The influence of electrolytes on the tactical movements of Chlamydomonas variabilis Dangeard

by

C. SPRUIT P.P.zoon.

PREFACE.

In my opinion, it is proved by the literature on the tactical movements of the unicellular organisms, how in physiology they have not been able to come into contact with chemistry and physics by a terminology of their own and a special method.

This investigation should be looked upon as an attempt to change this.

To begin with, it was necessary to find a sin table method. I think that the denoted way of working is a practical one. Moreover it can be proved by this investigation, that it is necessary to examine in detail the tactical phenomena.

There must not be put too great value on the contemplations which arose from the comparison of the phenomena investigated by me with *Chlamydomonas* and those, which occur in colloïd-chemistry.

To begin with the collected data are limited, though the number of experiments made amounts to two or three thousand.

It is also very difficult to get a clear insight into colloïd-chemistry. Besides the fact that many phenomena of it have not yet been examined systematically, there is another great difficulty, namely the difference of opinion between the colloïd-chemici in very important problems. It is sufficient to read something of the chemistry of proteins to be convinced of this. As far as the conclusions are concerned, I believe not to have gone too far, however.

It will give rise to some astonishment, perhaps, that in this dissertation, is not said a word of the investigations of R. S. Lillie (1904 etc.). The cause of this lies in the fact, that I thought it impossible to compare Lillie's data directly with mine.

CHAPTER I.

Contemplations on Chemotaxis.

§ 1. *The chemotactical phenomena caused by stimulation.*

The occasion to this investigation was a study of literature and some provisional experiments on chemotaxis.

As a rule, people are of opinion, that chemotactic phenomena appear, when a substance that works attractive or repulsive, forms zones of diffusion.

Swimming on the organism continually comes in another medium, as the chemotacticum is not always present in the same concentration. It reacts upon this change of concentration and alters its direction of movement.

We must accept that the chemotacticum affects elements of the cell, either local or as a whole. Perhaps by this affection, perhaps by changes which closely depend on it, a stimulation is called forth, the consequence of which is that the motion is changed.

The real stimulation process is considered to exist in the taking up of the stimulus (the perception), the conducting of the stimulus and after that unknown actions which lead to the reaction. The reaction is the criterium by which we judge the chemotaxis. We must pay attention to the appearance of the reaction, as well as to its intensity.

That concentration of the chemotacticum which is able

to form with pure water such a diffusion-zone, that the object distinctly reacts upon it, is called the threshold for the chemotacticum.

When comparing the definition of the threshold for a chemotacticum with the way, in which a threshold is fixed with the phototropical experiments, one perceives, how unsuspecting one acts with the chemotactical experiments. It has been known for a long time, that to acquire a just visible phototropical curvation, the time during which the stimulus must act, is often much shorter, than the time necessary to bring about the further changes, which lead to the curvation. It is said that the time of presentation is shorter than the time of reaction, that the curvation is formed by an after action. The complications that crop up when one goes on stimulating till the reaction appears, are very hard to judge. One has to face so-called changes of „condition". The plants used for experiments grow continually more insensible during the stimulation that it is even possible, that the reaction changes of sign by this. Though it becomes more and more probable of late that these complications are not so incomprehensible, as they seemed before (Arisz, 1914), it is necessary to reckon with them. Supposing that with the chemotactical phenomena we have to do with processes, which may be compared to those of phototropism, it is clear, that in our experiments we do not observe the phenomena in their simplest forms. Pfeffer pointed to the possible connection between chemotactical phenomena and phototropical ones: „Überhaupt liegen hier analoge Beziehungen vor. Die in anderen Reizwirkungen, z.B. im Heliotropismus" (W. Pfeffer 1888, pag. 624).

They try to learn more about perception, by working with combinations of chemotactica. They think to find out by this whether the stimulation, exercised by the composing elements of the mixture are percipiated every

one by itself or as one stimulus. Is the first suggestion the case, then they speak of various stimuli, a stimulus for substance a and one for substance b, which have no influence on each other. They have understood the fact, that this can be concluded only, if first the threshold is fixed of the substances a and b, after that they are combined in such a way, that of substance a as well as of substance b a too small concentration is present to bring about a reaction by itself. (Pfeffer, 1888, Kniep, 1905). So it is the question whether we can call up the two different stimuli. In phototropism and geotropism we work in the same way if we will find out whether different stimuli can be called up there. They have even succeeded there in combining a grantational stimulus with a light-stimulus by a simple addition. In that case, however, other things have to be taken into account besides the threshold (C. E. B. Bremekamp, 1915). It has come to light that the time which elapses between the beginning of the stimulation and the reaction, even if we work with threshold-values is shorter with a geotropical process than with a phototropical process. They say that the reaction times are not the same. If both stimuli are applied in such an order, that for both the reaction should begin at the same moment, a simple addition takes place. When they had not yet paid attention to the reaction-times, it was accepted, as it was proved by Mrs. C. J. Rutten Pekelharing (1910), that a light-stimulus and a grantational-stimulus could not be simply called up.

The possibility is not excluded, that two chemotactical stimuli do not make their influence felt in the same short time. If so we have to face the same difficulties with the addition of them, as we have with the addition of light- and grantational-stimuli. Though it is a matter of course to suggest, that the reaction-times in our chemotactical experiments are very short, in principle it does not make

any difference with the case before mentioned, when those times are not the same.

Of course it is not possible to say before-hand whether the drawbacks mentioned exist.

§ 2. *The drawbacks which belong to the use of the capillary-method.*

The method, followed till now for the experiments on chemotaxis, exists in this, that we put a capillary tube with one open end and filled with the chemotacticum in a preparation, holding the organisms, whose conduct we will examine. We wait now about ten minutes and look after that whether the organisms react, by controlling where they are. The great advantage of this method is that Pfeffer derived it from nature; so we can accept, that it will be able, at any rate as far as the spermatozoa of ferns are concerned, to denote which chemotactica could act a part biologically. Moreover the method is very demonstrative.

In course of time they have required too much of the capillary method. The more they worked at chemotaxis, the more they perceived that they had to handle a very complicated phenomenon. Meanwhile the method remained the same. Yet it is easy to point out, that this has been a pity. We must acknowledge, that some authors have worked wonders with it (Pfeffer, Kniep, Shibata). Kniep has made very important discoveries with this method. Kusano changed it in such a way, that he was able to fix the threshold in a much more exact way.

From the publication by Kusano (1909) it appears, where the difficulties are, For qualitative purposes the method is very dangerous. Stange (1890) had worked with the same organism as Kusano, namely with the

swarmspores of *Aethalium septicum*. Stange declares that these are positively chemotactical as regards the following free acids: lactic acid, butyric acid, valeric acid, propronic acid, malic acid and tartaric acid. Kusano, on the other hand, thinks the swarmspores positively chemotactical for nearly all acids. He sees the explanation of these various results in the fact, that Stange used much narrower tubes than he did. To make this clear, he gives a list (page 38). Capillary tubes with a diameter of 10 to 240 μ were all filled with $^1/_{20}$ normal solution of monokalium phosfate and put into a preparation of *Aethalium* swarmspores. After 5 or 10 minutes in the tubes of 100 μ or still smaller diameter there was nothing or very little to be seen of a positive reaction, while in the wider tubes a distinct reaction could be seen. Knowing moreover, that Stange worked with capillary tubes, which had a diameter of 13—15 μ, we can easily understand the various results of Stange and Kusano. Remembering that, the bigger the diameter becomes, the more diffusion plays a part and that by this during the experiment the concentration of the chemotacticum in the capillary tube must really change, we shall see that also the defining of a threshold with the capillary method has great drawbacks.

Kusano, in his investigation, gives us a clear representation of this (page 69). He works as follows. By bringing the swarmspores into acid-solutions of various concentrations, he fixes the minimum-concentration of every acid, by which action all swarmspores immediately are at rest and contract themselves, in consequence of the injuring action of the acid. On page 60 we find a table of this. When comparing these concentrations to those of the acid-solutions, which he has to do in the capillary tubes to obtain a just visible accumulation at the mouth, it appears that these concentrations are far more injurious than the solutions of page 60.

He now changes the method and puts the object into the capillary tube, which is filled with distilled water and puts this under the cover-glass in a drop which consists of the acid-solution. When he states, that a swarmspore that will leave the capillary tube makes the 'motorreflex-movement' at the month (Kusano points out that his object reacts exclusively phobochemotactically like *Paramaecium*) the acid-solution under the cover-glass must possess a concentration, which corresponds to the threshold-value of that acid. Thus he finds the threshold-value for sulphuric acid to be $^1/_{20000}$ molair, for hydrochloric acid $^1/_{10000}$ molair, for tartaric acid $^1/_{8000}$—$^1/_{10000}$ molair, for malic acid $^1/_{4000}$—$^1/_{6000}$ molair and for acetic acid $^1/_{1000}$ molair. It is very peculiar how he himself speaks of his changed method or rather of the found threshold values: „We cannot yet by all means determine this critical concentration but we will satisfy ourselves in accepting the following method, which may give, at least in my case, more accurate result for our purpose than by the usual capillary method."

I, myself, too have tried to get an insight into the dimension of the threshold. My experiment-organism was *Chlamydomonas variabilis Dangeard.* It reacts positively to all acids, which I have examined (strong inorganic acids, phosphoric acid, carbonic acid, acetic acid, malic acid). Into the capillary tubes I put, for example, 0,1 normal acid. Before the mouth of the capillary tube, I got a ring-shaped accumilation. In a similar preparation, but without the organisms, the water was provided with a certain quantity of a methyl-red solution. By this it got a rather clear, orange-yellow colour. Under te influence of the acid, which diffused from the capillary tube, a red ring was formed before the opening of it. The place of thing ring coincided almost with the ring, which was formed by the organisms in the other preparation. The

optimal acid-concentration for *Chlamydomonas* must lie near the turning over of methylred. This takes place at a hydrion concentration of about 10^{-5}. So this solution contains about 0,00001 gram-ion of hydrions. From all this it appears with what minimum concentrations we have to work.

I can make clear in another way, that for *Chlamydomonas* the threshold value must be very small. When I put the *Algae* into a solution, the hydrion concentration of which was fixed in an electrometrical way, it became clear to me, that with a hydrion concentration of 10^{-5}, the organism did hardly move, that they no longer reacted on the light, neither to gravity. During experiments with a capillary tube, filled with acid and in which one gets a ring-shaped accumilation, the algae at the inside of the ring, are inclined to be at rest. The greater part in the middle and at the outside of the ring does not show this inclination. The state of things, which I find with a H-ion concentration of 10^{-5}, probably lies above the optimum. How these experiments took place, will be proved further on. We arrive at the conclusion, that *Chlamydomonas* must be susceptible to acid concentrations smaller than $^1/_{100000}$ gram-ion of H-ions per liter.

From the above-mentioned statements we can draw the conclusion that we must n o t expect from the capillary method:

1°. that it can provide us with useful definitions of the threshold-values.

2°. that with the qualitative examination as regards the chemotactical activity of solutions, a negative result actually denotes insensibility for the solution.

If we use the capillary method for biological investigations, I believe, that it is very suitable for those. Thus it is a matter of course that with biological questions (for instance with the fructification) so small a sensibility for

some material, that it cannot be proved by the capillary method, is of no importance whatever. To solve theoretical problems, however, it seems to me, to be fit only under very much reserve. I again point here to the difference between the sensibility to acids with Stange and with Kusano. Of course we cannot say with certainty, that Kusano is really right with his explanation of the difference; however it is very probable that he is correct. But it is very useful to see what result this inaccuracy has had with Stange. Of course Stange did not come to the conclusion, that it was the hydrions, that worked attractively, but that it must be the anions of the acids Kusano proves that the contrary is true.

