MgSO_4$ solution was added to obtain a concentration of about 50 milli aequivalents.

Sources of error are: 1°. the uncertainty in the method of expressing the germination percentages amounted to ± 11%. 2°. in traces of electrolytes on the spore material or on the tube walls. Electrolytes in low concentration have a great influence on the germination. 3°. bacteria are present in large quantities in all experiments of longer duration.

B. Experimental results.

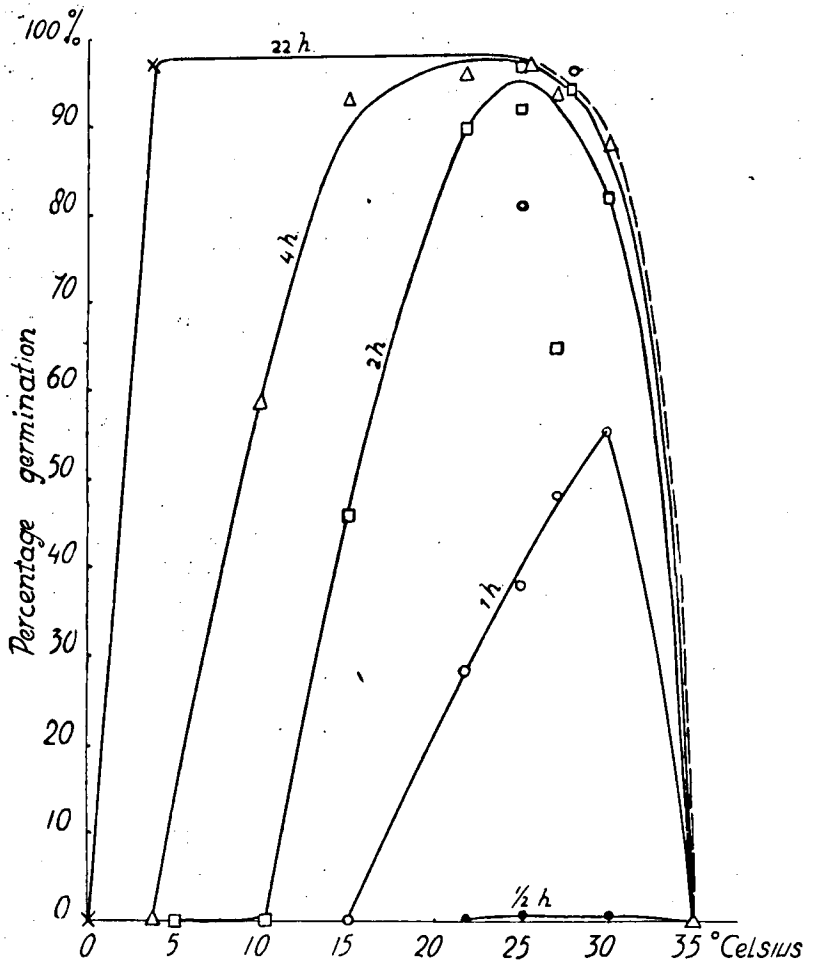
a. Spore-germination and temperature.

The method of spore germination of Reticularia Lycoperdon has been described bij Wilson and Cadman. Their results were confirmed by us and are mentioned in the introduction.

The germination took place from 2—5° to 30° C. At all temperatures nearly all the spores germinated, excepted at 30°. The rate of germination is greatly influenced by temperature. At 0° no germination was observed. At 2—5° germination is the slowest observed. When the germination-percentage is ascertained after one hour the optimal temperature is 30°. When it is determined after 2 and 4 hours the optimum is situated between 25° and 30°. So there is a damaging influence by high temperatures after a certain lapse of time. At 35° no germination was observed. Table 1 and

Text fig. 1 show the influence of temperature on germination in distilled water in relation to time.

Some experiments of the first series were taken later. Their



Text-fig. 1. Influence of the temperature on the germination of the spore of *Reticularia 1.*

results are indicated in the second row of each column of the table. The high values for the germination percentages found after one hour are probably due to electrolyte action.

TABLE 1.

Influence of the temperature on the germination of the spore.

Temp.	Percentage germination after:					
	one half hour	1h.	2h.	4h.	6h.	22h.
35° C.	—	—	—	—		
30	+	55	82	88		
28	+	96	94			
27	+	48	70	93		
25	+ —	38 81	97 92	97		
20—22	—	28	90	96		
15	—	—	46	93		
10	—	—	+	59		
2—5	—	—	—	—	+ —	97 87
0	—	—	—	—	—	—

The temperatures of 0° and 35° were not damaging to the plasma of the spore. A temperature of 0° could be tolerated for 24 hours without damage. When brought at room-temperature the spores germinated regularly. The temperature 35° proved to be innoxious after a duration of four hours.

In the second set of experiments made with *Reticularia 2* in KCl solution of 2 milli aequivalent the germination percentages were less than in the first set. The optimum was situated at 23.5° C. after two hours. The minimum resp. the maximum were situated at 17.5° and 30° after that time.

b. Division of the swarm-cell and temperature.

The method of cell-division in this species has been described in the introduction. In the experiments made in decoctions of maize the cell-divisions occur from 7°—26.5° C. They are always infrequent and only occur during a certain period after the sowing of the spores. At 0° and 30° no divisions were observed. Still these temperatures were not damaging for the swarm-cells, for after a sojourn of two days at 30° C. a large number of swarmers is alive.

The periods in which the divisions of the swarm-cell occur have a duration of some hours. At 7° this period is the longest, at least 8 hours. The period of cell-division begins late at that temperature; after about 16 hours. At 26.5° and 23.5° the duration is less then 6 hours and division starts after about four hours. In table 2 and 3 are shown the results of the observations made on the cell-divisions at different temperatures. It is evident that there exists a relation

between the temperature and the latency-time before the period of cell-division and also between the temperature and the duration of the period of cell-divisions.

TABLE 2.

Influence of the temperature on the cell-division of the swarm-cells.

Temp.	Cell-divisions after:		
	17h.	22h.	41h.
30° C.	—	—	
26.5	—	—	
23.5	—	—	
20 5	+	—	
18.5	—	—	
16	—	+	
14.5	+	—	
12	—	—	
10	++	+	—
7	++	+++	—

TABLE 3.

Influence of the temperature on the cell-divisions of the swarm-cells.

Temp.	Cell divisions after:						
	2h.	4h.	8h.	16h.	24h.	42h.	48h.
30° C.	—	—	—	—			
26.5	—	12	—	—			
23.5	—	4	—	—			
20.5	—	—	9	—			
18.5	—	—	8	—			
16	—	—	2	—			
14.5	—	—	—	—			
12	—	—	—	1	—		
10	—	—	—	4	—		
7	—	—	—	4	9	—	
0	—	—	—	—	—	—	—

In regard to the number of cell-divisions it was already observed that this never was large. The largest number seen was at 26.5° C. in the observation made four hours after the preparation of the spore-suspension. On about 300 swarm-cells 12 appeared to be

in division stage. The number of swarm-cells after the division-period was about twice the number of empty spore-walls in all experiments.

So it appears from these observations that divisions of the swarm-cell are not of much importance for the multiplication and the growth in this species.

c. Plasmodia-formation and temperature.

The method of plasmodia-formation has been described in the introduction.

It appears that the formation of plasmodia took place from 12° —26.5° C (table 4). Below 12° C. zygote formation was only observed at 10°, but this process was not followed by the coalescence of other swarm-cells. Above 26.5°, at 30° no zygote formation was observed. 18.5° and 16° C. are optimal for the coalescence, as measured by the growth per unit time of the plasmodia. At 23.5° and 26.5° the plasmodia remain small and are rounded off. They show only little amoeboid movements. At 20° they are already larger and show a greater variety in shape, also copious amoeboid movement.

TABLE 4.

Influence of the temperature on the plasmodia-formation in 50 milli aequivalent $MgSO_4$ solution.

Temp.	after 16h.		after 23h.		after 40h.	
	zygotes	plasmodia	zygotes	plasmodia	zygotes	plasmodia
30°C.	—	—	—	—	—	—
26.5	+	+				
23.5	+	+				
20.5	+	+				
18.5	+	+				
16	+	+				
14.5	—	—	+	+		
12	—	—	+	+		
10	—	—	—	—	+	—
7	—	—	—	—	—	—

The number of hours has been counted from the incubation of the swarm-cells in the salt solution.

Summary. We summarize the results of the experiments on the influence of temperature in a table:

Process	Range	Optimum
germination	2-30° C.	+ 25°
division	7-26.5°	26.5°
zygote-formation	10-26.5°	
plasmodia formation	12-26.5°	16-18.5°

So with each step in the development the temperature range has become narrower.

CHAPTER III.

THE INFLUENCE OF ELECTROLYTES ON THE DEVELOPMENT.

a. The influence of H^+ and OH^- ions on germination.
b. The influence of salts on germination.
c. Behaviour of the swarm-cells in solutions of one salt.
d. Behaviour of the plasmodia in solutions of one salt.

A. Experimental methods and sources of error.

The influence of H^+ and OH^- ions on germination has been determined in buffer solutions. The following buffer solutions were prepared:

HCl—KCl mixture of Clark and Lubs; for pH 2.
Phthalate—HCl mixture of Clark and Lubs; for pH 3.
Na_2HPO_4—NaH_2PO_4 mixtures of Gyemant; for pH 4.5, 5.9, 7.3.
KH_2PO_4—NaOH mixtures of Clark and Lubs; for pH 6, 7.2, 8.
Boric-acid, KCl—NaOH mixtures of Clark and Lubs; for pH 8, 9, 10.
Glycocoll—NaCl—NaOH mixture of Sørensen; for pH 10.2.

In the standard concentrations the germination was very scant. Therefore the solutions were diluted to one-fifth of their standard strength (one part of buffer solution and four parts of distilled water). Controls of the diluted mixtures were made potentiometrically or colorimetrically with the "Universal Indicator". In some cases both methods were used.

The influence of salts on germination. The action of several cations was studied in solutions of NaCl, KCl, NH_4Cl, $MgCl_2$, $CaCl_2$, $BaCl_2$, $CuCl_2$ and $FeCl_3$. To investigate the action of anions solutions were taken of $MgCl_2$, $MgBr_2$, $Mg(NO_3)_2$, $MgSO_4$ and

$MgCO_3$. The salts were purified by recrystallisation. "Purissimum" salts were recrystallised twice, the ordinary salts three times. From these stock solutions of 0.1 mol. were prepared. The concentrations of these solutions were controlled with the refractometer. The stock solutions were diluted to several concentrations.