Of course we arrive now at the question which ions act the greatest part in the various chemotactica. In this point there is a great deal of discordance I will not try to give a review of this. It seems to me, that the various authors are wrong in their opinions of the influence of the hydrions or the anions being thus predominant, that they are right to disregard the influence of the other ions. When perusing the various treatises one gets the impression, that every ion has its stare, though it is not always clear for a limited number of cases.

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§ 3. *A colloïd-chemical conception of the influence of electrolytes with chemotactical phenomena.*

The drawbacks we have thus far been discussing, are such, that the possibility is not excluded that we have got a wrong idea of the influence of the chemotactica by a defective method. The older investigations gave a wrong impression of the active concentrations. Moreover the number of active solutions seems to be very limited for each object. The result of all this is, that as yet, they have never

compared the influence of the chemotactica with the action of electrolytes on colloïds. Now it is my intention to prove, that when studying chemotaxis we may not forbear to examine the colloïd-chemical phenomena.

The very first argument for colloïd-chemical opinion, is that one generally accepts, that the protoplasm is colloïdal. Then the suggestion follows that the influence of the chemotactica is a colloïd-chemical one. The active concentrations may be very small. When we find acid-concentrations of 0,0001 normal and still smaller chemotactically active on numerous organisms, we can best form an opinion of it, when we accept, that we have to do with phenomena from colloïdchemistry. A further argument might be found in the nature of the substances. It is known, that particular special ions often have a great influence on colloïds. That is the case with the hydrions and the hydroxylions. We have already seen, that the hydrion plays a great part in chemotaxis too; provided we may accept, that with the activity of acids the hydrion exercises the greatest influence. For the hydroxylions we can also find an example Shibata states, that the spermatozoa of *Isoëtas* are attracted by the hydroxylions. Besides these two ions there are a lot of others, which are very active sometimes. As regards these, however it is still more difficult to find conformities in colloïdchemistry and with the chemotaxis. This is brought about by the fact, that we cannot very well make out with what colloïds we have to compare the protoplasm. In colloïdchemistry we distinguish two large groups of colloïds. The suspensoïds are susceptible to very small quantities of neutral salts. The valence of the ions acts a great part; polyvalent ions have an immense effect. The suspensoïds are charged either positively or negatively. Especially ions with an antogonistic charge have a great influence on the statility; we cannot see much of the influence of the other ions.

For the greater part it is anorganic substances which in a colloïdal state, belong to the suspensoïds. The emulsoïds have absolutely different properties. Only very great saltconcentrations have an influence on the stability. The precipitation of these colloïds is called „salting out" anions and cations are both active. The valence of the ions does not act a great part, anions and cations can, as regards their influence, be put down in series (for the anions the series of Hofmeister). The charge of emulsoïds changes its sign if the solution is acid or alkaline. To the emulsoïds belong all sorts of organic substances for instance proteïns.

When we ask to which group the plasma-colloïds should be reckoned to belong, we cannot give a satisfactory answer to that. From the few things that have come to light in this respect, it appears, as if, for the greater part, we have to do with emulsoïds which, possess however, some properties of the suspensoïds. So with substances between emulsoïds and suspensoïds. The charge of the plasmacolloïds depends on the reaction of the surrounding fluid. Small salt concentrations have an influence. The valence of the ions too plays a part.

At present it is impossible to say, whether the reaction of phosphates or malates must be ascribed to the particular place, which these substances take up in the series of Hofmeister, or if we must pay a special attention to the polyvalence of the anion.

Still another argument in favour of the colloïd chemical opinion can be found. The publication of Kniep (1905) denotes by numerous examples, how the chemotactical influence of a salt can be changed through the presence of other salts. We find there that KCC and NH_4Cl can paralyze each others effect. The same with K_2SO_4 and $(NH_4)_2SO_4$. However the sulphates are unable to diminish the effect of the chlorides. Peculiar is the effect of the mixtures of KCC and $CaCl_2$. KCC has but little influence

on the effect of $CaCl_2$; on the other hand $CaCl_2$ has an enormous influence on KCC. $CaCl_2$ has no influence on K_2SO_4. It is clear that the valence and the sign of the ions defines their mutual influence. In calloïd chemistry we can find back a similar state of things. If we do not see any connection between the colloïdchemical phenomena and the chemotaxis we cannot but side with Kniep in his opinion, that the organism for different chemotactica holds different sensibilities unless one accepts that different chemotactica need a different reaction-time.

I believe that by this is sufficiently proved the right claim of a colloïd chemical treatment of the chemotactical phenomena.

If indeed colloïd chemical processes act such a great part with the chemotaxis, it is a matter of course to suggest, that there are no specific chemotactica. It is problable that all electrolytes exercise their influence, though those, called chemitactica, shall have a very great effect. Kniep's data agree with this opinion. So do his discoveries with *spirillum rubrum*. *Spirillum rubrum* did not react to nitrates. Meanwhile nitrates were able to diminish the effect of chlorides.

Starting from the opinion that there are no specific chemotactica, we begin with controlling the conduct of experiment-organisms towards solutions of electrolytes, which act on all sides. If we know all about this, we must try to make out, whether the conduct shows peculiarities towards the electrolytes, defined as chemotactica.

CHAPTER II.

Chlamydomonas variabilis Dangeard.

§ 1. *The culture-method used and the determination of the Alga obtained.*

For my experiments I used an unicellular motile Alga, *Chlamydomonas variabilis Dangeard.* For representatives of the *Chlamydomonas* and of some narrowly related Algae there is a suitable culture-method. The latter is stipulated by Jacobsen (1910) In a vessel of glass one puts some fibroïn in water. We moculate with a great quantity of good garden-ground. After bacteria have affected the fibroïn at first, the Alga gradually begins to develop itself. With a favourable course of events the whole fluid becomes green in course of time. If once a good culture has been obtained, we can soon acquire a new culture by inoculating in water with fibroïn and sterilized ground. After I had been using fibroïn for some time, I added, according to Buder's advice, dried white of eggs. By this the time, necessary to develop a culture, is considerably shortened. Sometimes we obtain very dense cultures, in other cases, however, it is much more difficult to have good material at our disposal. This is especially the case in autumn and in the beginning of winter.

Of all cultures a part was set aside and fixed with formaline. The determination of the material proved that I had been working almost exclusively with the same

species. A determination-table of Wille (1903) pointed out that I had to do with *Chlamydomonas variabilis Dangeard.* The description, which Dangeard gives of it (1898, page 135), and also his picture confirm this. The description and picture which Jacobsen gives of *Chlamydomonas variabilis* agreed less with it. Therefore it is well perhaps to give a short description of the Alga.

The swarmspores have a size up to 20 μ, as a rule the cell is cylindric-ovoïd. There are two cilia, which spring up at the base of a distinct wart. The eye-spot is disciform, and lies in the middle. Generally the chloroplast fills the whole cell. There are often many big granules of starch. A pyrenoid is not to be seen. Division stadia were often found. I did not find more than four daughtercells. The first division takes place transversely. Occasionally I found capulation stadia.

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§ 2. *Some remarks on the nature of the reactions, which we observe with living beings.*

We will now speak about the various reactions, which *Chlamydomonas variabilis* can exercise. Especially we will pay attention to those reactions which were of great importance to my further experiments. Thus we are going to speak of the phototaxis, the geotaxis, the thigmotaxis, the chemotaxis and the aërotaxis.

Before beginning with this, however, it is necessary that we get a clear idea of the nature of these reactions. We might have to do with physical, chemical and with physiological processes. Among the physiological phenomena the reactions caused by stimulation take up a particular place.

These differences are not of a great value. In the first place it is impossible to draw a distinct line of demarca-

tion between pure physical or chemical processes and physiological phenomena. Most probably physiological phenomena are nothing but physical or chemical phenomena. This is sufficiently proved by the circumscription of the idea physiological process. It is used to denote, that, as yet we have not been able to reduce the process to a physical or chemical process, or rather, that it is not a *simple* physical or chemical phenomenon. When, besides with the more complicated, the physiological reactions of the living organisms we distinguish between actions caused by stimulation and processes with a different result, the difference is not very marked. Truly speaking, it is hardly possible to give a definition of a stimulation-process. It seems to one that this is chiefly caused by the fact that many so-called stimulations are complicated reactions. With a chemotactical reaction we have to do with a change of an existing motion. As regards geotropism and phototropism we have to do with reactions of growing, that are altered under the influence of one-sided geotropical and phototropical stimuli. When we do not look upon these reactions, we receive a very complicated representation of them. Though we have no definition of a stimulation, yet we are convinced of the existence of it, when a connection is noticed between stimulus and reaction, which reminds us of the relation, stated by Weber's law, for stimulations from the physiology of animals and the experimental psychology. Also the inversion of the reaction by an increase of the stimulus would be characteristic of stimulation-processes.

I think it better not to set too much value on both peculiarities, which can arise during stimulations. Weber's law concerning phenomena from plantphysiology, as far as it has been examined, goes into circumstances which do not differ too much. Besides this, the connection, stated by this law, needs not at all point to a physiological

or psychological process, at any rate not when the connection only exists in some degree.

Also the meaning of the inversion of the reaction is entirely unknown to us. It is sufficiently clear that the term stimulation is very vague.

The terms physical process, chemical process, physiological process and stimulation-process are not suitable to keep the various phenomena of apart.

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§ 3. *The Geotaxis of Chlamydomonas.*

There is not much literature, treating of geotaxis. The negative geotaxis of *Paramaecium* is well-known, owing to Jennings' book. It is easy for every-body to observe, that *Paramaecium* swims upwards in a tube with pure water, often many decimeters without getting disturbed or to rest for a little while. Of the geotaxis of *Chlamydomonas pulvisculus* we can read in Schwartz (1884, page 51). What is told there is not very convincing. The greater part of the experiments are done in wet sand. In water the phenomena are not very distinct. If we mix wet sand with water, which holds *Chlamydomonas* and let this stand for a time (for instance during six hours) in the dark, so we see that many Algae have come above the sand. Schwartz concludes that we have to do with negative geotaxis and bases his conclusion upon the fact that under the influence of slight and avarage centrifugal forces, *Chlamydomonas pulvisculus* behaves itself as towards the gravitation, and that when the acceleration of the centrifugal force becomes greater than $8^1/_2 \times$ g., the object, under the influence of that force turns its motion.

Then we find something about *Chlamydomonas pulvisculus* in Massart (1891, page 148). He, too, concludes that *Chlamydomonas pulvisculus* is negative geotactical

in the dark. There is always a part of the organisms that does not mount in the vertical tube. These individuals hardly more. With Massart the experiment generally takes an hour.

We also find something of geotaxis of *Chlamydomonas pulvisculus* with P. Jansen (1893, page 428). He, too, finds that the object is negative geotactical; that we have to do the experiments in a tube, no longer than about four centimeters and that often individuals are found, which sink down to the bottom, apparently passive.

From all this we see, that the individuals of *Chlamydomonas pulvisculus* did not react so very nicely in the state, in which the examiners quoted, use them.