The behaviour of the swarm-cells in solutions with one salt. For these experiments the same salts were used in concentrations varying from 100 milli aequivalents to 0.5 milli aequivalents. When a certain concentration proved to be toxic higher dilutions were prepared.

From a dense suspension of germinated spores in distilled water a drop was placed on a slide, and also a drop of salt solution of approximately the same volume. The drops were covered with a coverglass and sealed with paraffin-oil.

The behaviour of the plasmodia in salt solutions has been studied in the solutions in which they were formed, and in solutions of $CaCl_2$ and $BaCl_2$. They were placed in the latter solutions after their development in $MgCl_2$. The original culture fluid was removed by washing with distilled water and centrifuging. They were studied on sealed drops as described for the swarm-cells. In this way several plasmodia are present in each drop together with swarm-cells and empty spore-walls. All the experiments described in this chapter are made at room-temperature (15—20° C.).

Sources of error are: 1°. substances diffusing from the spores into the liquid. These substances exert, when present in sufficient quantity (e.g. in dense suspensions), a protecting action against chemical substances dissolved from glass. (cf. chapter V). All experiments on salt action have been performed with dense suspensions of spores. So in all these experiments these substances have exerted their influence. 2°. As a second source of error bacteria and their dissimilation products should be mentioned. The spores of Reticularia used for the experiments were but little infected with bacteria. By plating from a dense suspension of spores on yeast autolysate-agar (pH 8), bouillon agar (pH 8) and malt agar no colonies of bacteria or fungi appeared after 36 hours. The petri dishes were incubated at 30° C. and at room-temperature (18—20° C.). After eight days on the plates incubated at 30°, only on malt agar, one colony of bacteria was present. At room-temperature one colony appeared on yeast autolysate-agar. The bacteria from this medium were spore forming bacteria belonging to the genus Bacillus. 3°. A third source of error may arise from the inaccuracy in mixing a certain quantity of spore suspension with an equal amount of salt-solution. This was performed by means of calibrated capilla-

ries, but as the final concentrations were not checked, small deviations may have occurred.

B. Experimental Results.

a. The influence of H^+ and OH^- ions on germination.

A pH of 6 to 8 can be withstood without damage. Beneath pH 6 there is still germination down to pH 3. No germination occurs, however, at pH 4.5 and 5. This is evidently due to other ions, for in all solutions of Na_2HPO_4—NaH_2PO_4 from pH 4.5 to pH 7.3 no germination took place. Probably the $HPO_4^=$ ions are toxic, the other ions do not show any toxicity when used in the other buffer-mixtures.

Above pH 8 germination takes place, but the germination percentage could not be stated because the spores did not remain suspended. In the boric-acid, KCl—NaOH mixture of pH 9 and in the glycocoll NaCl—NaOH mixtures of pH 9 and 10 more than 1% germinated. In the boric-acid, KCl—NaOH mixture of pH 10 the germination is less than 1%, the swarm-cells are soon rounded off, in all other solutions they remain active.

Table 5 shows the influence of H^+ and OH^- ions and also the specific action of the buffer-mixtures.

Table 5.

Germination in different buffer-mixtures.

Buffer-mixtures	pH	Buffers controlled by:	Percentage-germination after: 1 hour	2h.	4h.
HCl—KCl	2	c.	—	—	—
Phthalate—HCl	3	c. p.	8	19	19
Na_2HPO_4—NaH_2PO_4 ..	4.5	c.	+	—	—
„ „	5.9	c	—	—	—
Na_2HPO_4—NaH_2PO_4 ..	6.0	c. p.	87	90	90
„ „	7.2	c. p.	76	90	92
Na_2HPO_4—NaH_2PO_4 ..	7.3	c.	+	+	1
KH_2PO_4—NaOH	7.7	c. p.	87	87	90
Boric-acid, KCl—NaHO	7.8	c p.	74	84	88
„ „ „	8.5—9	c.			more than 1%
„ „ „	10	c.	+	+	+
Glycocoll—NaCl—NaOH	10	c.			large number

In the column for buffers controlled by; c. means controlled colorimetrically, p. means potentiometrically.

b. The influence of salts on the germination.

The action of cations. For these experiments highly-dilute

solutions were used, to exclude osmotic action. In the first set of experiments the concentration was 2 or 4 milli aequivalent. In the second one 20 milli aequivalent. The salts used were NaCl, KCl, NH_4Cl, $MgCl_2$, $CaCl_2$, $BaCl_2$, $CuCl_2$ and $FeCl_3$. The results in table 6 show a markedly-favourable influence of KCl, also NaCl seems favourable for the germination. NH_4Cl, $MgCl_2$, $CaCl_2$, BaCl, $CuCl_2$ and $FeCl_3$ are damaging, the toxicity increases in the following sequence:

$[NH_4Cl, MgCl_2] < CaCl_2 < BaCl_2 < CuCl_2 < FeCl_3$.

With NH_4Cl and $MgCl_2$ the values for the germination percentages prove to be variable.

TABLE 6.

Germination in salt solutions; chlorides with varying cations.

Solution	Concentr. in milli-aequivalent	Percentage germination after:		
		1 hour	2h.	4 h.
NaCl	2	46	91	98 89
KCl	2	79	95	94 89
NH_4Cl	2	18	60	90 53 16 36
$MgCl_2$	4	24 8	33 55	34 75 18 15
$CaCl_2$	4	25	51	48 29
$BaCl_2$	4	5	11	7 2
$CuCl_2$	4	—		—
$FeCl_3$	6	—		—
$MgCl_2$	20			1
$CaCl_2$	20			—
$BaCl_2$	20			—
Distilled water		16	88	95 92 80 50

The second set of experiments in which germination in solutions of 20 milli aequivalents $BaCl_2$, $CaCl_2$ and $MgCl_2$ were compared, showed that $CaCl_2$ is more damaging than $MgCl_2$.

In $CuCl_2$ germination is 1% after 7 hours. The swarm-cells are directly rounded off and become very large with large granules in Brownian movement.

In $FeCl_3$ there is no germination, here the low pH may have showed that $CaCl_2$ is more damaging than $MgCl_2$.

The cations seem to act therefore on the germination in the following sequence:

K^+ Na^+ $[NH_4^+$ $Mg^{++}]$ Ca^{++} Ba^{++} Cu^{++} Fe^{+++}

germination is favoured — germination is inhibited
(as compared with distilled water)

The action of anions has been studied in solutions of $MgCO_3$, $MgSO_4$, $MgCl_2$, $Mg(NO_3)_2$. The concentrations used were 4 and 20 milli aequivalent. The results are shown in table 7.

TABLE 7.

Germination in solutions of Mg salts, with varying anions.

Solution	Concentr. in milli-aequivalent	Percentage germination after: 1 hour	2 h.	4 h.
$MgCO_3$	4	54	94	85
$MgSO_4$	4	30	78	93 83
$MgCl_2$	4	24	33	34 18 75 15
$Mg(NO_3)_2$	4	8	41	57 24
$MgBr_2$	4	10	37	50 17
$MgCO_3$	20	+	90	94
$MgSO_4$	20	—	21	26
$MgCl_2$	20	+	1	1
$Mg(NO_3)_2$	20	—	+	2
$MgBr_2$	20	—	+	1
Distilled water			84	96

$MgCO_3$ acts favourably, also $MgSO_4$. The other solutions are damaging. From the results it is evident that the series for the anion action on the germination becomes:

$CO_3^=$ $SO_4^=$ Cl^- Br^- NO_3^-

germination is favoured — germination is inhibited
(as compared with distilled water)

c. Behaviour of the swarm-cells in solutions of one salt.

A dense suspension of swarm-cells in distilled water is able to stay alive for about fourteen days between slide and coverglass closed with paraffin-oil. The swarm-cells arrange themselves in

a zone situated at some distance from the edge of the coverglass. Probably this is due to aerotaxis. In the centre of the slide only some large, rounded and degenerate swarm-cells are present. Observations were made after one hour, 24, 48 hours and longer.

Action of NaCl. A solution of 50 milli aequivalent is toxic, from 5 experiments only in one experiment the swarm-cells stayed alive after 24 hours. In the other four experiments the swarm-cells remained motionless, were without flagella, and were rounded off. After about 24 hours the rounded off cells become hardened, later on they appear more liquified with granules in Brownian movement. A solution of 25 milli aequivalent is less toxic. From four experiments in two cases living swarm-cells could be observed. In the lower concentrations (12.5, 5 and 0.5 milli aequivalent) the swarm-cells stayed alive in nearly all experiments. Plasmodia were formed in all concentrations (one case in each concentration).

Action of KCl. This salt has been used in the same concentrations 50, 25, 12.5, 5 and 0.5 milli aequivalent. In none of these concentrations it appeared to be toxic, only in one out of five experiments with 50 milli aequivalent solution the swarm-cells had rounded off after 20 hours. The plasmodia-formation took place in all experiments at the concentration of 50 milli aequivalent. In the lower ones less.

Action of NH_4Cl. This salt always appeared harmless to the swarm-cells. The concentrations used were the same, except that the lowest concentration used was 1 milli aequivalent. The plasmodia-formation was very frequent in all concentrations used. Many vacuoles were observed in the plasmodia after 48 hours. Finally the whole endoplasma was vacuolised, and the plasmodia rounded off. The swarm-cells did not show any vacuolisation.

Action of $MgCl_2$. In all concentrations used (100, 50, 25, 10 and 2 milli aequivalent) $MgCl_2$ was harmless. The plasmodia-formation took place in all solutions except in 2 milli aequivalent. It was most frequent in 100 and 50 milli aequivalent.

Action of $CaCl_2$. In a solution of 100 milli aequivalent a slight toxicity was observed. In all experiments only few swarmers were motile after 24 hours and nearly all were rounded off. The protoplasm seems to be hardened, no granules in Brownian movement could be distinguished. A 50 milli aequivalent solution proved to be harmless. However no plasmodia-formation took place when dilutions were made to concentrations much lower than 2 milli aequivalent.

Action of $BaCl_2$. A 100 and a 50 milli aequivalent solution are toxic, the swarm-cells are rounded off after 24 hours; they are of the same volume as in $CaCl_2$, with no Brownian movement. No plasmodia-formation was observed.

Action of $CuCl_2$. $CuCl_2$ is toxic in concentrations down to 0.2 milli aequivalent. The swarm-cells begin to form buds, soon they become rounded off with granules in Brownian movement. No plasmodia-formation was observed.

Action of $FeCl_3$. This salt is less toxic than $CuCl_2$. In solutions of 0.3 milli aequivalent the swarm-cells remain alive. In 0.03 milli aequivalent solutions even small plasmodia were formed.

Action of $MgSO_4$. $MgSO_4$ was non-toxic from 100 to 2 milli aequivalent, in all concentrations used. Plasmodia-formation took place in all concentrations used and in nearly all the experiments.

Action of $Mg(NO_3)_2$. $Mg(NO_3)_2$ was also non-toxic in the same range of concentrations (100—2 milli aequivalent). The plasmodia-formation did not occur in the 2 milli aequivalent solutions. In the other concentrations the behaviour was equal to that in $MgSO_4$.

Action of $MgCO_3$. As $MgCO_3$ is little soluble, the highest concentration which could be obtained was 25 milli aequivalent. This proved to be toxic. After 24 hours the swarm-cells are swollen, there was less movement, after 48 hours many are rounded off and contain granules in Brownian movement. In 10 milli aequivalent they are also swollen, but rounded off to a lesser extent. In 2 milli aequivalent solution no swelling occurs and no rounding off. Plasmodia-formation was never observed.

Action of $MgBr_2$. In 100 and 50 milli aequivalent solutions a slight toxicity could be observed. After 48 hours many cells were rounded off. In 25 and 10 milli aequivalent solutions the picture is much the same. In 2 milli aequivalent the effect could not be observed. Plasmodia had been formed in concentrations of 25, 10 and 2 milli aequivalent.

The results of the action of the salts on the swarm-cells are shown in table 8. Each column has been divided in three rows; the numbers in the first row show the numbers of experiments performed, the second row indicates the numbers of experiments in which the swarmers remained in good condition, while the third row gives the number of experiments in which plasmodia were formed.

TABLE 8.

Action of the salts on the swarm-cells of Reticularia Lycoperdon.

Conc. n m. aeq.	NaCl	KCl	NH_4Cl	$MgCl_2$	$CaCl_2$	$BaCl_2$	$CuCl_2$	$FeCl_3$	$MgSO_4$	$Mg(NO_3)_2$	$MgCO_3$	$MgBr_2$
100				4 4 2	3 1 0	3 0 0	1 0 0		3 3 2	3 3 2		2 1 0
75								1 0 0				
50	5 1 1	5 4 4	3 2 2	5 5 3	4 3 0	3 0 0	1 0 0		3 3 2	3 3 3		2 1 0
37 5								1 0 0				
25	4 2 1	4 4 3	3 3 2	4 4 1	3 2 0	3 2 0	1 0 0		3 3 3	3 3 2	2 0 0	2 2 2
15								1 0 0				
12.5	4 3 1	2 2 1	3 3 1									
10				4 4 1	3 2 0	3 2 0	1 0 0		3 3 2	3 3 2	2 1 0	2 2 2
5	4 3 1	3 3 1	3 2 2									
3								1 0 0				
2				4 4 0	3 3 0	3 3 2	1 0 0		3 3 3	3 3 0	2 2 0	2 2 1
1			2 2 2				1 0 0					
0.5	3 3 1	3 3 1										
0.3								2 2 0				
0.2							1 0 0					
0.15								2 2 0				
0.1							2 2 0					
0.03								2 2 2				
0.02							2 2 0					
0.003								2 2 0				
0.002							2 2 0					

Comparing the action of the salts on the swarm-cells it was found that NaCl, $CaCl_2$, $BaCl_2$, $CuCl_2$, $FeCl_3$, $MgCO_3$ and $MgBr_2$ are toxic. The other salts KCl, NH_4Cl, $MgCl_2$, $MgSO_4$ and $Mg(NO_3)_2$ did not show any toxic action on the swarm-cells.

The toxic effect of $CaCl_2$ and $BaCl_2$ is a rounding off of the swarm-cell and the loss of the flagella, while the protoplasm seems to be hardened. Possibly this occurs by a diminition of the permeability by the Ca^{++} and Ba^{++} ion. In the other salt solutions the toxic effect is a liquefaction of the protoplasm shown by the marked Brownian movement, the cells are likewise rounded off and lose the flagellum.

Plasmodia are formed in NaCl, KCl, NH_4Cl, $MgCl_2$, $FeCl_3$, $MgSO_4$, $Mg(NO_3)_2$ and $MgBr_2$; in NaCl, KCl, NH_4Cl and $MgSO_4$ plasmodia are formed in alle concentrations used. In $MgCl_2$ and $Mg(NO_3)_2$ plasmodia are formed only in the higher concentrations, while in $MgBr_2$ and $FeCl_3$ they occur only in the lower concentrations.

In the table below we summarize the results of the action of the salts on the swarm-cells. In the column "Toxicity for swarm-cells" the numbers mean the concentrations in milli aequivalents

which are toxic. "Toxicity" is assumed when in 50% of the experiments toxic action had been observed. In the column "Plasmodia-formation" are indicated the percentages of the experiments in which plasmodia-formation was found in the given concentrations.

Electrolytes:	Toxicity for swarm cells:	Plasmodia-formation:	
	conc. in m aeq.	concentr.	%
NaCl	25	50— 0.5	25
KCl	50	50—25	78
		12.5— 0.5	37
NH_4Cl	50	50— 1	64
$MgCl_2$	100	100—50	55
		25— 2	16
$CaCl_2$	100	100— 2	0
$BaCl_2$	50	100—10	0
		2	66
$CuCl_2$	0.2	100— 0.002	0
$FeCl_3$	3	75— 0.15	0
		0.03	100
		0.003	0
$MgSO_4$	100	100— 2	80
$Mg(NO_3)_2$	100	100—10	75
		2	0
$MgCO_3$	10	25— 2	0
$MgBr_2$	50	100—50	0
		25— 2	83

This peculiar action of the salts on the developmental cycle of *Reticularia Lycoperdon* Bull. seemed to me most remarkably. In order to see whether the effects observed were of more general applicability the action of salts on other Mycetozoa was investigated.

Stemonitis fusca Roth. NaCl proved to be equally toxic as for Reticularia. KCl also acted in the same way. With NH_4Cl a different action was observed. The salt proved to be toxic in all concentrations used. In solutions of 50 milli aequivalent there is no movement, all swarm-cells are rounded off after some hours. In 25 and 5 milli aequivalent solution they are rounded off after 24 hours. The rounded off swarm-cells have large vacuoles. $MgCl_2$ and $CaCl_2$ are untoxic. $CuCl_2$ and $FeCl_3$ have the same toxicity as for Reticularia. Plasmodia were never formed.

Stemonitis splendens Rost. var. flaccida List. NH_4Cl is non-toxic for this species. $BaCl_2$ is toxic. No plasmodia were formed in solutions which gave positive results with Reticularia.

Fuligo septica (L.) Gmel. To this species NH_4Cl was toxic in

the same way as for Stemonitis fusca. In $CaCl_2$ 50 milli aequivalent all swarm-cells become rounded off, their protoplasm has been hardened after 24 hours. $BaCl_2$ is also toxic. Plasmodia-formation has not been observed.

Other species on which the plasmodia-formation by the swarm-cells was investigated gave negative results.

d. Behaviour of the plasmodia in solutions of one salt.

The behaviour of the plasmodia of Reticularia Lycoperdon in salt solutions was studied in the solutions in which they were formed and also in $BaCl_2$ and $CaCl_2$. In the latter solutions the plasmoida were rounded off by the manipulation before they are placed in the solution. In $BaCl_2$ 200 and 100 milli aequivalent they remain in this condition. After 48 hours their protoplasm is disintegrating into a liquid hyaline substance on one side of the rounded off plasmodia while on the other side a mass of coagulated protoplasm appears. In 50 milli aequivalent solution the plasmodia are still moving after 24 hours. After 48 hours there is no more movement. In 10 milli aequivalent solution the plasmodia are still moving after 48 hours.

In $CaCl_2$ the plasmodia are moving in all concentrations from 200—10 milli aequivalent. After 48 hours they are still alive. So the toxic action of $BaCl_2$ is not an osmotic action, for the $CaCl_2$ solutions have nearly the same osmotic pressure. The action of $BaCl_2$ is, therefore, a specific salt action.

In regard to the protoplasm of the plasmodia, it seems to be more fluid in the lower concentrations ($CaCl_2$ 50 and 10 milli aequivalent and in $BaCl_2$ 10 milli aequivalent). In these solutions the plasmodia are spread out thinner, whereas in the higher concentrations they are more rounded off.

CHAPTER IV.

THE INFLUENCE OF THE OSMOTIC PRESSURE ON THE DEVELOPMENT.

The influence of the osmotic pressure has been studied on the germination of the spores. A few observations have been made on swarmers and plasmodia.

A. Methods of experimentation.

Two series of experiments were performed. The first set was

taken with *Reticularia 1* (see introduction). The spores were suspended in solutions of saccharose from 0.1 to 0.2 mol, the several concentrations differring by 0.02 mol.

The suspensions were kept in tubes at room-temperature. After one-, two-, four-, six- and twenty-four hours samples were taken out and examined for the germination percentage following the method described in chapter II.

For the second series *Reticularia 2* was used. In saccharose-solutions of concentrations varying between 0.001 mol. and 0.3 mol. the germination percentage was ascertained after one half-, two-, four- and twenty-four hours. This set of experiments was performed at 25° C. in the optimum range of the germination-rate.

B. Experimental results.

a. The influence of osmotic pressure on the germination.

With *Reticularia 1* germination seemed to be inhibited in all concentrations used; e.g. a concentration of 0.1 mol. is damaging in a high degree (Table 9).

Table 9.

Influence of the osmotic pressure on the germination of the spores of Reticularia 1.