With *Chlamydomonas variabilis Dangeard* I noticed a very distinct motion towards the bottom of the vessel. Within ten minutes all the organisms had reached the bottom. In the dark the phenomenon, as a rule, took place in the same way. In some cases the experiment did not succeed in the dark. Whether this was a consequence of the sudden change light-dark, I cannot judge. It is not so easy to find out that here we have to do with positive geotaxis; as the Algae react at the same time negatively phototactically. In bright light we get by the two movements, when we put the algae into distilled water, into a thin glass tube placed vertically, a dense green stripe on the shadow-side, which sinks to the bottom comparatively fast (see fig. 1 B. and C). With a weak objective we can follow the motion of the algae with a microscope. Then it seems that *Chlamydomonas* moves downwards absolutely passive, that means we see the organisms swim forwards, backwards, downwards and upwards. When working in very weak white light or in red light, we perceive, that the general direction of the movements is downwards. I speak of a positive geotaxis, but will only say, that *Chlamydomonas variabilis* moves

actively downwards under the influence of the gravitation. The fact that in strong light, we get the impression of a passive motion, is caused by the negative phototaxis. By this the objects always remain near the glass wall. Under the influence of the phototaxis, the geotaxis and of the impediment of the glass, the direction of the movements becomes rather irregular.

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§ 4. *Electrolytes can prevent the reaction to gravity.*

I have been astonished at the fact, that I did not find anywhere a word about this positive geotaxis of *Chlamydomonas* and think this is caused by the circumstance, that this property only crops up, when the fluid is neutral and there is not too much salt in the solution. In distilled water the phenomenon has a splendid course. At last the organisms form green grounds on the bottom of the tube (see fig. 1 C. page 34). I used for these experiments narrow glass tubes, which were tapering at the bottom and had been closed by melting. So the grounds could be seen very distinctly, it made the impression of a settled precipitation (see fig. 1 C. page 34). With the microscope we could notice that the movement had not at all stopped, the algae continued very movable and swarmed in the point of the tube. If we put the tube with the settled algae in the dark and if we let this stand for half an hour or an hour, then we perceive, that the sediment has been enlarged, in course of time the algae spread diffusely in the lower part of the tube.

Led on by statements in literature, I thought, that this diffusely spreading might have something to do with want of oxygen or with aërotaxis. In stead of common distilled water, I now used boiled out, distilled water. If the want of oxygen played a part, the moving downwards should,

at least, not go smooth. Meanwhile it was of no use at all. The only thing we could think of then, was the increase of carbonic acid in the fluid, in consequence of the respiration, while assimilation of CO_2 was excluded. So I added to the water small quantities of aerated water, from which the excess of CO_2 had disappeared. It was proved that the quantity of carbonic acid could cause the algae to move downwards no longer. (See fig. 1D, pag. 34). We might suppose perhaps, that the salts from the aerated water, might be the cause of this phenomenon (aerated water is prepared from tapwater). This cannot be the case, however, since the algae in tapwater behave in about the same way as in distilled water; at any rate they give there a splendid show of the moving downwards. Further on I saw, that all sorts of acids are capable of annulling the positive geotaxis, moreover diluted bases and all kinds of salts which were active however in a somewhat higher concentration.

With the microscope can be observed, that the motility of the algae has greatly diminished, when they move downwards any longer.

I got the impression that the change motile—immotile does not crop up all of a sudden, that on the contrary the decrease of the motility gradually changes with the increase of the concentration. Meanwhile this cannot be stated with certainty, since complications act, which will be discussed more in detail further on. (See Chapter IV, § 7). When examining with the microscope transition states in a weak white or red light, then the direction of the movements for the various individuals of a preparation is different. The number of those that swim upwards is probably rather great. There often takes place an accumulation in the upper layer of the fluid. Whether this is perhaps an indication of negative geotaxis, I dare not decide. It is remarkable, that with phototaxis, similar

strange phenomena can be observed in the transition state of negative phototaxis to insensibility as regards light.

§ 5. *The Phototaxis of Chlamydomonas.*

Of late there appeared a publication of Buder (1917), in which there is said a good deal of *Chlamydomonas variabilis*. The data, which can be found there about the phototactical movements of this Alga, coincide absolutely with what I observed of it. In distilled water and solutions in which there is not too much acid, base or salt *Chlamydomonas* reacts negatively to the light. Buder observed that the Alga did not always react with the same certainty. For the case, in which the reaction did not run very smooth, he used the term "Streuung".

I have found that "Streuung" takes place when acid, base or salt is added to the water. This goes so far, that the sensibility to light absolutely disappears. Just as with the geotaxis this is accompanied by a strongly diminished motility. The concentration necessary to cause, that the object no longer reacts to the light, well nigh corresponds to the concentration, that annuls the geotaxis. Besides the fact that the Alga reacts negatively phototactically the object can also be positively phototactical. I am unable to call up this situation arbitrarily. However I can assure, that the positive phototaxis only occurs in the transition-case of negatively phototactical to unsusceptible to light. In the cultures we very often meet with this state of things and sometimes some days at a stretch in the same culture. Before that time the organisms react negatively to the light after that they can become unsensible to light. It is a matter of fact that want of oxygen is not able to bring about the positive phototaxis.

§ 6. *Thigmotactical phenomena.*

Under special circumstances *Chlamydomonas variabilis Dangeard* has a strong inclination to attach itself to the glass wall with the cilia. (See fig. 1. A). This phenomenon is seen with many unicellular organisms. An easy object, to learn a little more about it, we find in *Paramaecium* (Jennings 1914, page 87). The sticking takes place with this Protozoön at the close of a number of changes of movement. The animal swims up against a firm object, makes the motor-reflex movement, now swims up against it with a diminished rapidity and stops its motion against the object, while it sticks to it with some cilia. The whole phenomenon is sometimes called thigmotaxis. With *Chlamydomonas*, I was able to observe, of the whole number of phenomena, only that the Alga attached itself. Probably this takes place at the ends of the cilia. After the attaching the motility needs not to stop. With individuals, that still move, after they are attached, we can observe, that the space in which the body moves, is much greater than the volume of it, from which we may conclude, that the object is not attached with any part of the surface of the body, but with one or with both the, cilia. The movement to and fro, which can be made yet, proves, that the cilia still move. It is probable that the attaching takes place at the ends of the cilia.

In this attaching I think to see a pure physico-chemical process. Small particles of dead matter can also stick to the wall of the vessel under some circumstances. Perrin (1914. page 139) made use of this, to count the number in a suspension of particles of catgum. To this purpose he added to the water a small quantity of acid. Every particle, that was driven against the side by the Brown movement, stuck there. In colloïd chemistry too the phenomenon of sticking is known; there the term "sticking-

chance" is used and it is thought that this sticking-chance is closely connected with the electric charge. The sticking of *Paramaecium* is promoted for a good deal by a rather high concentration of carbonic acid of the medium, *Chlamydomonas* too can attach itself very well in very weak acid solutions. Before the concentration runs so high that the motility has diminished a great deal, there is found with numerous acids a region of concentrations where the inclination to go on sticking is very strong. The reaction on the light is very well executed then, besides the motility is great enough to react to gravity. The fact that no sinking takes place in the meantime is caused by the algae get attached when in contact with the glass. In those circumstances we get to see a green stripe, which does not sink to the bottom. (See fig. 1 A, pag. 34). This phenomenon appeared very nice in my experiments, when I worked with solutions of potassium salphate, made sour by small quantities of sulphuric acid. In solutions of known composition I never found the inclination to stick with a distinct alkaline reaction.

In the cultures of *Chlamydomonas* too the inclination to stick often appears. For days the culture-fluid can possess the composition fit for that. I have been unable to make out whether in such a period the culture-fluid was also slightly acid. This is caused by the presence of all sorts of substances in the culture-fluid, which cause that the hydrion concentration of it electrometrically cannot be easily defined. How great the sticking-force is may appear from the information, that often the attaching we can suck away the culture-fluid with a pipette without the algae being taken along by the fluid. I will just draw the attention that the before-mentioned state of the algae only appears with concentrations, which come very close to the concentrations, that make the mobility stop; we always are then just before the line of

demarcation of negatively phototactical to unsensible to light and of positively geotactical to unsensible to gravity.

Besides the fact that the individuals of *Chlamydomonas* stick to the glass side with the cilia, it can also occur, that the cilia of various individuals are attached to one another. Under the microscope we often see algae that stick to each other in this way. With the experiments on the reaction to gravity this mutual sticking together was often so general, that it became with the naked eye visible. In the green stripe which was formed under the influence of the light on the shady side of the tubes, we saw then dense green spots, which moved down much more quickly than the rest of the stripe. With the microscope it could be seen, that the green spots consisted of aggregations of the algae.

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§ 7. *The Chemotaxis and Aërotaxis of Chlamydomonas.*

With the help of the capillary-method I found the Alga positively chemotactical for all sorts of acids (inorganic acids, carbonic acid, acetic acid, malic acid, phosphoric acid), and positively chemotactical for acid and alkaline phosfates; not for malates. To alkaline solutions the algae reacted exclusively negatively.

Again and again we find in literature statements about complications with the reactions of *Chlamydomonas* which are ascribed to positive chemotaxis to the oxygen of the air (aërotaxis). I tried to make out, whether we may ascribe to *Chlamydomonas* aërotactical sensibleness. For this we must try the method whether we can get an „oxygen-line" under the cover-glass, according to Engelman's data (1881). Since however, we have to do here with a green Alga, that assimilates carbonic acid in the

light and consequently secerns oxygen I worked in the dark. Indeed, then was formed after some time a splendid „oxygen-line" by *Chlamydomonas*. Owing to my experiences with the indifferent conduct of the Alga with geotaxis with regard to boiled out water, I was not quite convinced. I have always doubted the fact that the „oxygen-line", which form Bacteria and Protozoa under a coverglass, should be caused by want of oxygen. When examining more closely the circumstances, under wich the line is formed, we must confess, that there is not only a diminishing of the oxygen but that continually other substances must change their concentration.

Especially the carbonic acid might play a great part, as the disappearance of oxygen must coincide with the formation of carbonic acid. The same observation is made by Pringsheim (1912, page 275), I tried to prove that the carbonic acid played a great, if not the only part in the formation of oxygen-lines. To that purpose I made culturus of *Spirillum* species I took a drop of the culture-fluid, covered this with a cover-glas, that was on one side a little further away from the object-glass by a wire of platina and let this preparation stand for some time. After a clear line had been formed, composed of the greater part of the Bacteria, the object-glass with the preparation was turned upside down and used as a lid for a little glass room; with a couple of rubberrings and a little vaseline the lid was shut air-tight. Through the little room gas could be conducted. At my disposal I had carbonic acid, oxygen and nitrogen. The carbonic acid was prepared in an apparatus of Kipp out of marble and hydrochloric acid, the oxygen came from a bombe. The nitrogen was obtained from nitrite. As far as necessary the gasses were purified by washing with the required reagentia, Only with carbonic acid I found it possible to get motion in the line formed by the bacteria.

Was carbonic acid led through, then the line changed its place to the middle of the preparation, was soon after that air or another gas led through again (oxygen or nitrogen), then the line removed to the outside. In some cases I succeeded three times at a stretch with the same preparation to remove the line to the inside and then again to the outside. This removing took place very quickly.

These experiments were done with *Spirillum* species. With *Bacillus butyricus* it was impossible to effect a distinct removing of a once formed oxygen-line under the influence of carbonic acid. I will not make use of the negative result of the experiments with *Bacillus butyricus* to ascribe to these organisms a different conduct towards carbonic acid from *Spirillum*. We should remember that the experiment in a little glass room was more intricate, than it looked perhaps. We have to do with the loss and the taking up of gases by the culture-fluid. These processes are rather complicated. The experiment with *Bacillus butyricus* is made more difficult by the fact that these latter bring butyric acid into the culture-fluid. It will be understood that the presence of this acid can have a great influence on the effect of the carbonic acid on the place of the bacteria-line.

Meanwhile I am of opinion that the experiments with *Spirillum* prove, that for this organism at any rate, the existence of airotaxis is very improbable.

By the data I obtained about the geotaxis, the phototaxis and the inclination to stick fast of *Chlamydomonas*, it is proved how much these phenomena are influenced in a corresponding way by the quantities of electrolytes resolved in the surrounding fluid. Especially the sticking on the glass side and the positive phototaxis, which both occur with high concentrations of electrolytes, though those concentrations are not so high to bring about

immovability, show, that we see an important phenomenon in the influence of the electrolyt-concentration of the milieu on the motility. We can conclude with certainty, that here an injurious action does not play a predominant part.

CHAPTER III.

The systematic investigation of the influence of electrolytes on the motility of Chlamydomonas variabilis Dangeard.

§ 1. *The judgment on the motility of the organisms.*

In the preceding chapter we have seen that the electrolyt-concentration of the watery medium, in which *Chlamydomonas* is, is of a very great influence on its activity, i. e. the power to react to light, gravity, chemotactica and contact.

To learn a little more about this influence of the electrolyt-concentration, it seemed desirable to me to follow systematically the motility of *Chlamydomonas* in solutions of electrolytes. This has chiefly been the aim of my experiments.

Firstly we tried to find a method to observe the influence of the concentration of electrolytes in an easy, if possible macroscopic way. The motility of the cilia gradually diminishes with the increase of the concentration of the solution. To express the grade of motility for every case, by a number did not seem easy to me. Meanwhile it was very simple to observe two states of motility, namely such a motility, that the reactions on the light, to gravity etc., could be distinctly observed and a state of immotility or a thus diminished motility, that these reactions were no longer carried out. We are now looking for the concen-

tration in which the reaction can yet clearly be observed and the concentration, in which there is no question of a distinct reaction. Between these two limiting concentrations there is one, denoting the point, on which, theoretically the transition from one state of motility to the other must take place. We call this concentration the critical transition concentration or the critical concentration. It is calculated from the limiting concentrations. All concentrations, which are between the limiting concentrations we call transition concentrations. Whether for the stating of the limiting concentrations we make use of the reaction to gravity, on the light or on chematactica does not matter practically. The reaction to gravity can easily be observed macroscopically. By a sufficient motility there is soon formed a distinct amassing of the Alga on the bottom of the vessel. As limiting concentrations we now adopted the concentration, with which again exactly a distinct amassing took place in the lower layers of the fluid, (See fig. 1 B and C, page 34). and the concentration, in which no amassing took place any longer. (See fig. 1 D, page 34). It was supposed that with the critical transition-concentration the state of motility was always the same.

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§ 2. *Precautions, in order that the differences observed can be exclusively attributed to the changed quantities of electrolytes.*

We must take care that the circumstances under which the experiment takes place are such, that the differences which are observed, can be ascribed exclusively to the different concentration of electrolytes. We must pay attention to the temperature and strength of the light,

but besides this, it is desirable to work with glass vessels, which have always the same diameter. With experiments, that took place six-fold, it struck me that a difference in diametre of the tubes gave rise to deviating results. I worked with narrow glass tubes, tapering at the bottom and shutted there by melting. (See fig. 1, pag. 34), By observing that the various tubes were filled alike, when they contained the same quantity of fluid, I had a guarantee, that the diametre had the required size.

It is not probable that the temperature has a great influence on this phenomenon we are studying.

When still engaged with preliminary experiments on the various reactions of *Chlamydomonas*, I controlled the influence of the temperature. By means of tapwater, saturated with carbonic acid, I obtained by a mixing up with boiled out tapwater solutions, which changed regularly, as far as the concentration of carbonic acid, was concerned. In the way, which was also applied later on I examined the motility of the Alga to the reaction to gravity and so it was possible to state the limiting concentrations for the carbonic acid solutions. The experiments were done by 25° C. and by about 0° C., to obtain the latter temperature, the tubes, in which the reaction was to take place, were steeped in ice-water. For the low temperature the same limiting concentrations were found as for 25° C.

It was observed that with the low temperature the algae do not attach themselves in the same solutions in which they did it very fine by 25° C. I am of opinion that my preliminary experiment proves sufficiently, that the influence of the temperature on the motility of *Chlamydomonas* in solutions of electrolytes is not great. This opinion is the same as Voegler's (1891) or the influence of the temperature on the value for the threshold of chemitactica for spermatozoa of *Dicksonia antarctica*.

Between 14° C. and 28° C. the threshold-value remained unchanged.

Since the results of my further experiments denote a clear agreement between the phenomena which I studied of *Chlamydomonas* and the flocculation and solution of colloïds, it is of importance to point to the fact, that for instance the influence of the temperature on the stability of suspensoïds is of little importance (Freundlich, page 368). The temperature, in which my experiments were done, changed between 14° C.—28° C. The temperature was always noted down. For all experiments with the same salt the temperature varied much less. It was for my statements with KNO_3 20.5° C.—24° C. In the beginning it seemed better to do the experiments in the dark. Possible changes under the influence of a changed strength of light would then be excluded. Very soon it was proved, however that is was not desirable, to experiment in the dark. So it struck me directly, that the conduct of the Alga in distilled water in the dark, was very uncertain. Sometimes in this milieu the sinking took place in a splendid way; in other cases it might happen however, that the reaction to gravity, under those circumstances, did not appear at all in distilled water.

Meanwhile we must not forget, that we must always expect a change of the concentration of carbonic acid during the experiment. In diffuse daylight we find as well assimilation of carbonic acid as respiration, by which the concentration can diminish, increase or remain unchanged. In the dark an increase of the concentration of carbonic acid can only take place under the influence of the respiration. Now it can be derived from the graphic representations which shall be given later on, that the conduct in distilled water by the addition of small quantities of acid is changed considerably.

However the increase of the concentration of carbonic

acid cannot alone be responsible for the uncertain conduct of the Alga in the dark. In the first place the experiment takes but little time (ten minutes). Moreover this can be seen from the following example: For solutions which contain besides 0,00010 n. KOH also KNO_3, one day the limiting concentrations of the KNO_3 were stated for objects from a definite culture. The limiting concentrations were 0,08 n. KNO_3 and 0,09 n. KNO_3.

The next day the same limiting concentrations were stated with algae from the same culture for solutions, which contain besides 0,00010 n. KOH, KNO_3.

It appeared now, that even in 0,005 n. KNO_3, could not be observed a reaction to gravity. Other experiments too from the series of the preceding day were then repeated and the conduct of the Alga was entirely changed.

After the drawbacks of working in the dark had thus distinctly come to light, I examined whether in the light were obtained less irregular results. This proved indeed to be the case. As far as I worked with algae from the same culture, I have, with my experiments in the light, never observed anything of similar sudden changes. The tubes were thus placed, that they were exposed to one-sided diffuse day-light. Naturally the strength of the light was rather different.

§ 3. *In which manner the algae were brought into the solutions of electrolytes.*

We have now found a suitable method to judge of the motility. Before, however, we can go further and can examine the influence of various solutions, a means must be sought, with which we can transport the algae, free from bymixtures and together with as little fluid as pos-

sible, in the solutions, of which we will examine the influence. This transporting of the algae is very easy, when we take advantage of the inclination to attaching and the reaction to gravity. The inclination to sticking to the side was very great in cultures, in which the object was present in a sufficient number.

The tubes were filled with the culture-fluid by means of a pipette. Now I let them stand upright for about ten minutes, exposed to one-sided diffuse day-light. Under the influence of the light the algae moved to one side of the glass tube, where they attached thimselves (see 1. A). After ten minutes the culture-fluid was sucked away with a pipette. The algae remained fixed to the side. As there always remained behind something of the culture-fluid to the sides of the tube, I washed with a definite quantity of distilled water. After this water had been put into the tubes the algae were shaked with it. After that the tubes were placed vertically again. The inclination to sticking had now disappeared, but the reaction to gravity took care, that in a short time all algae were closely amassed on the bottom of the tube (see 1. B. and C.). By means of a pipette the above washing-fluid could be easely taken away. The staying for a short time in distilled water was of no influence on the sensibility of the algae for the solutions. Only when the staying in it lasted longer than half an hour, I could perceive, that the algae could bear a smaller concentration. After these preparations the tubes were filled with the solutions, the influence of it had to be controlled. 0,6 cM^3 of these solutions was brought to the organisms. By using more or less of culture-fluid for the filling of the tubes, the number of the algae that partook of each experiment, could be taken the same to the eye.

§ 4. *The mutual proportion of the different ions, which are present in the fluids and the use of the solutions in series.*

The elements of the solutions deserve a further discussion. In the preceding chapter we have seen, that small quantities acid or base and solutions of neutral salts in a somewhat greater concentration have an influence on the motility of the Alga. The electrolytes are divided into ions for a great part in weak watery solutions. It is a matter of course to suggest, that the influence, exercised by the electrolytes, is excercised by the presence of the ions. In every watery solution are, besides the ions of the dissolved substance, also hydrions and hydroxylions, since the water has always been for a small part dissociated into H-ions and OH-ions. In really neutral solutions the number of H-ions and the number of OH-ions is equally great. However small quantities of carbonic acid, which easily dissolves in water, are the cause, that it is very difficult to obtain that the number of H-ions and of OH-ions is equally great in a solution. Moreover the quantity of dissolved carbonic acid is sometimes greater and then again smaller. The result of this is that the water, with which we make our solutions does not contain a definite concentration of H-ions and OH-ions.

It is generally said, that solutions which are made from neutral salts with distilled water, are neutral. However for these solutions the acidity varies, as the acidity of the distilled water varies. For the phenomenon studied by us these small changes of acidity are of great importance. Therefore it is desirable with a systematic examination of the influence of the electrolytes on the motility of *Chlamydomonas*, to make use of series of solutions, which not only vary as regards the concentration of the electrolyte, which we examine, but also as regards the

concentrations of the H-ions and OH-ions. The solutions are thus composed, that they form series. All solutions from one series contain the same hydrion concentration that means the same acidity or the same alkalinity, as the H-ions or the OH-ions are present in a greater number. The successive solutions from every series contain an increasing quantity of salt. The series succeed one another in such a way, that the hydrion-concentration of one of them is smaller than that of the next.

We can also arrange the solutions in such a way, that in each series the amount of salt is constant, whilst the hydrion-concentration of the following solution regularly changes.

By the experiment we state from a series, in which the hydrion concentration is constant the limiting concentrations of the salt. From the limiting concentrations we calculate the critical concentration for the salt with that definite hydrion concentration. In a similar way we can find the limiting concentrations and the critical concentration of the hydrions with the help of the series with a fixed amount of salt. Both proceedings must have the same results, for a solution, which contains the critical concentration for a salt by a fixed hydrion concentration can also be considered to contain the critical hydrion concentration by a constant concentration of salt.

In most of our experiments we worked with series of constant hydrion concentration. The limiting concentrations which are fixed by that, represent the limiting concentrations of the salt and must be used to calculate the critical salt concentration by a definite amount of hydrions. Occasionally this gave rise to difficulties. In those cases the limiting concentrations found for salt were used as limiting concentrations of hydrions and they were used to calculate the critical H-ion concentration. In a series with constant H-ion concentration we find one critical concen-

tration. Within the series of constant salt concentration we find two, one for acid and one for alkaline solutions (hydrion concentration large, hydrion concentration small). That in one case we find one, and in the other case two critical concentrations will be understood, when we consider the proportion of the concentrations of the various ions in a solution.