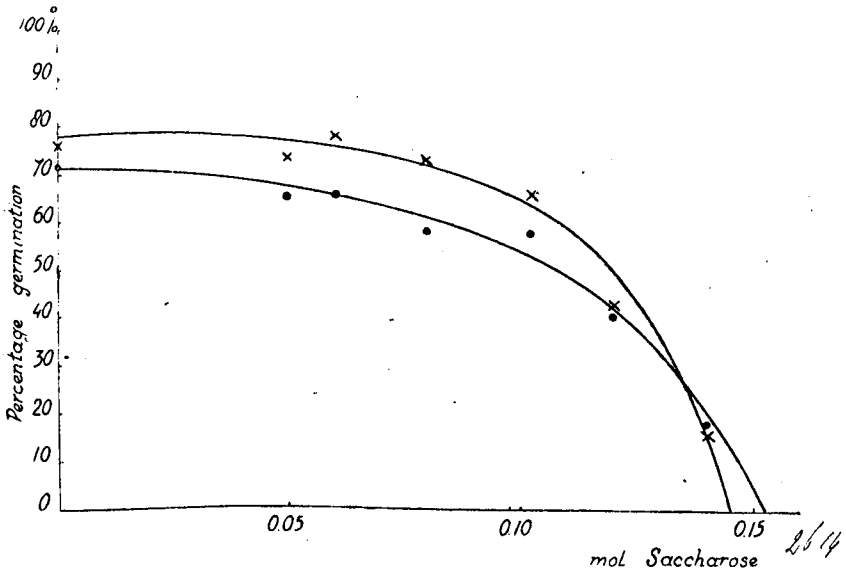
Concentration in mol.	Germination after:				
	1 hour	2 h.	4 h.	6 h.	24 h.
0.2	—	—	—	+	+
0.18	—	—	—	+	+
0.16	—	—	+	+	+
0.14	—	+	4	3	5
0.12	—	4	11	10	11
0.10	—	9	9	12	11

In the second set the concentration-range was taken somewhat wider. It appeared that concentrations of 0.1 mol. and lower are practically harmless. The germination rate is nearly like that in distilled water. At 0.12 mol. nearly 50% of the fertile spores are able to germinate, while at 0.14 mol. a marked hindrance is present. At 0.185 mol. no germination appears. (Table 10 and text-fig. 2).

TABLE 10.

Influence of the osmotic pressure on the germination of the spores of Reticularia 2.

Concentration in mol.	% Germination after:		
	one half h.	2. h	24 h.
0.185	—	—	—
0.14	18	16	
0.12	40	41	
0.102	58	68	
0.08	59	68	
0.06	64	79	
0.05	63	74	
distilled water	72	75	



Text-fig. 2. Influence of the osmotic pressure on the germination of the made of *Reticularia* 2.. Indicates the observations made after half an hour, × after 2 hours.

So the osmotic pressure of the spore-plasma in saccharose solutions is from 2.45 to 3.43 atm. (aeq. 0.10—0.14 mol. saccharose solution).

The results of the experiments on the influence of osmotic pressure on the germination of Reticularia are in accordance with

those of J a h n. He found that at 4% saccharose or 0.12 mol. the germination is inhibited.

b. The influence of osmotic pressure on the swarm-cells and plasmodia.

The swarm-cells when brought in 0.2 mol. solution of saccharose soon cease movement and are hardened, later on they round off and lose their flagella, showing the harmful influence of this concentration.

The plasmodia are damaged in the same way. When the culture-medium in which they had developed was replaced by 0.2 mol. solution of saccharose the streaming of the protoplasm soon ceased. The plasmodia did not become rounded off, they retained their form (even vacuoles stayed), while the protoplasm was hardened.

This sensitiveness of the plasmodia to osmotic pressure had already been remarked in some preliminary culture experiments. Culture experiments made on solid media of agar always failed, even when the plates were kept in moist chambers. The plasmodia when brought on the plates always soon ceased movement and became hardened.

So the spores, swarm-cells and plasmodia are equally sensitive to osmotic action in their environment.

CHAPTER V.

ON THE CAUSES OF THE FAILURE OF SINGLE-CELL CULTURES.

In some culture experiments, carried out in order to obtain pure cultures from the spores, the method of hanging drops was used. When few spores (from 1—10) were present in a drop of nutrient solution or distilled water the swarm-cells soon died, while in drops with more spores they stayed alive. *(Reticularia 1* and *2).*

In the cultures with few spores the swarm-cells degenerated in 24 hours, after 48 hours they had disappeared. In cultures with more spores the swarmers stayed alive for about ten days. Experiments in which the number of spores present in a drop differred from one to about thousand evidently show a graduel increase in viability of the swarmers. (Table 11 and 12).

The results of the first set are given in table 11.

TABLE 11.

Survival of the swarm-cells in drops of distilled water with different numbers of spores.

Number of drops	Number of spores in a drop	Alive after 24 h.
2	5	0
1	10	0
2	16	1
2	50	1
1	100	0
2	300	1
3	500—1000	3

In drops with 5 and 10 spores the swarm-cells had died within twenty-four hours, while with 300 and 500—1000 spores in a drop the swarm-cells had survived after the same time. The intermediate experiments with drops containing 16, 50 and 100 spores did not show a relation between the number of the spores and the viability of the swarm-cells. In this set the cover-glasses on which the drops were placed were cleaned with alcali and potassium bichromate H_2SO_4 and rinsed with distilled water. In this method I did not always obtain quite fat-free slides.

For this reason more attention was paid on the cleaning of the cover-glasses in the second set of experiments. The results of this set more regularly show the relation between the number of the spores and the viability of the swarm-cells. (Table 12).

TABLE 12.

Survival of the swarm-cells in drops of distilled water with different numbers of spores.

Number of drops	Number of spores in a drop	Alive after 24 h.
4	1—10	0
2	25	0
1	100	1
3	300	3
1	500	1
2	500—1000	2

In drops with 1—10 spores and 25 spores the swarm-cells have died, while in drops with 100, 300, 500 and more spores the swarm-cells have survived after the same period (24 hours).

From these experiments we have concluded that for the viability of the swarm-cells the number of the spores in a drop is of im-

portance. At the same time was remarked an effect of fatty substances on the glass.

The same phenomenon was observed by Wilson and Cadman (58) in still a higher degree. They stated that when a small number of spores is present the germination either does not take place or, if it does, the swarm-cells never produce flagella and round off almost immediately. Besides they found that the larger the number of spores present, the more satisfactorily did the germination proceed. From this they conclude that for the germination of the spores and for the development the presence of other swarm-cells is essential. In connection with this phenomenon they refer to the theory of autocatalysis of Robertson and allude to similar phenomena with cultures of bacteria and yeasts. No further investigations about the question were made by them.

In the following pages a number of experiments are described which attempt to contribute to this question for Reticularia Lycoperdon. In the first place it should be investigated whether the presence of living cells themselves is necessary to the swarm-cells or some substance derived from the spores. For this purpose spore-extracts were made in distilled water. They were prepared by heating a dense suspension of spores, in distilled water, to about 40° C. and filtering this suspension after half an hour (with S & S paper No. 595). In these extracts few spores stayed alive longer than in the control-drops of distilled water. After 24 hours they always were alive in the drops of spore-extract, while in the blank experiments in distilled water the swarm-cells had died after that time.

So it appears that the immediate death of the swarm-cells in drops with few spores is prevented by substances from the spores. Still it appears from the results in table 13 that the number of spores in a drop is of importance to the "span of life" of the swarm-cells.

TABLE 13.

Survival of the swarm-cells in spore-extracts.

Number of drops	Number of spores in a drop	Alive after:			
		24 h.	2 d.	4 d.	6 d.
7	1—10	7	0		
2	—40	2	0		
2	40	2	2		1
4	25—100	4	4	4	

In the above table (table 13) we see that in drops containing less than forty spores the swarm-cells had died after two days, while

in drops with 40 spores and from 25—100 the swarm-cells were alive after two days and in nearly all cases still after a longer time.

Experiments were performed to investigate the action of the substance in the spore-extract. The following possibilities were taken into account:

1°. The substance is necessary to the swarm-cell to form a part of some structure in the cell e.g. of the ectoplasm-membrane. Or it consists of some regulating substance, "autocatalyst" or "bios".

2°. The substance protects the swarm-cell from some toxic influence of the environment, in this case of the glass. The toxic influence emanating from the glass may be a physical or a chemical one. Physical influences which may act on the swarm-cell are the negative electrical charge in the boundary between glass and water, or some interfacial tension in the drop. A chemical influence from the glass may occur by solution of substances from the glass. The possibility of a chemical damage due to dissolved silica was suggested by Prof. H. G. Bungenberg de Jong, to whom I wish to express my hearty thanks for this suggestion and for some directions he kindly extended to set about this problem.

The necessity of the substance for the swarm-cells was soon eliminated form our considerations, as the "substance" could be replaced by substances of quite varying chemical nature. When a layer of paraffin-oil was placed on the cover-glasses single spores in drops stayed alive. Films of lecithin acted in the same way. To prepare these films a drop of an alcoholic solution of lecithin (from Merck) was placed on the cover-glass. After the evaporation of the alcohol a film stayed on the glass. On these films single spores in a drop also stayed alive. Later on it appeared that the results with the lecithin films were only positive when old cover-glasses had been used. With new cover-glasses the swarm-cells died within 24 hours just as on glass. Used cover-glasses although cleaned with alcali and potassium bichromate H_2SO_4, aceton and aether still show traces of fatty substances.

So it was found that the "substance" could be replaced by paraffin-oil and by lecithin together with traces of fatty substances.

Under these circumstances the single swarm-cells, when in drops with nutrient solutions stayed alive as long as in dense suspensions of swarm-cells. This result was used in some culture experiments. Before cleaning the cover-glasses were rubbed with the fingers, and after cleaning with alcali and acid and rinsing with distilled water films of lecithin were placed on it. The action of the fatty substances from the skin was not investigated further.

The following experiments showed that a toxic action of the

glass exists, which is probably chiefly of a chemical nature. The cover-glasses were replaced by other surfaces differring from the glass in electric charge, surface tension and chemical composition. The results of the experiments are shown in table 14. The table has been divided in five columns. In the first column are the surfaces which replace the glass. For quartz surface quartz cover-glasses (from Heraeus-Quarzglas-Ges. M.B.H. Hanau a. M.) were used. Also for mica surface mica cover-glasses were used. Collodion surface was prepared from an ether solution of commercial collodion (from N.V. Koninkl. Pharmac. Fabrieken). The starch surface was prepared from a 2% solution of amylum solubile in water. From this solution some wire-loops were placed on glass cover-glasses. After evaporation of the water films of starch remained on the cover-glasses. Copper was used as plate-copper. The paraffin-oil used in this experiment had been purified. It was put in ether solution on cover-glasses. The films were dried by half an hour's presenvation at 100° C. From zein and cetylamin also films were prepared respectively from an alcoholic solution and from a solution in water. I am much indebted to Mr. G. Th Philippi who suggested the use of these substances to me and kindly put his preparations at my disposal. Through the kindness of Prof. Ir. P. D. C. Kley a sample of steel plate V2a was obtained.