In a solution of a salt (with univalent anion and univalent cation) the number of negative is as great as the number of positive ions. As regards the salt we have to do with the combined influence of an equal number of anions and cations. In a series of solutions, where the H-ion concentration changes, we always have the combined influence of H-ions and OH-ions. The mutual relation of these ions is now such, that the concentrations of it give a constant product, when we work by a constant temperature. Are the concentrations expressed in gram-ions per liter, then the constant product, by 18° C. is about 10^{-14}.

Now we can find the case, that the H-ion concentration is great and the OH-ion concentration is small. Under those circumstances it is especially the influence of the H-ions, which will act a part. Is the number of OH-ions great as regards the concentration of the H-ions, we chiefly see the influence of the OH-ions. The rather complicated connection between the H-ions and the OH-ions gives us an opportunity to examine the influence of every ion separately. Hence that we find two critical concentrations for the H-ions in a series with constant salt concentration. The constant product of H-ions and OH-ions causes that the concentration of the hydroxylion can be found from the concentration of the hydrions. As, moreover of a solution the H-ion concentration can be measured immediately, and the OH-ion concentration not, it has become a habit

to denote by the H-ion concentration, also the reaction of an alkaline fluid. When we say that for a series with a constant salt concentration we find two critical concentrations as regards the H-ions, this does not mean, that it is the H-ions in both cases, which cause the phenomenon studied by us.

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§ 5. *How the results are represented graphically.*

The results of the experiments obtained with the series, can easily be represented graphically in a flat level (see 2 page 51; 4 page 56; 5 page 57). On the abscissa-axis of a rectangular bi-axial coördinate-system we plot the concentration of the salt. On the ordinate-axis is denoted the H-ion concentration or the acidity and the alcalinity of the solutions. The points, representing the critical concentrations, are connected one with the other; the curve obtained contains all the combinations of salt and H-ions and OH-ions, with which *Chlamydomonas variabilis* has a certain motility. The curves which are obtained by connecting the low limiting concentrations or the high limiting concentrations, denote each separately a certain state of motility. At the origin we put on the abscissa-axis the figure zero; the points on the ordinate-axis denote the solutions, which contain no salt. From the origin is denoted on the ordinate-axis upwards the concentration of the added acid and downwards the concentration of the added base. Only in the figure for sodium acetale-acetic acid-mixtures (see fig. 3 page 52) is denoted on the ordinate-axis the acidity by the hydrion concentration. At the origin we place there 10^{-7}, that means the hydrion concentration of very pure water.

When the concentrations are denoted in this way, the three curves, which we get by connecting the low limiting

concentrations, the critical concentrations and the high limiting concentrations cut twice the ordinate-axis and once the abscissa-axis. The points of intersection of the ordinate-axis agree with the limiting concentrations and the critical concentrations of solutions, which contain only acid or base; the point of intersection with the abscissa-axis agree with the limiting concentration and the critical concentration of solutions, which contain only salt. Between the lines connecting the limiting concentrations, we find a region, containing all the points, which represent transition concentrations. The region between the curves for the low limiting concentrations denotes all the solutions in which the Alga is so movable, that during the experiment they clearly show the reaction to gravity. Outside of the curves for the high limiting concentrations we find all solutions of the salt, in which the algae do not show the reaction to gravity.

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§ 6. *The preparation of the solutions.*

The preparation of the solutions requires a nearer discussion. We can be short, as regards the obtaining of the desired concentrations of the neutral salt. To this purpose we make normal solutions (is that not possible, solutions with the normality $^1/_2$). The concentrations of the successive solutions of a series differed, as a rule, 0,01 normal. Thus were prepared solutions of 0,01 normal to 0,20 normal, by taking with a pipette 1—20 cM^3. of the normal fluid and adding to this quantity distilled water up to 100 cM^3. Properly speaking, it would be necessary, in stead of denoting the concentration in the normality, to define the concentration of the ions. For the various neutral salts the ionisation however is equally strong for every definite concentration. This holds for the small

concentrations which we used. Therefore we have not made the definition of the concentration of the salt-ions. To obtain solutions with different H-ion concentrations we can use various methods. The simplest way is to add to the solutions small quantities of acid or base. This manner of working is only suitable, when the solutions do not contain anything but neutral salt. I used this means. However we must make allowance for all sorts of details. When, in this way, we will make a series of solutions with increasing amounts of hydrions, we have to make, for example, a solution of 0,001 normal acid. It must thus be preserved, that the strength of the acid can change as little as possible. Of this solution we must have enough to compose all solutions with the same salt. Solutions with different H-ion concentrations are now obtained by adding per 100 cM^3. fluid, 5, 10 etc. cM^3. 0,001 normal acid. Thus we obtain solutions which contain f. i. 0,00005, 0,00010 etc. normal acid. The distilled water, which is used, may not vary too much as regards the amount of carbonic acid. When working in the stated way, the method can be used. The drawbacks of the method are the following. The thus obtained solutions are not reproducable, that means, if we make the same solutions with another solution of 0,001 n. acid, the H-ion concentration can deviate a good deal from that of before-made. Small mistakes denote very great differences as regards the acidity. The H-ion concentration of these solutions cannot be reckoned out. Moreover it cannot be defined electrometrically in this. Only by means of indicators we can find here an amount for the acidity. However, I did not apply this method.

The cause of the difficulties must be sought in the fact, that small quantities of acid or base enormously change the H-ion concentration. Moreover, small quantities of carbonic acid can also bring about a great change. For

the influence of the acidity on the movability of our organisms the method was suitable, when at least we are content with the knowledge of the course of the changes. All my curves, except those for sodium acetate are obtained by means of this method. They can only be of service to examine the qualitative course of the phenomenon. Conclusions about the absolute value of the H-ion concentrations, must be considered under a certain reserve.

A method, which permits, to make reproducable solutions with small H-ion concentrations, is the using of so called buffer solutions. These are obtained by adding to solutions of salts of weak acids with strong bases and of strong acids with weak bases, the according free acid or free base.

A well-known buffersolution is sodium acetate since the sodium acetate is split up hydrolytically, we must add to a solution it, rather much free acetic acid to cause a slight change in the H-ion concentration of the solution. The acetate makes go back for a great deal the dissociation of the free acetic acid in H-ions and axions of the acid. This causes the effect of a buffer. In buffer-solutions the H-ion concentration can be easely stated electrometrically. The drawbacks of this method are, that te number of buffer-solutions is limited, and that, as a rule they can only be used for solutions of which the H-ion concentration lies in a rather limited region. Moreover there are always ions in the solutions, which can also have an influence on the phenomenon and of which the concentration is so high, that their influence cannot be entirely excluded.

CHAPTER IV.

Contemplations and conclusions, to which the experiments give rise.

§ 1. *The conduct of Chlamydomonas in solutions with a slight, constant salt concentration and a gradually increasing H-ion concentration.*

In the first place I have pointed out as well as possible, that the faillure of the reaction to gravity can be caused by a change of the amount of hydrions or of hydroxylions. To this purpose a series of solutions was made, which contain all 0,01 normal sodium acetate and changing quantities of free acetic acid or free sodium hydroxyde. In these solutions we can cause the H-ion concentration to change regularly, while the number of the other ions remains well-nigh the same. The not dissociated molecules of acetic acid are not always present in the same number. We must here rely on experience, which teaches, that such small quantities of not dissociated molecules have no important influence.

If we put the algae in these solutions we find, that they do not show the reaction to gravity in those numbers of the series, which are most acid, neither in those solutions, which are most alkaline. It is a matter of course, that it cannot be said beforehand, whether a series of solutions which one has made, contains such solutions the first time

already, that the experiment goes smooth. If one has obtained a suitable series, then one must look for the acid solution, which yet permits to the algae to show the reaction to gravity, and the solution within which the reaction yet does not occur. The same thing is repeated with the alkaline solutions. Of these solutions the H-ion concentration is fixed electrometrically. For the acid solutions it was found, that with a hydrion concentration of $10^{-5.9}$ the reaction just took place yet while with a concentration of $10^{-5.5}$ gramion hydrions per liter the reaction took no longer place. For the alkaline solutions these figures were $10^{-10.7}$ and $10^{-11.2}$.

By this it is pointed out, that, at least partly, it depends on the H-ion concentration of a solution, whether or no the reaction to gravity is executed by the individuals of *Chlamydomonas variabilis Dangeard.*

A repetition of this experiment with mixtures of sodium malate and free malic acid or free sodium hydroxyde gave the following results. In the acid solutions of 0,01 molair sodium malate the limiting concentrations of H-ions were respectively $10^{-5.1}$ and $10^{-4.9}$. For the alkaline solutions the figures were $10^{-10.6}$ and $10^{-11.1}$. It appears, that in the acid solutions of sodium malate the acidity was greater than in the solutions of sodium acetate. In the alkaline solutions the boundaries were not changed. We see from this, that besides the H- and OH-ions, also other ions have an influence on the reaction; even in the slight concentration of 0,01 molair. It is of importance that the limiting concentrations in the alkaline solutions are not (at any rate very little) changed. This will be discussed further on.

§ 2. *Measures, to obtain reliable results.*

Now that it has become clear that H-ions, OH-ions,

anions and cations of salts have an influence on the power of reaction of the object, we can proceed to the discussion of the conduct of the algae in acid and alkaline solutions of potassium nitrate, potomum chlorid, sodium acetate and potassium sulphate. Besides the various quantities of the dissolved salts these solutions contain regularly increasing or decreasing quantities of hydrions and hydroxylions. In the preceeding chapter we find stated, how the solutions were composed, there is also told which drawbacks belong to the working with these solutions. Moreover there is pointed to the fact, that always by the transport of the Alga into the fluids, the influence of which is examined, small quantities of moisture mix with the solutions, by which an inaccuracy crops up.

Not only by the precautions, of which is spoken in the preceding chapter, the results have become much more certain, but also by the fact that all experiments, were carried out six fold. Each of the results is therefore an average from six experiments. An example can throw a clearer light on this proceeding. A series of solutions was prepared, which contained all 0,00040 normal potassium hydroxyd and different quantities of potassium nitrate namely:

0,07, 0,08, 0,09, 0,10, and 0,11 normal. We call these solutions successively A, B, C, D and E.

Solution A gave in six little tubes cleargrounds. With the solutions B, C, D and E, we could see a distinct amassing in 4, 3, 1, 0, of the six tubes. In the graphical representations an amassing in six tubes is denoted by ●; when in none of the tubes was observed a clear accumulation at the bottom, the mark O is used. For all cases where a part of the experiments gave accumulation and the remaining part no amassing, this result is denoted by a ×. In the example we get:

Number of the solution.	A.	B.	C.	D.	E.
Amount of KOH × 100 000.	40.	40.	40.	40.	40.
Amount of KNO_3 × 100.	7.	8.	9.	10.	11.
Number of tubes in which an amassing is seen.	6.	4.	3.	1.	0.
result for the graphical representation.	●	×	×	×	○

We must draw the attention to the fact, that by the judging of the result in each of the tubes an absolute amassing and a rather distinct amassing at the bottom were looked on as a positive result, while the formation of a slight amassing and no amassing at all were judged to be a negative result. With the help of the example we can now easily denote how the average was reckoned out. Solution A with 0.07 normal KNO_3 gave a positive result, solution E with 0,11 normal gave a negative result, while B, C and D denoted transition-cases; 0,07 normal and 0,11 normal are the limiting concentrations, while. $\frac{0{,}07 + 0{,}11}{2} \times 1$ normal = 0,09 normal is considered to be the critical concentration.

Now we must tell beforehand that we should not put too great a value on the absolute amount of the concentration. In the preceding chapter we already pointed to this fact in connection with the way, in which the solutions were composed. With the exception of this way of preparation of the solutions, the absolute value of the limiting concentrations is of comparatively small importance, since for various cultures, especially for cultures from periods, which differ greatly as regards light and temperature, the limiting concentrations for the same salt and at the same acidity can run widely apart. In the preceding chapter I drew special attention to the fact, that, with the proceeding followed for the same culture, I was not troubled by

sudden changes of the algae, as regards the sensitiveness to salts, when I worked in the light. If a new culture had to be used however, then it was necessary always to repeat the latest series of experiments, which were done with the old culture, with the new culture, to see whether the results with the new culture succeeded to those with the old culture. In most cases the difference was so slight, that the new culture can be used to continue the statements, without an interruption in the curve. In some cases however the new culture gave greatly deviating values. This case is made more clear by the curve, for potassium chloride (4, pag. 56). With 0,00065 normal potassium hydroxyde. I did experiments with a new culture. The organisms of this culture appeared to be less susceptible to KCl than those of the old culture. The critical concentration was removed from 0,10 normal to 0,14 normal. Probably the removal has not only taken place horizontally, but also vertically. I think that a part of the new piece of the curve corresponds to the last part of the old curve. The corresponding pieces were connected by dotted lines. Examples of less important removals gives the curve for mixtures of acetate and acetic acid (fig. 2). In this case algae from three different cultures were used. The critical concentrations obtained with organisms from each culture were mutually connected. Thus were formed the curves I, II and III.

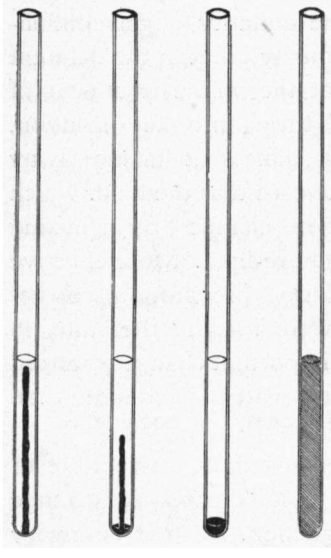

Fig. 1.

When we ask for the cause of the different behaviour of the algae from different cultures, we can be sure, that the electrolyte concentration of the various cultures is not always the same. It is true the irregularities in this respect were diminished by preparing the cultures fluids with a special quantity of sterilized earth, white of egg and tapwater. The fact, however that the putrefaction of the eggwhite did not always take place with the same quickness, must cause, that for example the quantity of ammonium-carbonate has not always been equally great in the different cultures. To this is added, that the whole treatment of the algae, before transporting them into the solution, whose influence was examined, aimed at taking away superficial defilement and to take care that the fluid, which had to be examined, would not be changed by a mixing up with small quantities of the culture-fluid. More effective changes were always avoided during preparation, so that we can accept, that the algae were used in the state, in which they were in the cultures. Important changes cropped up, when the stay in the distilled water lasted more than half an hour.

The organisms then became much more susceptible to salts. Perhaps one is inclined to look upon this change by distilled water as an injurious action. It is not necessary for us to go against this opinion. However we can observe, that the expression "injurious action" does not bring us much further. I imagine the action of distilled water, to be totally different. All we know as yet of the conduct of *Chlamydomonas* in regard to electrolytes points to the fact, that the influence of the dissolved substances on the protoplasm is a colloïdchemical one. With acids and bases the plasmcolloïds form compounds which are chemical compounds probably. With neutral salts are probably formed absorption compounds Starting from these suggestions, we think that the changes by a long washing

with distilled water is founded on a partial taking away of the absorbed salts and the different conduct of the algae from the various cultures is based on differences between the quantity and the nature of the substances absorbed by the plasmcolloïds.

A phenomenon which should also be mentioned, crops up, when the objects are left for more than ten minutes in a solution, in which no amassing at the bottom takes place. Is the solution not too strong, the algae become movable again in course of time. This phenomenon may also be a result of absorbation processes, which have a slow course. However we shall not go further in to these contemplations; but we will point to the fact that here also we have to do with "condition phenomena".

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§ 3. *A conformity between the different influence of the combination acid and salt and of base and salt on the motility of Chlamydommas and the entering again into solution of globulins in acid and alkaline salt solutions.*

Now we shall discuss the graphical representation. Only the curve for potassium sulphate is about complete (see fig. 2). We see from it that the course is very complicated. It strikes us at once, that the shape of the curve in the utmost acid solutions (from 0,00015 n. H_2SO_4) is quite different from that in most alkaline solutions (from 0,00100 n. KOH). A course of the curve as in most alkaline solutions can arise when the influence of base and salt is additive. For the shape of the curve in the most acid solutions a similar supposition is not possible. Here it seems, as if the salt diminishes the influence of the acid, while, on the contrary the H-ions also diminish the influence of the salt. When, namely, we follow the curve from the

ordinate axis into the most acid region we see, that the line makes two bends. The first bend takes the curve

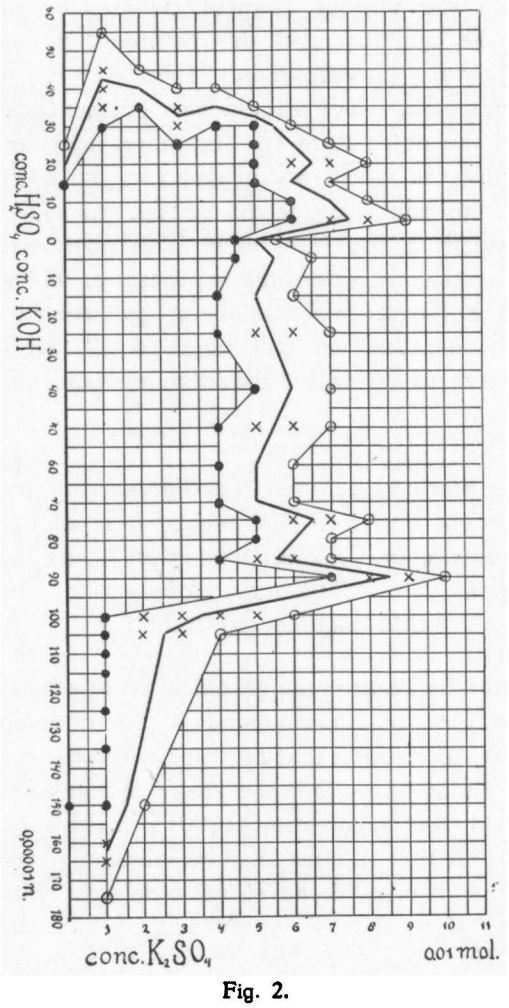

Fig. 2.

higher into the acid region (at 0,01 molair K_2SO_4); here, under the influence of small quantities of neutral salt the

susceptibility to the acid is diminished. After that the curve goes back, to make another bend soon afterwards (till 0,00015 n. H_2SO_4, 0,06 m. K_2SO_4), which we may think to have arisen from the opposition, which the salt gets from the acid.

The curve for K_2SO_4 is not the only argument for the different conduct of acid and salt and of base and salt mutually on the motility of *Chlamydomonas*. The course of the line for sodium acetate (fig. 3) in the most acid

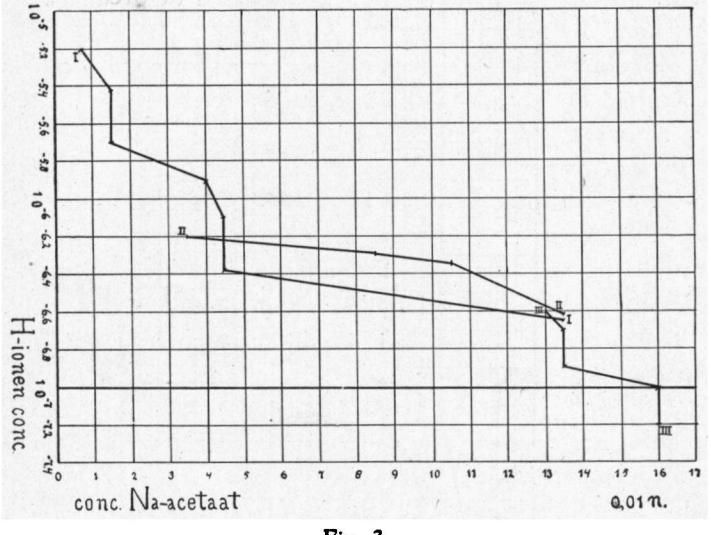

Fig. 3.

solutions (between [H] = $10^{-5.2}$ and [H] = $10^{-6.4}$) agrees entirely with the opinion, that the influence of acid and salt is not additive.

Though the point, where the curve in the acid solutions reaches the ordinate axis, was not fixed, yet we can observe, that the line in the denoted region, just as the line for K_2SO_4, makes two bends. If we follow the curve from the ordinate axis; then the first bend illustrates the

resistance which the H-ions experience from the small quantities of sodium acetate, while the second bend can be attributed to the resistance of the salt by the H-ions.

It is a very important fact that in alkaline solutions there is found the additivety of the influence of base and salt while this does not hold for the combined influence of acid and salt. A corresponding phenomenon is observed in the conduct of proteïns towards base and salt and acid and salt. In a publication of Hardy on colloïd solutions of globulins this phenomemon is discussed at great length (Hardy, 1905).

Globulins are ampholytes, they can form compounds with acid and base. Globulin solutions can be floceulated by neutral salts.

In this respect they show characteristic qualities. Every salt gives a precipitation by a slight concentration and also by a great concentration. By a moderate concentration the salts cause solution. By a slight concentration the salts only act on globulin combined with base or acid.

The action of the salts is in this case corresponding to that on suspensoïds; the colloïdal parts are charged electrically and are discharged by the ions of the salt, which contain an opposite charge. Plurivalent ions have more influence than univalent ions. By great concentrations the salts act upon combinations of globulin with salt, which give colloïdal parts without charge.

In this case we can make a comparison with the salting out of emulsoïds. The concentrations which Hardy used when studying the flocculation of the globulins by small quantities of electrolyte and their dissolving again by average salt concentrations can be compared to the concentrations, which have an influence on the motility of *Chlamydomonas*.

It is with the experiments on the dissolving of the globulins, that Hardy noticed the different conduct

of the combinations acid and salt and base and salt. He expresses this on page 323, as follows:

"there is however one feature of fundamental importance which is never obscured, and that is the antagoniom between the solvent actions of salts and acids and the additive nature of the combined solvent actions of salts and alkali".

One of the facts, which brought Hardy to this conclusion, was, that with the dissolving of globulin in the presence of salt, the quantities of acid necessary variegated strongely with the nature of the salt, while the quantities of base changed very little for the various salts. The concentration of the salts was rather different in these experiments. At first 1,5 cM.^{3} normal salt solution, was added to 10 cM.^{3} globulin suspension, by which was formed a concentration of about 0,13 n. After that was added the required quantity of acid or base by which, especially in the most acid solutions the concentration of the salt was yet considerably diminished (0,06 n. or less). A similar result we obtained with the experiments on the conduct of *Chlamydomonas* in acelate solutions and malate solutions (from 0,01 molair), in which the H-ion-concentration regularly changed. We then observed that the limiting concentration for H-ions in the acid malate solutions had been considerably raised with regard to those in the acid acelate solutions. The limiting concentrations in the alkaline malate solutions had not removed with regard to those in the acelate solutions. Here we see another argument for the different conduct of acid and salt and of base and salt with regard to the motility of *Chlamydomonas* and moreover once more the correspondence between the influence of electrolytes on the dissolving of globulins and on the motility of the organisms used by us.