In the second column are enumerated the sign of the electrical charge between the surface and distilled water. In the third column it has been indicated whether the surface was easily wetted by water or not: ++ means it becomes wetted very well, — the surface is difficult to wet, + is intermediate.

The fourth and fifth column contain respective the numbers of experiments made and the numbers of experiments in which no toxic action occurred.

TABLE 14.

Survival of the swarm-cells in drops with few spores on different surfaces.

Surfaces used	electr. charge	wetting	number of drops	untoxic after 24 h.
quartz	neg.	++	17	3
mica	neg.	++	6	1
collodion	neg.	+	20	19
starch	neg.	++	11	11
copper	neg.	+	5	0
paraffinum	indifferent	—	12	9
zein	pos.	+	16	16
cetylamin	pos.	+	10	0
steel V2a		+	3	3

Quartz, mica, copper and cetylamin were toxic. Untoxic were collodion, starch, paraffinum, zein and steel V2a. It is evident that the toxicity does not go parallel with the electrical charge. Collodion and starch which posses, like glass, a negative electrical charge, are non-toxic. The action of the surface-tension could be ruled out, for the small depth of the drops on glass and quartz was not damaging in itself. Drops made on paraffinum with the same depth were harmless.

So there only remains the supposition of a chemical damage. The existence of such a damage could be proved by placing the spores on a harmless surface in drops consisting of distilled water with water soluble substances from glass. For this reason pulverised cover-glasses were suspended in distilled water and the suspension shaken during thirty-six hours and filtered. Drops of the filtrate, placed on films of paraffin-oil, showed a highly toxic action, comparable to that of glass or quartz.

CHAPTER VI.

CULTURE.

A. Theoretical part.

a. Data from the litterature.

Data on the methods for culture are given by Bruck (3), Cienkowski (4), Constantineanu (7), Howard (19), Lister (29), Nadson (37), Potts (42), Pinoy (41), Schünemann (47), Skupienski (48) and Watanabe (56).

Cultures have succeeded with Dictyostelium mucoroides, several species of Didymium, Badhamia utricularis, Physarum polycephalum.

Nadson (37) cultivated Dictyostelium mucoroides in a solution containing dextrose, peptone and inorganic salts. He found that a symbiosis existed between Dictyostelium and Bacterium fluorescens liquefaciens. He also obtained pure cultures of Dictyostelium, without bacteria. In these he got fructifications but the growth was anomalous.

Potts (42) cultivated the same species. He mentions an extracellular digestion of the bacteria, for he did not succeed to observe bacteria within the amoebae. He found that also dead bacteria are fit as food material. He did not succeed to obtain bacteria-free cultures.

Pinoy (41) also tried to obtain pure cultures of this species. He found that the spores were even unable to germinate without bacteria. He used sterilised carrots and agar with linseed as media. The amoebae of Dictyostelium are parasitic on the bacterial colonies, the bacteria are digested intercellular in vacuoles, visible after coloration with neutral red or vesuvin.

Pinoy also cultivated other Acrasiales: Dictyostelium purpureum and Polyspondylium violaceum on agar with linseed in the presence of bacteria. He also performed experiments with some endosporic species: Didymium effusum and Didymium difforme. Cultures were made on agar with decoction

of decomposed wood in the presence of Bacillus luteus. Pure cultures failed. As they were attempted by the use of 5 year old cysts, which were uncapable of development even with addition of bacteria, this experiment does not prove the necessity of bacteria for the development of these species.

Skupienski (48) also found that Didymium difforme is symbiotic with bacteria. The bacteria present in the cultures on potato- and carrot agar 2% belonged to the group Bacillus vulgaris. In these cultures the agar is liquefied and fructifications are formed within 14 to 16 days. He also obtained cultures without bacteria by sowing 4 year old spore-material. The spores germinated and plasmodia were formed but these plasmodia were very weak and never fructificated. The agar liquefaction was not pronounced. After addition of fresh bacteria to these cultures growth appears like in the other mass-cultures, with agar liquefaction, while fructifications are formed soon. The addition of dead bacteria was helpful for growth, but it did not cause agar liquefaction and formation of sporangia. Other microorganisms could serve as symbionts as well, for instance Aspergillus glaucus, Torula glutinis, Sarcina lutea, Bacterium prodigiosum.

Constantineanu (7) cultivated several species in decoctions of plant-material and in solutions with organic and inorganic compounds. He also used solid media; agar and pumice-stone. Several species formed plasmodia also on solid media for instance Aethalium septicum. Of Reticularia he observed only plasmodia-formation in small moist-chambers, where these plasmodia soon encysted. No information is given by Constantineanu on the occurrence of other organisms in the cultures.

Cienkowski (4) tried to culture a number of species: Physarum album, Didymium leucopus, and Licea pannorum. He made his cultivations on slides. The spores were sown in water in which small fragments of plants were present. From Physarum album he obtained, in this way, fructifications after five days.

Schünemann (47) cultivated Didymium nigripes, Didymium difforme, Didymium squamulosum and Physarum leucopus in hay decoction. After development of plasmodia in drops, he placed these on agar with hay decoction and in this way he obtained fructifications. The whole cycle from spore to spore took about 20 days. He also made monospore cultures of these species.

Information on the culture of some mycophageous species is given by A. Lister and later on by Howard.

Lister (29) cultured Badhamia utricularis from spores and from sclerotia. He sowed the spores in watch-glasses in boiled water on slices of scalded Stereum. The plasmodia which spread over the Stereum were removed on a fresh plate and fed with Stereum. The plate was covered with a bell-jar. He found a more easy method by starting with the sclerotia. The sclerotia revive soon when placed on pieces of soaked Stereum. Every morning a fresh supply of Stereum must be given on which the plasmodia spread. To obtain fructifications the food supply was stopped and the plasmodia were led over sticks, which frees them from contaminations.

Howard (19) has cultured plasmodia of Physarum polycephalum, viride and other mycophageous species on agar with several food substances of which rolled-oat agar and corn agar were most succesful. When gathering the plasmodia they first were placed on clean agar, after some transfers they were brought on the agar with food substances.

Watanabe (56) investigated the feeding of plasmodia with bacteria. All plasmodia tried were able to feed on several bacterial species. He tried 17 species of plasmodia with 16 species of bacteria. The bacteria were placed in a ring-shaped figure on clean sterilised agar and the plasmodium was placed

within. From the chemotactic movements and the growth the preference of the plasmodium to the bacteria was determined. The range in which the bacteria were preferred by the plasmodium was nearly the same in all species. Bact. Zopfii, Staphylococcus aureus and some Sarcina's are most suitable. Bacillus megatherium always repelled the plasmodia. Of all plasmodia used Didymium nigripes var. xanthopus shows the best growth with the bacteria.

b. Data from the experiments on environmental conditions.

From the experiments on environmental conditions described in this paper some results may be used towards a method for the cultivation of *Reticularia Lycoperdon.*

The experiments on the influence of the temperature showed that 16—18.5° C. is optimal for the coalescence of the swarm-cells. This will probably be the temperature optimum for the growth of the plasmodia.

In the experiments on the influence of salts it was found out which salt solution was optimal for plasmodia-formation. $MgSO_4$ and KCl solutions proved to be the most suitable.

It was found, furthermore, that solutions of relatively low osmotic pressure are harmful for the germination of the spores and for the swarm-cells and plasmodia.

Finally when drop-cultures are used the cover-glasses should be covered with some indifferent substance in order to protect the swarm-cells from the noxious effect of the glass.

B. Experimental part.

The culture-experiments were performed during the research on environmental conditions. Attempts were made at pure culture and at mass culture, also with some other species. The pure cultures were obtained by sterilisation of the spores from bacteria and fungi and by the isolation of plasmodia. The sterilised spores, respectively the isolated plasmodia, were put into sterile culture media.

The methods used for mass culture are:

1°. The method given by Constantineanu, by sowing the spores in decoctions of maize and bean stalks in petri-dishes.

2°. The method of hanging drops, few spores were suspended in several nutrient solutions and from these suspensions drop cultures were made.

3°. By starting with plasmodia, care being taken to establish optimal environmental conditions, as appears from the experiments described above.

The results of the five methods are given below.

a. Attempts at pure culture.

Pure culture starting from spores. The spores of *Reticularia Lycoperdon, Didymium effusum* and *Stemonitis splendens* var. *flaccida* were treated with antiseptics as indicated by Klein und Kisser (28) for the sterilisation of seeds. The spores were placed in little sacs of filterpaper which were submerged by aid of a looped wire in the solution and rinsed in sterile water before inoculation in the culture medium.

The antiseptics used with succes were:
sublimate 0.1% aqueous solution during one minute, the dry spores must be submerged first into alcohol, otherwise they are not moistened by the sublimate.
bromine 1% aqueous, during one to two minutes or a stronger solution during half a minute.

Only the spores of *Stemonitis splendens* var. *flaccida* could be sterilised with above-mentioned methods. The spores of Reticularia Lycoperdon were very sensitive to the antiseptics and those of Didymium effusum were too heavily infected with bacteria. In both cases the bacteria also survived whenever the treatment was harmless for the spores of the slime-mould.

The sterilised spores of Stemonitis were inoculated in yeast autolysate 5% and in maize decoctions. In both media the spores germinated and the swarm-cells were actively swimming, in yeast autolysate even after six weeks. Growth was never observed, neither in the fluid in which they were first inoculated nor in further inoculations.

Pure culture starting from plasmodia. Young plasmodia of *Reticularia Lycoperdon,* formed in $MgCl_2$ solution some 24 hours after the suspension of the spores were isolated with the micromanipulator (Janse—Peterfi) and placed seperately in a sterilised suspension, consisting of particles of Norit in distilled water in Erlenmeyers of 50 cc. containing about 2 cc. of the fluid. The Norit particles had been saturated first with yeast-autolysate. From mass cultures between slide and coverglass it appeared that the fluid was harmless to the plasmodia and that the particles could be ingested by the plasmodium.