So far there was found a difference between the influence of electrolytes on the motility of *Chlamydomonas* and on

the entering again into solution of globulin, that the H-ion-concentration, by which the phenomena took place for the globulins were somewhat bigger, by which processes took place chiefly in very weak acid solution while our phenomena were principally observed in very weak alkaline solutions.

The correspondence in the conduct of *Chlamydomonas* and of the globulins with regard to acid and alkaline solutions of salts, leads us to the suggestion, that the influence of electrolytes on the motility of our object is caused by the fact that the electrolytes work in upon the colloïds, which are present in the cell of *Chlamydomonas*, probably the plasmcolloïds. This hypothesis is not unexpected; we have already seen that there are many facts, which can be understood by it. Moreover one is universally of opinion, that the protoplasm is colloïdal.

§ 4. *Description of the middle part of the curves.*

After we have examined the influence of the salts in the most alkaline and most acid solutions, we shall discuss the middle most parts of the curves. We are of opinion, that in the region which will now be discussed, we have chiefly to deal with the influence of the salt, while the H-ions and the OH-ions have a less great effect.

For K_2SO_4 (fig. 2 page 51) this part runs from 0,00015 n. H_2SO_4 to 0,00100 n. KOH. For KNO_3 (fig. 5 page 57) and for KCl (fig. 4) the curves as far as they have been drawn belong entirely to this region.

The course of these lines is somewhat capricious. This is caused by the presence of summits.

With K_2SO_4 (fig. 2 page 51) we distinguish five (0,00005 n. H_2SO_4, 0,00005 n. KOH, 0,00040 n. KOH, 0,00075 n. KOH and 0,00090 n. KOH). With KCl (fig. 4,

p. 56) we see also five (0,00010 n. HCl, 0,00025 n. KOH, 0,00045 n. KOH, 0,00065 n. KOH for the upper part and 0,00085 n. KOH for the lower part and with 0,00115 n. KOH), when we accept at least, that the lower part can

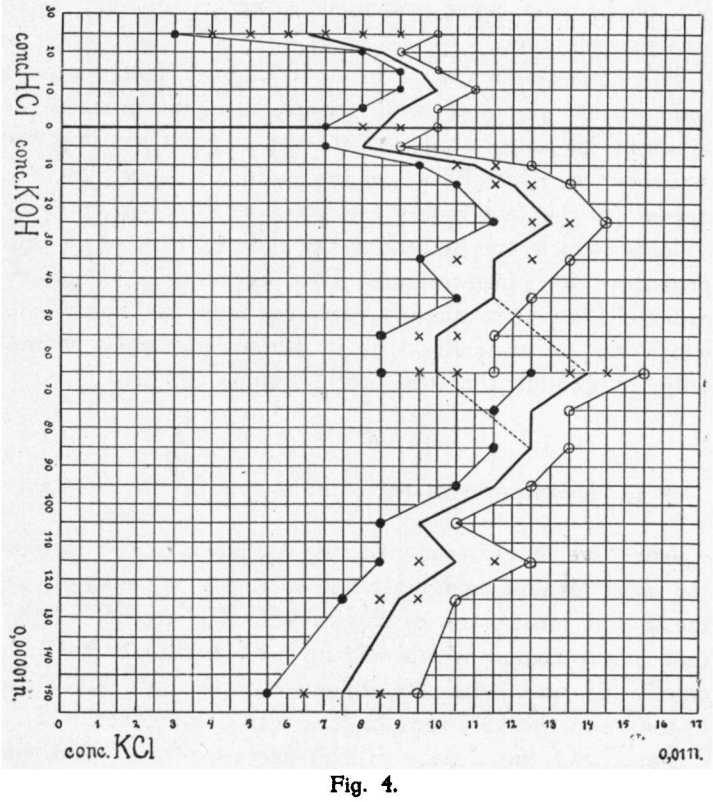

Fig. 4.

be fitted close to the upper part by a removal in a vertical and horizontal direction. With KNO_3 (fig. 5) it seems at first as if we have to deal with one broad maximum. If we look closer and pay also attention to the lines which connect the limiting concentrations, then there are also indications for five tops (with 0,00010 n. HNO_3,

0,00015 n. KOH, 0,00030 n. KOH, 0,00050 n. KOH and 0,00065 n. KOH).

Especially with K_2SO_4 we see that these tops are arranged all but symmetrically, we can namely draw an axis of symmetry along the line, denoting a concentration of 0,00040 n. KOH. For KNO_3 (fig. 5) the symmetry can also be well observed. The axis of symmetry here cuts the curve at 0,00030 μ KOH. It does not run horizontally

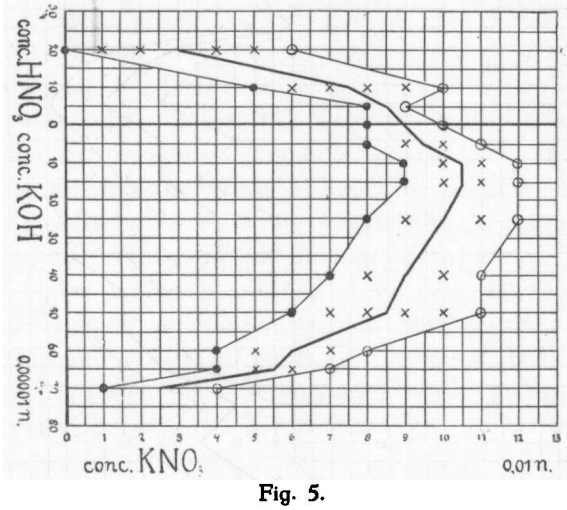

Fig. 5.

here. Of KCl (fig. 4, page 56) the point of intersection of the axis of symmetry lies (at any rate for the topmost part, which is lengthened with the removed lower part) at 0,00045 n. KOH. The axis of symmetry does not run horizontally here either.

The symmetry in question is for us an indication, that the capriciousness of the figures cannot be attributed to inaccuracy. The sixfoldedness of the experiments, besides the fact, that for the denoting of a point of the curve we generally did 18 or still more experiments, are a very good warrant for that. The meaning of the symmetry

can be sought in the fact, that we have to do with the influence of ions. With a positive charge of the plasmcolloïds the anions of the salts will exercise most influence in this case, while with a negative charge the cations are most active. As a rule, a symmetry of figures will be the result of such an action of anions and cations.

Is the action of one ion much stronger than that of the other one, the figure may become oblique.

For K_2SO_4 (fig. 2, page 51) a bivalent anion is placed opposite to a monovalent cation, Here the chief direction of the curve is vertical. With KCl (fig 4, page 56) and with KNO_3 (fig. 5, page 57) the chief direction of the line runs from above obliquely downwards to the left. Here a monovalent anion is placed opposite to a monovalent cation. Definitions made for $Ca(NO_3)_2$, with a monovalent anion against a bivalent cation showed, that for the curve for $Ca(NO_3)_2$, the direction would be yet much more oblique as for the curves for KCl and for KNO_3 and again in the same direction. The succession K_2SO_4—KCl and KNO_3—$Ca(NO_3)_2$ agrees with the opinion that the oblique direction of the lines is the result of the valence of the anions and cations present in the salt solution.

In the colloïd-chemical literature I have not been able to find figures, which showed much likeness to the curves, which we discussed just now. The cause of this might be found perhaps in the fact, that, generally, the influence of salts by a different hydrion concentration on colloïds, has not often been examined systematically, while in those cases, where this really happened, the concentrations of the acid and of the base increased far stronger than in my experiments.

The presence of the tops in our figures may be caused, by the fact that the plasmcolloïds, which we have made responsible for the conduct of *Chlamydomonas* with regard to acid, base and salt, are proteïns.

Of the proteïns it is known that they can combine with various quantities of base or of acid. According to their taking up one molecule of acid or an other number, various compounds are formed. People do not agree about the stability of the thus formed compounds. T. B. Robertson thinks to have proved, that they are stable with a set H-ion concentration. Besides these compounds greatly differ in their conduct towards salts. Is Robertson's opinion right, then the H-ion concentrations, where the maximums or minimums in our figures are to be found, agree perhaps with the H-ion concentrations, by which the colloïdal substances from the protoplasm form stable compounds with various quantities of acid or base. To make things still clearer, we can say, that the phenomena, which we study of *Chlamydomonas*, take place in a region of H-ion concentrations, lying between $[H] = 10^{-5.5}$ and $[H] = 10^{-11.2}$. In a book by Robertson it is stated, that a compound of caseïn at neutrality against litmus ($[H] = 10^{-7}$) contains 1,5 % CaO, while caseïn combines with 2,4 % CaO at neutrality against phenolphthaleïn ($[H] = 10^{-3}$).

Though it is probable, that in both cases, we have to do with mixtures of different compounds of caseïn and calciumhydroxyde we can state from the data, that in the region of the H-ion concentrations, in which *Chlamydomonas* is movable different compounds of plasmaproteïns and base and acid can be expected.

§ 5. *The influence of the salts in the motility of Chlamydomonas can only be compared with its influence on suspensoïds.*

The representation which we have obtained of the conduct of *Chlamydomonas* under the influence of salts

with a different H-ion concentration is only acceptable, when the supposed plasmcolloïds behave towards salts as suspensoïd colloïds. The suspensoïd properties of the plasmcolloïds of *Chlamydomonas* come to light by the influence of small salt concentrations. In our experiments were necessary very small quantities of phosphate to make the organisms immovable. The highest concentrations were required for $Ca(NO_3)_2$ namely 0,40 n. With binair salts the concentrations asked for, were from 0,10 n. to 0,20 normal. From these data appears the influence of the valence of the ions.

The plurivalent anions of the phosfate required the lowest concentrations, the pluvalent cation of the calcium nitrate the highest concentration.

The influence of the valence of the ions also points to suspensoïd properties. For the case, that there was a greater agreement between the phenomena by *Chlamydomonas* and the salting out of emulsoïds, the anions and the cations of the salts, should exercise more or less influence according to a fixed order. The anions and the cations form, as regards their influence series. For the anions the series (the series of Hofmeister) is as follows:

SO_4, acetate, Cl, ClO_3, NO_3, Br, J, CNS.

In order to examine whether the influence of the salts on the motility of *Chlamydomonas* decreased or increased according to this series, experiments were done in KCl, KNO_3, KBr, KJ and KCNS. All these experiments were done with individuals of *Chlamydomonas* from one culture. The following order was stated:

J, NO_3, Cl, Br, CNS.

It is clear that this order is not at all like that in Hofmeister's series. Meanwhile this result does not prove. much. The series of the ions show a subverse order, when the milieu becomes of alcaline acid. In neutral solutions we get transition-series, in which the order is

changed. The experiments with *Chlamydomonas* were executed in a weak alcaline milieu, so the chance was not great to find back the series. However it was necessary to work in a weak alcaline milieu, since, otherwise the possibility had been great, that we compared the salts with a H-ion concentration, where for one salt a maximum and for another salt a minimum was to be found in the curve, which connects the critical concentrations. With the influence of salts on the motility of *Chlamydomonas* we have not been able to find an argument, which gives use to a comparison with the salting out of emulsoïds. All details, which we observed, make us think of phenomena, as they are observed with suspensoïds. Since, however, we have to deal with ampholytes, we are obliged to compare the plasmcolloïds with colloïdal substances, which, it is true, must be reckoned to the emulsoïds, by their positive or negative charge, according to their staying in acid or alcaline milieu, but, in connection with other properties they show, behave like suspensoïds. There is no great choise left. To the few colloïdal substances, which may be considered for a comparison, belong the globulins, to which we already drew the attention. The properties of the plasmcolloïds of *Chlamydomonas* show, with regard to acid, base and salt much likeness to the properties of the globulins.