In all eight cultures made in this way no growth appeared. Still the possibility remains that the plasmodia are unable to take in the particles under these circumstances (in hanging drops particles were not ingested) or that they are not able to digest the yeast autolysate.

Further attempts on pure culture by this method were not made.

b. Attempts at mass cultures.

In *the first tentative experiments* which we performed with the spores of several species the spores were sown in decoctions of maize and of bean stalks in petri dishes and kept at room-temperature (18° C.). Of these cultures only *Didymium effusum* formed plasmodia and fructifications. The plasmodia, when brought on agar with nutrient substances, were kept for nearly one year by reoculating once a month on a new plate. The growth of the plasmodia always started after the agar was covered with bacteria. Before this bacterial development took place the plasmodium was only moving comparatively quickly in all directions over the agar. A vigourous growth was always concommittant with a liquefaction of the agar. From the other species *Reticularia* and *Stemonitis* the spores germinated well but plasmodia did not develop and the cultures deteriorated.

From these experiments it appears that, from the species tried, Didymium effusum is best adopted for ordinary laboratory culture.

Cultures in hanging drops. For this purpose the cover-glasses were covered with lecithin to protect the cultures from the substances dissolved from the glass. The results were always negative, in all culture media used a multiplication of the swarm-cells was never observed (performed with *Reticularia).*

The following culture media were used:

Culture solution for *Reticularia* of Wilson and Cadman;

tapwater
lactic acid 0.01%
sugar 2%

the same modified;

tapwater
lactic acid 0.01%
sugar 1%
peptone 1%

Culture solution for *Polytoma* of Pringsheim; (Kufferath, 28a)

water	100 gr.
K_2HPO_4	0.02
$MgSO_4$	0.01
K_2CO_3	0.5
glucose	0.2
glycocoll	0.2
Na acetate	0.2

Culture solution for *Infusoria* of Lwoff; (Kufferath, 28a)

water	1000
NaCl	0.5
KCl	0.01
$CaCl_2$	0.02
$MgSO_4$	0.01
peptone (Witte)	10

Culture solution for *Euglena gracilis* of Zumstein; (Kufferath, 28a)

water	100
$MgSO_4$	0.02
KH_2PO_4	0.05
peptone	0.5
glucose	0.5

The latter two solutions were also used with some modifications.

The culture solution of Pringsheim appeared to be unsuitable, as the spores did not germinate in it.

In all other solutions the spores germinated and the swarm-cells stayed alive for some weeks.

Mass cultures starting from plasmodia. The plasmodia of *Reticularia* which were formed in magnesium sulphate solution of 0.025 to 0.01 mol. were placed in maize decoctions and kept in watch-glasses in petridishes to prevent drying. They were kept at several temperatures, 9°, 15° and 19° C. After a week growth could be observed in several cultures; also at 9° C. Some of the plasmodia were thread-like, attached probably to the cotton fibers, in others spread on the glass. The thread-like plasmodia were transferred into fresh culture media. In these media no more growth occurred, but the plasmodia degenerated, bacteria were growing vigourously and after one or two weeks they had died. In the first cultures the plasmodia had also degenerated after some weeks.

Therefore by neither of the five above-mentioned methods cultures of Reticularia Lycoperdon could be obtained.

CHAPTER VII.

A. DISCUSSION OF RESULTS.

On the temperature influence. It was found that the spore-germination of Reticularia is possible from about 2° to 30° C.; the division of the swarm-cells is possible from 7° to 26.5° C.;

and the formation of plasmodia from 12° to 26.5° C. These results show that the development in this species is impossible at low temperatures, below 7° C., and at temperatures above 30° C.

The lowest limit seems reasonable for an organism but the upper limit seems very low. The sensitivity to temperatures > 30° C. may be the cause of the chiefly extra-tropical distribution of the species, as appears from the geographical enumeration given in the introduction of this paper.

The results of the experiments on germination are not in contrast with those of Jahn with the same species (22).

Contrary to our findings, however, Constantineanu found (7) that the spores still germinated at 35° C. And at 2—4° C. he observed a damage by which the germination percentage was influenced.

Besides this difference between the Reticularia of Constantineanu and the forms with which these experiments were made, there exists also a difference in the behaviour of the swarm-cells. Constantineanu describes them as follows: "Die sehr beweglichen Zoosporen bewegen sich 3—5 Tage und verwandeln sich dann in Amoeben, die sich abrunden. Bei einer Temperatur von 3—4° leben die Zoosporen bis zu 10 Tagen, ein Verhalten welches man bei den Zoosporen anderer Myxomyceten Arten nicht findet".

Our swarm-cells, on the contrary, are very slow in comparison with those of other species. When moving they always become attached to the glass or other wall with their posterior end. Only in some food-solutions old swarm-cells move more or less freely. Furthermore they never changed into myxamoebae.

On salt influence. The salts used partly consisted of chlorides of several metals, in order to ascertain the action of several cations. From the study of various magnesia salts the action of the anions could be established.

The results of the experiments on the influence of the cations on the germination are: potassium and sodium promote germination, while ammonium, magnesium, calcium, barium, copper and iron seem to inhibit germination. The experiments are taken in 0.002 mol. solution (also some in 0.01 mol. solution) as compared to distilled water. The promoting action was shown by a shortening of the germination-time and the inhibiting action by a hindrance of the germination of a number of the spores.

In $FeCl_3$ no germination takes place. In $CuCl_2$ germination does take place but in small number, while the swarm-cells soon die.

Of the other cations Ba^{++} was most toxic, followed by Ca^{++}. NH_4^+ and Mg^{++} are equally harmful.

Accordingly the series for the action of the cations on germination may be written:

$$K^+ \quad Na^+ \quad [NH_4^+ \quad Mg^{++}] \quad Ca^{++} \quad Ba^{++} \quad Cu^{++} \quad Fe^{+++}$$

favoured — inhibited

For the anionic influence the following series was found:

$$CO_3^= \quad SO_4^= \quad Cl^- \quad Br^- \quad NO_3^-$$

favoured — inhibited

Carbonate and sulphate promote the germination. Bromide, chloride and nitrate are injurious in the same grade.

An explanation of the action of the ions on the germination of the spores may be attempted on the basis of their influence on the swelling of the protoplasm and furthermore on their specific toxic properties. As vacuoles are absent in the spore-plasma in its first stages of germination, it is evident that the germination takes place by swelling pressure more than by osmotic pressure. Now the cationic series mentioned above is a lyotropic series. It differs from the original series which Hofmeister found for the influence of cations on the swelling of gelatin in the mutual places of the alcali metals and between the earth alcalies. Many similar variations occur in the literature on the swelling of colloids.

The anionic series found for the spore-germination is quite different from the lyotropic anionic series. In these the sulphate ion counteracts the swelling, whereas the chloride, bromide and nitrate ions promote the swelling. Cases of inversion of the direction of the lyotropic series are found by Höber (18). He found an inversion of the series of alcali metals dependant on the acid or alcalic reaction in the precipitation of egg-albumen by chlorides. And also an inversion of the anionic series of K, Na, Li and NH_4 salts by a change in the salt concentration for the coagulation temperature of egg albumen.

The harmlessness of distilled water and of several one-salt solutions seems explainable. All these experiments were made with dense suspensions of spores, therefore, the solutions must have

contained a certain amount of cell-substances. The same may be said for the behaviour of the swarm-cells.

Toxic to the swarm-cells are Na^+, Ca^{++}, Ba^{++}, Cu^{++}, Fe^{+++}, $CO_3^=$ and Br^-. The toxic effect always appeared as a rounding off of the swarm-cell with loss of the flagellum, except in $FeCl_3$ in high concentrations, where the swarm-cells preserve their form. In $CaCl_2$ a regular hardening of the whole protoplasm was observed. In $BaCl_2$ the hardening becomes irregular. In $MgCO_3$ the rounded-off swarm-cells are swollen. In NaCl granules in Brownian movement appear in the rounded-off cells.

The peculiar action of the salts on the developmental cycle appears from the following. In NaCl, KCl, NH_4Cl, $MgCl_2$, $FeCl_3$, $MgSO_4$, $Mg(NO_3)_2$ and $MgBr_2$ solutions plasmodia may been formed. In NaCl, KCl, NH_4Cl and $MgSO_4$ in all concentrations used. Also in NaCl, KCl and NH_4Cl from 0.5—50 milli aequivalent, and in $MgSO_4$ from 2—100 milli aequivalent. In $MgCl_2$ and $Mg(NO_3)_2$ only in the high concentrations, from 10—100 milli aequivalent. In $MgBr_2$ from 2—25 milli aequivalent and in $FeCl_3$ only in 0.03 milli aequivalent solution. Especially KCl and $MgSO_4$ solutions are suited as media for plasmodia-formation.

In regard to the action of the salts on the plasmodia the following can be said; from the solutions in which they were formed only NH_4Cl was toxic. The movement of the protoplasm soon ceases, probably due to a penetration of the ammonium ion. Furthermore it appeared that $BaCl_2$ was toxic to the plasmodia, while $CaCl_2$ proved to be innoxious.

On the osmotic pressure. The osmotic pressure in the environment was found to be of great importance for the development of this species. The germination of the spores is impossible in 0.18 mol. solution of cane-sugar; the swarm-cells cease moving and round off in 0.2 mol. solution of cane-sugar; the plasmodia also stop moving in 0.2 mol. solution of cane-sugar.

So when the osmotic pressure in the environment equals one of a 0.2 mol. cane-sugar solution no development is possible. The development of Reticularia is limited, therefore, to environments of an osmotic pressure between 0 and 0.2 mol. cane-sugar solutions or from 0—5.29 atm. of or a relative vapour-pressure from 100—99.6%.

We compare these values with those of other organisms. Other species of Mycetozoa are investigated on this question by J a h n (22). The lowest value for the concentration limit by which germination still occurred he found for Reticularia. The highest value he found

for Didymium difforme; the concentration limit for the germination amounted to 25% cane-sugar or 0.73 mol. (equals 22 atm. pressure and a relative vapour-pressure of 98.3%).

Plant-like organisms, which resemble Reticularia in their behaviour with regard to osmotic pressure are the thallous liver-worts. Bender (55) found their osmotic values to be from 5—9 atm., when under active conditions of life.

Of the algae Nostoc punctiforme is the most hygrophilic species. It still showed growth in a cane-sugar solution of 8 atm., according to Harder (55). Values for the plasmolysis limit for several species of algae found Bottazi (55) being from 8.7—21 atm.

The fungi and bacteria, so far known, are able to live under conditions of much higher osmotic pressure. The so-called hygrophile species can live at vapour-pressures from 100—97.5%, so in 33.8 atm. pressure. (Walter 55).

On the failure of cultures with few cells. In chapter V it was observed that a substance coming from the glass was damaging the swarm-cells when derived from few spores. The same damaging influence exists in drop-cultures on quartz and mica. Therefore it seemed probable that the damaging substance is silica.

An argument for this supposition is: in a filtrate of glass-suspension (made from cover-glasses) which had been shaken during 36 hours silica has been determined with ammonium molybdate reagent according to the method of Diénert and Wandenbulcke, modified by Thayer (52).

The protection of the swarm-cells in dense cultures against the silica is thought to be by means of a connection of the silica with substances derived from the spores. The investigations of Bungenberg de Jong and Nieuwenhuyzen (38) showed that positive albumens like clupeine and gelatine beneath their iso-electrical point are able to flocculate silica solutions. Further investigations about the properties of the substances which occur in the dense suspensions are not made.

Another damage which perhaps may have been caused by silica I found in the formation of plasmodia. The plasmodia, used in the culture experiments had been formed in solutions of $MgSO_4$ or KCl. With a two year old solution of $MgSO_4$ no plasmodia were formed. This has occurred four times. A determination of the silica in KCl solutions of the same age, showed that the old solutions contained 5.9 mgr. SiO_2 per liter (according to the method of Diénert and Wandenbulcke modified by Thayer). By repeating this experiment later on I could not

reproduce this damaging effect, the plasmodia formed in the old solutions as well as in freshly prepared ones.

From the literature some cases of silica damage are known.

1°. Damage of the lung tissue by inhaled quartz. Collis, Mavrogordato, Gardner, etc. (38) found a dying of the "dust-cells" in the lungs after the inhalation of quartz particles by guinea-pigs. They presumed that this was caused by silica dissolved from the quartz.

The experiments of Nieuwenhuyzen (38) have strengthened this opinion. He found that silica is dissolved from a suspension of quartz particles in different buffer solutions. At the same time he observed in tissue cultures of embryonic chicken-lungs vacuolisation in the quartz-containing cells, the quartz particles being present in the vacuoles. This observation points, according to Nieuwenhuyzen, to a dehydration of the protoplasmic proteins under the influence of colloid silica.

2°. Gue, Kettle and Purdy (38) stated that silica acts like a "tissue-toxic" on the subcutaneous connecting membrane in mice and rabbits, also by means of dissolving in the cells.

It is possible that not only leucocytes (Nieuwenhuyzen) and Reticularia are sensitive to silica. This would imply a revision of our microscopic method, in which glass covers and slides are universally used.

On culture. The results of the culture experiments with Reticularia were negative. In the mass-cultures sometimes growth of the plasmodia occurred, but the cultures deteriorated after some weeks. In the pure cultures obtained from isolated plasmodia growth was never observed.

Of the other species tried, Didymium effusum and Stemonitis splendens var. flaccida, mass-cultures only succeeded with Didymium effusum. Pure cultures were attempted with both species (also with Reticularia) by starting with spores, which had been treated with antiseptics. The sterilisation of the spores, without damaging them, only succeeded with the spores of Stemonitis. In the cultures made with these spores a multiplication of the swarm-cells was never observed.

Our results are in accordance with the culture experiments known from the literature.

Mass-cultures with Didymium species have succeeded to several authors: Bruck (3), Cienkowski (4), Constantineanu (7), Pinoy (41), Skupienski (48, 49, 50) and Schünemann (47). Skupienski also states to have got a slight growth

of the plasmodia of Didymium difforme, when in pure culture.

Cultures with Reticularia Lycoperdon and Stemonitis splendens var. flaccida, tried by Constantineanu, failed (7). Our culture experiments with Stemonitis splendens var. flaccida and Reticularia Lycoperdon are very incomplete. Besides the described cultures of Reticularia some others were made, but I did not get equal results in the duplicate experiments. A discussion of these experiments is therefore omitted.

B. SUMMARY.

1. Investigations were made on the influence of temperature electrolytes and osmotic pressure on the development of the endosporic, acalcareous slime-mould, Reticularia Lycoperdon Bull. Experiments were made to investigate the question of the failure of single-cell cultures with the same species. Attempts have been made for pure culture and for mass culture with Reticularia and with some other species of Mycetozoa.

2. The germination of the spores takes place in a temperature range from 2—30° C. The division of the swarm-cells from 7—26.5° C. The plasmodia-formation from 12—26.5° C. The temperature has great influence upon the rate of the life-processes. The optimum temperature for the germination and for the cell-division was found at about 25° C., when the rate of the process is considered. The optimum for the plasmodia-formation was found at 16—18.5° C., the plasmodia becoming the largest at those temperatures.

3. As to the influence of electrolytes it was found that the cations acted on the germination according to their place in the Hofmeister series. The series for the usual anionic action was found to be reversed.

H^+ and OH^- ions were non-toxic from pH 6—8. The swarm-cells when placed in solutions with one salt sometimes formed plasmodia, in other solutions they never fused. The solutions in which plasmodia were formed were: NaCl, KCl, NH_4Cl, $MgCl_2$, $FeCl_3$, $MgSO_4$, $Mg(NO_3)_2$ and $MgBr_2$. In $CaCl_2$, $BaCl_2$, $CuCl_2$ and $MgCO_3$ plasmodia were never formed.

4. The osmotic pressure limits much the development of this species. In 0.18 mol. solution of saccharose germination has stopped. The swarm-cells and plasmodia are equally sensitive. When placed in 0.2 mol. solution of saccharose they stop their activity, the swarm-cells become rounded off, while the plasmodia are hardened in their habituel form.

5. In single-cell cultures made on glass the swarm-cells are damaged by substances which dissolve from the glass. Indications were found that the damaging substance is silica.

6. The results of the culture experiments with Reticularia were negative. Pure cultures were attempted by starting with isolated plasmodia. Growth was never observed in a culture medium containing yeast-autolysate and particles of Norit. In mass cultures, made in maize decoctions, growth was observed, but the cultures could not be kept under healthy conditions.

From the other species tried the pure-culture of Didymium effusum by starting with spores failed, as the spores could not be freed from bacteria. It was cultured in mass-culture on a medium of agar with decoction of maize for about a year. From Stemonitis splendens var. flaccida the pure culture was attempted by starting with spores which had been treated with antiseptics. A multiplication of the swarm-cells was not observed in media of yeast-autolysate and maize decoction. Mass-cultures of this species failed.

C. CONCLUSIONS.

The development of this species is influenced much by temperature, salts and osmotic pressure.

The temperature-maximum lays between 26.5° and 30° C.

The plasmodia-formation is a salt action. In media without salts no plasmodia were formed.

The organism is extremely hydrophylic during a large part of its development.

SiO_2 was found to be the most probable cause of the failure of the single-cell cultures.

The foregoing investigations were carried out in the Botanical Laboratory of the University of Leyden. I am very much indebted to Professor Dr. L. G. M. Baas Becking for his suggestions and valuable help, wherefore I am thanking him particularly.

LITERATURE.

1. Boedijn, K. B. 1928. Verzeichnis der von Sumatra bekannten Mycetozoa. Ann. Myc., 26, p. 450.
2. Brandza, M. 1914. Myxomycètes de Roumanie, Ann. Sci. de l'Univ. de Jassy, 8, p. 259.
3. Bruck, W. F. 1907. Beiträge zur Physiologie der Mycetozoen. Ztschr. f. Allg. Physiol., 8, p. 505.
4. Cienkowski, L. 1863. Das Plasmodium. Jahrb. wiss. Bot., 3, p. 400.
5. Cleland, J. B. 1927. Notes on a collection of Australian myxomycetes. Transact. Proc. R. Soc. South Australia, 51, p. 62.

6. Clark, W. M. 1928. The determination of hydrogen ions. 3rd Ed.
7. Constantineanu, J. C. 1906. Uber die Entwicklungsbedingungen der Myxomyceten. Ann. Myc., 4, p. 495.
8. Emoto, Y. 1931. The Malayan myxomycetes. Journ. Bot., 69, p. 38.
9. ——— 1931. Javanische Myxomyceten. Bull. Jardin Bot. Buitenzorg. Sér. 3, 11, p. 161.
10. ——— 1931. Die Myxomyceten der Südmandschurei. Bot. Mag. Tokyo, 45, p. 229.
11. Freunlich, H. 1930. Kapillarchemie. Leipzig, 4e Aufl.
12. Gilbert, F. A. 1927. Myxomycetes from Eastern Massachusetts. Rhodora, 29, p. 165.
13. ——— 1928. Myxomycetes from British Guiana and Surinam. Mycologia, 20, p. 27.
14. ——— 1929. Factors influencing the germination of Myxom. spores. Amer. Journ. Bot., p. 280.
15. Greene, H. C. 1929. Myxomycetes of Western Washington. Mycologia, 21, p. 261.
16. Hagelstein, R. 1927. Mycetozoa from Porto Rico. Mycologia, 19, p. 35.
17. ——— 1929. New Mycetozoa from Long Island. Mycologia, 21, p. 297.
18. Höber, R. 1926. Physikalische Chemie der Zelle und der Gewebe. Leipzig, 6e Aufl.
19. Howard, F. L. 1931. Laboratory cultivation of myxomycete plasmodia. Amer. Journ. Bot., 18, p. 624.
20. Iwanoff, N. N. 1925. Uber den Eiweiszstoff des Protoplasmas der Myxomyceten. Biochem. Ztschr., 162, p. 441.
21. ——— 1925. Uber die Trehalose und Trehalase bei Myxomyceten. Biochem. Ztschr., 162, p. 455.
22. Jahn, E. 1905. Myxomycetenstudien 4. Die Keimung der Sporen. Ber. d. deutsch Bot. Ges., 23, p. 489.
23. Jarocki, J. 1924. The Myxomycetes of the Great Bialowieza Forest. Acta Soc. Bot. Pol., 2, no. 3, p. 183.
24. ——— 1931. Mycetozoa from the Polish Eastern Carpathians. Bull. de l'Acad. Polonaise des Sc. etc., Sér. B. p. 447.
25. Kauffman, C. H. 1928. A study of the Fungous Flora of the Lake Superior region of Michigan, with some new species. Papers Mich. Acad. Sci. Arts, L., 9, p. 169.
26. Kiesel, A. 1925. Untersuchungen über Protoplasma I. Uber die chemischen Bestandteile des Plasmodiums von Reticularia Lyc. H.—S. Z. ph. Ch., 150, p. 149.
27. ——— 1925. Beitrag zur Kenntnis der chemischen Bestandteile der Myxomycetenfruchtwandung. H.—S. Z. ph. Ch., 150, p. 102.
28. Klein, G. und Kisser, J. 1924. Die sterile Kultur der höheren Pflanzen. Bot. Abh. Ht. 2.
28a. Kufferath, H. 1930. La culture des algues. Paris. 8°.
29. Lister, A. 1901. On the cultivation of Mycetozoa from spores. Journ. of Bot., 39, p. 5.
30. ——— A. and G. 1905. Mycetozoa from New-Zealand. Journ. of Bot., 43, p. 111.
31. ——— A. 1911. A Monograph of the Mycetozoa. London.
32. Macbride, Th. H. 1926. A bit of polynesian mycology. Mycologia, 18, p. 125.

33. Maire, R., Patouillard, N., et Pinoy, E. 1926. Myxomycetes de l'Afrique du Nord. Bull. d. Hist. Nat. d. l'Afrique du Nord, 17, p. 38.
34. Meylan, Ch. 1929. Recherches sur les Myxomycetes en 1927—28. Bull. Soc. Vaud. Sci. Nat., 57, p. 39.
35. Minakata, K. 1927. List of the Japanese species of Mycetozoa. Bot. Mag. Tokyo, 41, p. 41.
36. Muenscher, W. C. and Wann, F. B. 1926. Myxomycetes of the Lord Cornell Reservation. Bull. Lloyd Library, 27, p. 68.
37. Nadson, 1899. Des cultures du Dictyostelium mucoroides Bref. et des cultures pures des amibes en général. Extr. Scripta Bot. fasc. 15. Saint—Petersbourg.
38. Nieuwenhuyzen, F. J. 1933. Over het biochemisme van de Silicosis. Thesis. Utrecht.
39. Overeem—de Haas, C. und D. van, 1922. Verzeichnis der in Niederlandisch Ost-Indien bis dem Jahre 1920 gefundenen Myxomycetes, Fungi und Lichenes. Bull. du Jardin Bot. Buitenzorg, Sér. 3, vol. 4, p. 1.
40. Penzig, O. 1898. Die Myxomyceten der Flora von Buitenzorg.
41. Pinoy, E. 1907. Rôle des bactéries dans le développement de certains myxomycetes. Ann. de l'Instit. Pasteur, 21, p. 622 and 686.
42. Potts, G. 1902. Zur Physiologie d. Dictyostelium mucoroides. Flora, 91, p. 281.
43. Ruinen, J. 1933. Life-cycle and environment of Lochmiopsis sibirica. Woron. Thesis. Leyden.
43. Raunkiaer, C. 1928. Myxomycetes from the West-Indian Islands St. Croix. St. Thomas, St. Jan. Dansk Bot. Archiv. 5, p. 16.
45. Schinz, H. 1920. Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweisz. Abt. 10.
46. Scholte, A. M. 1926. Inheemsche Myxomyceten. Nederl. Kruidk. Arch., p. 155.
47. Schünemann, E. 1930. Untersuchungen uber die Sexualität der Myxomyceten. Planta, 9.
48. Skupienski, F. X. 1928. Etude bio-cytologique du Didymium difforme. Acta Soc. Bot. Pol., 5, no. 3, p. 255.
49. ——— 1930. Influence de la température sur la fructification de Didymium nigripes Fr. Act. Soc. Bot. Pol., 7, no 2, p. 241.
50. ——— 1933. Influence du milieu de culture sur le développement des Myxomycetes. Act. Soc. Bot. Pol., 10, no. 1, p. 113.
51. Smith, E. C. 1931. Ecological observations on Colorado Myxomycetes. Torreya, 21, p. 42.
52. Thayer, L. A. 1930. Colorimetric Determination of Silica in the Presence of Phosphates and Iron. Industr. and Engin. Chemistry, 2, p. 276.
53. Toro, R. A. 1926. Mixomicetos de Santo Domingo. Estacion Agaron Dominican Rep. Haima, 6, p. 3.
54. Vouk, V. 1932. Uber Skelett- und Membranstoffe bei Myxomyceten (Sammelreferat) Protoplasma, 16, p. 478.
55. Walter, H. 1931. Die Hydratur der Pflanzen. Jena.
56. Watanabe, A. 1932. Uber die Bedeuting der Nährbacterien fur die Entwicklung der Myxomyceten Plasmodien. Bot. Mag. Tokyo, 46, p. 247.

57. Wettstein, F. von, 1921. Das Vorkommen von Chitin und seine Verwertung als System. Phylogenetisches Merkmal in Pflanzenreich. Sitz. Ber. d. Acad. d. Wiss. Wien, Math. Nat. Kl. Abt. 1, Bd. 130, p. 3.

58. Wilson, M., and Cadman, E. 1928. The Life-history and Cytology of Reticularia Lycoperdon Bull. Transact. Roy. Soc. Edinburgh, 55, pt. 3. p. 555.

59. Zelle, M. 1925. Beiträge zur Schleimpilz Flora der Ukraine. Bull. Jard. Bot. Kiew, 2, p. 31.

A more complete bibliography (± 300 papers) on the subject Mycetozoa will be deposited in the library of the Botanical Institute at Leyden.

EXPLANATION OF THE PLATE.

Figure 1. Germination of the spore. × 890.

Figure 2. Formation of pseudopodia. × 890.

Figure 3. Mature swarm-cells. By the use of strong light the protoplasm has become hardened and the flagella are distinctly seen. × 890.

Figure 4. Fusions of the swarm-cells in pairs. The left fusing pair shows one vacuole. × 890.

Figure 5. Another fusion. × 890.

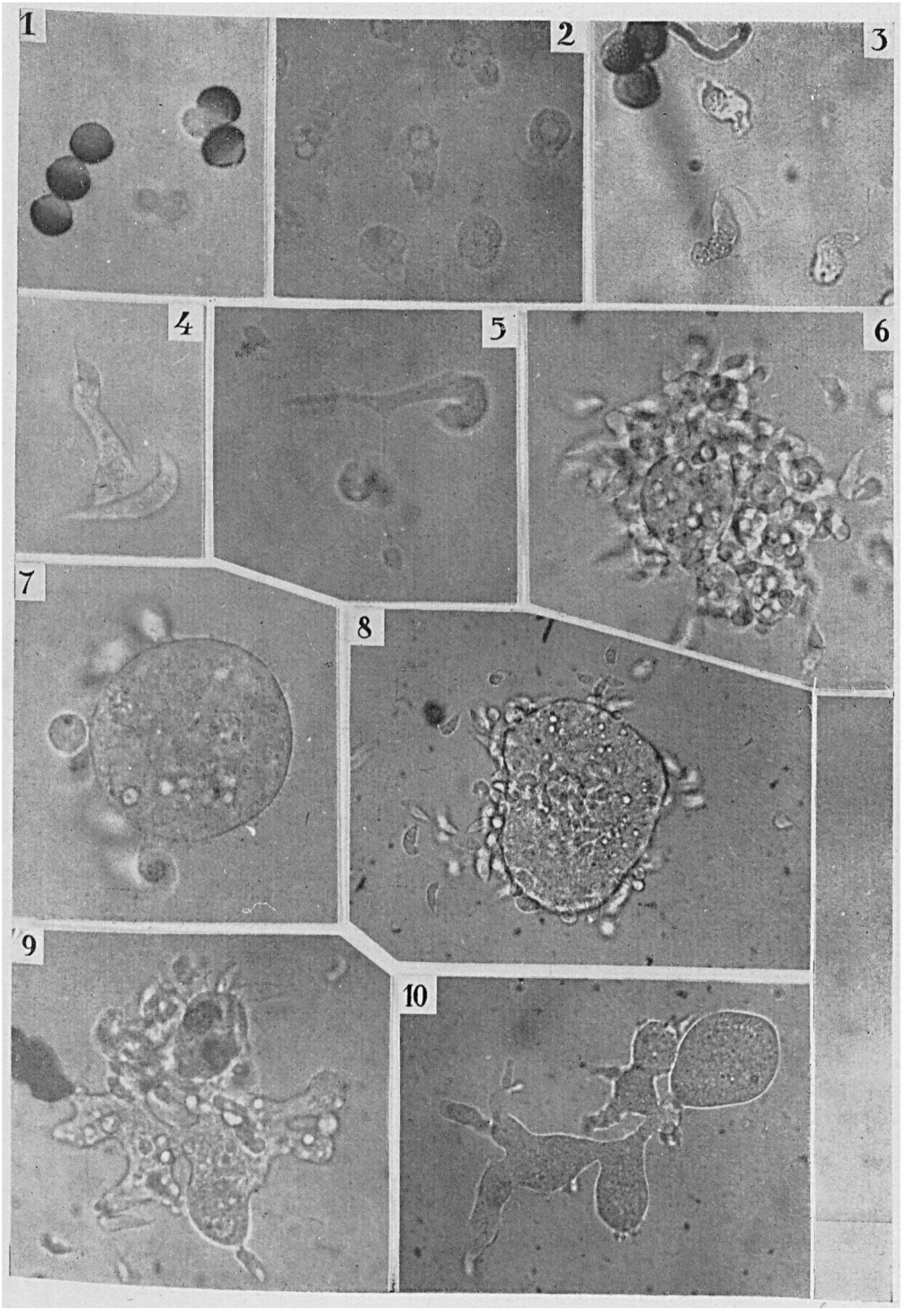
Figure 6. Large collection of swarm-cells. Fusion has already taken place to a large extend. × 625.

Figure 7. A rounded off mass of protoplasm. × 625.

Figure 8. A protoplasmic mass in which seperate swarm-cells are still seen. × about 400.

Figure 9. A plasmodium, in the film showing amoeboid movement and streaming of the protoplasmic contents. × about 400.

Figure 10. Idem figure 9.


1
2
3
4
5
6
7
8
9
10