§ 6. *Indications of a weak alkaline reaction, in the isoëlectric point of the plasmcolloïds.*

The H-ionconcentration, in which a colloïdal substance, is not combined with acid or base, is called the isoëlectric point. According to Michaëlis (1914, page 39) ampholytes are isoëlectric, when by a special concentration of the ampholyte, the sum of anions and cations of it has reached

a minimum, if we add acid, the substance behaves like a base, when we add base like an acid. In many cases it can be stated that the isoëlectric point is reached, when the colloïdal particles do not move with cataphoresis or are driven partly to the anode partly to the cathode.

The isoëlectric point can also be fixed indirectly. In the isoëlectric state, we often meet with flocculation. With the swelling of colloïdal substances the isoëlectric point often coïncides with the minimum of swelling. Of a colloïdal solution the inner friction would be a minimum in the isoëlectric point.

In many cases the H-ions and the OH-ions are chiefly of influence on the electric state of the colloïdal particles. Then they fix the place of the isoëlectric point. However, if the ampholyte has suspensoïd properties, the ions of the salts have a great influence on the situation of the isoëlectric point. It was impossible to fix immediately the isoëlectric point of the plasm-colloïds of *Chlamydomonas*. The great tension between the electrodes, which would have been necessary, to submit the whole organism to cataphoresis, would have caused the death of the algae. An attempt to find out bij means of the galvanotactical reaction in solutions of various H-ion concentration, something about the situation of the isoëlectric point, miscarried, since *Chlamydomonas* did not react, to weak electric currents. Indirectly we can fix the place in the curves, by which the plasmcolloïds are isoëlectric.

Is our opinion on the symmetry of the middle most part of the curves right, then the isoëlectric point must undoubtedly be found in the place, where the axis of symmetry cuts the curve. For K_2SO_4 (fig. 2, page 51), with 0,00040 n. KOH, for KCl (fig. 4, page 56) with 0,00045 n. KOH and for KNO_3 (fig. 5, page 57) with 0,00030 n. KOH.

Even if we do not agree with the representation, which

we have given of the course of the middle part of the curves, yet the possibility is great, that in the solutions of KNO_3 and of KCl the plasmcolloïds, respectively by 0,00010 n. KOH (fig. 5, page 57) and by 0,00025 n. KOH (fig. 4, page 56) are isoëlectric. We see namely, that the salts in these concentrations of potassium hydròxyd have the least influence. In both cases we find the isoëlectric point in a weak alkaline solution. The fact that the isoëlectric point is not found in the same place with the various salts, will be chiefly caused by the fact that the ions of the salts have an influence on the electric state of the plasmcolloïds. Moreover it is told in chapter III, that the results for the solutions of different salts may only be compared with reservation. Meanwhile I think that the figures point out that the isoëlectric state is to be found with a weak alkaline reaction.

§ 7. *Electric phenomena at the surface of the glass cause complications at the reactions of Chlamydomonas.*

There are some phenomena, which might help to find the isoëlectric point. Discharged or nearly discharged particles stick together, when they touch one another. They can also attach themselves to a firm object, that is not or but little charged. The cilia of *Chlamydomonas* consist of naked protoplasm. When the plasmcolloïds contain a certain charge, the cilia can also show that charge. In a solution, in which the plasmcolloïds are isoëlectric, we might expect, that the algae with the cilia stick to the side. Both phenomena act indeed, under special circumstances. We already discussed in chapter II the sticking of *Chlamydomonas* to the glass side. We called the phenomenon a thigmotactic reaction. With the different salts it did not always appear with the same

distinctness. With KCl a vague thigmotactic reaction was observed in some solutions. In the acetate solutions the phenomenon was not at all seen. For K_2SO_4 and KNO_3 it is denoted, in the figures 6, page 66 and 7, page 68, by means of the sign $\times$, by which concentrations the phenomenon could clearly be seen.

From the figures can be seen, that the solutions asked for were for the greater part weakly acid. At any rate the solutions are always more acid than the solutions in which we held the plasmcolloïds to be, isoëlectrical. This difference can however be explained. If we put water in a glass vessel, the glass gets a charge. Freundlich thinks, that the charge is caused by the fact that the glass absorbs ions. Electrolytes, which are dissolved in the water, have an influence on the charge of the glass. In alkaline, neutral and extremely weak acid solutions, that contain no salt the charge of the glass. as regards the water, is a negative one. In the other acid solutions the charge strongly decreases.

We have now probably the following case: the cilia are isoëlectric, so discharged in weak alkaline solution; the glass has a slight charge in acid solution. It is not strange that the phenomenon takes place in weakly acid solution.

For K_2SO_4 (fig. 6) where we also observe the phenomenon in solutions that contain little salt, we see that these solutions contain 0,00020—0,00045 n. H_2SO_4.

For a comparison we can mention the conduct of the catgumparticles from the suspensions of Perrin (conf.: les Atomes, page 139).

These suspensions behave as solutions of negative suspensoïds. They are discharged in acid solutions, stronger than 0,01 normal. Here the particles stick together. However they attach themselves to the glass already in less acid solutions. In these solutions the charge of the

glass has diminished very much already. So we find here under the influence of the electric phenomena at the surface of the glass, a removal of the fastening to less strong acid solution.

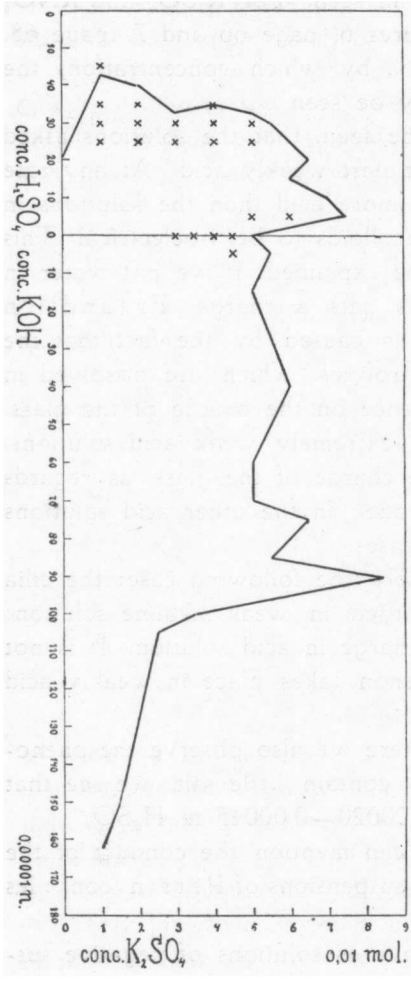

Fig. 6.

With the attaching of *Chlamydomonas* the removal was in inverse sense; there the cilia were discharged in weakly alkaline solution.

We may suppose that the thigmotactic reaction of *Chlamydomonas* with regard to the glass side takes place in all probability under the influence of electric phenomena at the surface of the glass in acid solutions.

The second phenomenon, which may be connected with the isoelectric state of the plasm-colloïds, namely the mutual sticking together of the individuals of *Chlamydomonas*, appeared in the experiments, because the algae at the reaction to gravity often formed, aggregates (conf. chapter II, § 6).

In which solutions the sticking together took place, was not noted down; however I think to have ob-

served, that in most alkaline solutions it did not occur.

The formation of agregates because the algae with their cilia stick together, and the fastening to the side are phenomena, which are undoubtedly connected with the electric state of the plasmcolloïds.

However these phenomena have not provided us with a means, to control the place of the isoëlectric point. Therefore, in the judging of other phenomena in connection

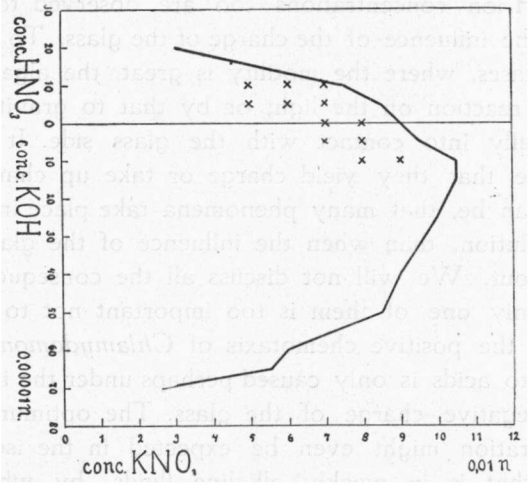

Fig. 7.

with the electric state of the plasmcolloids, we must be very careful. Of the other phenomena we have observed, two are of great importance, in connection with what precedes. Firstly we observed in chapter I, § 2, that there exists an acid-optimum for the chemotactic reaction of *Chamydomonas*. This must lie in the proximity of $[H] = 10^{-5}$. Moreover we observed (chapter II, § 5 and 6), that in solutions where sticking to the glass takes place positive phototaxis can appear, whilst in all other solutions, in which the motility is great enough, negative phototaxis is observed.

The H-ion concentration, which the algae seek by themselves (the optimum-H-ion concentration or the optimum acid concentration), and the H-ion concentration, in which the transition of positive to negative phototaxis takes place, lie, just like the concentration, where the fastening to glass is most clearly seen, in the most acid solutions. The possibility exists, that just like the H-ion concentration, where the fastening is very clear, the two other H-ion concentrations too are observed too large under the influence of the charge of the glass. To be sure, in all cases, where the motility is great, the algae either by the reaction on the light or by that to gravity come continually into contact with the glass side. It is very probable that they yield charge or take up charge; the result can be, that many phenomena take place in a more acid solution, than when the influence of the glass were linked out. We will not discuss all the consequences of this. Only one of them is too important not to be discussed; the positive chemotaxis of *Chlamydomonas* with regard to acids is only caused perhaps under the influence of a negative charge of the glass. The optimum-H-ion concentration might even be expected in the isoëlectric point, that is in weaklv alkaline fluids, by which the positive chemotaxis would change with regard to acids into a positive chemotaxis with regard to bases.

The charge of the glass might also have had an influence on the values which we found for the limiting concentrations. By this might be caused a removal of the curves to a greater H-ion concentration. However this removal cannot have been very great, since the motility of *Chlamydomonas* in the solutions with the limiting concentrations was not great, by which collisions with the glass side did not occur at all or only in a small number.

SUMMARY.

Chapter I.

§ 1. The way in which one works when studying chemotactical phenomena is compared to the proceeding for the examination of phototropical and geotropical processes. By this it is possible to call the supposition concerning the separated sensibilities for different chemotactic active substances, premature.

§ 2. With the help of data from literature is pointed out, that the capillary method is not a very suitable one to get a closer insight into the nature of the chemotactical phenomena.

§ 3. Here is discussed, that a colloïd-chemical representation can be formed of the influence of chemotactica.

Chapter II.

§ 1. It is denoted how the algae, which served for the experiments, were cultivated. By determination was proved that the Alga used was *Chlamydomonas variabilis*.

§ 2. Here is discussed, that the terms physical process, chemical process, physiological process and stimulation process are unfit to keep apart the various phenomena from physiology.

§ 3. For *Chlamydomonas variabilis Dangeard* was observed a very clear positive geotaxis while it appears from literature, that some investigators have found *Chlamydomonas pulvisculus* to be negativily geotactical.

§ 4. Here is discussed that the reaction to gravity takes no longer place after addition of small quantities of acid, base or salt to the water, in which the algae are.

§ 5. Here is told, that the susceptibility of *Chlamydomonas* to the light, just like that to gravity under the influence of added electrolytes is diminished. Besides a negative phototactical reaction a positive reaction can occur. The solutions in which the positive phototaxis acts, always contain so great an amount of an electrolyte, that a very slight increase of concentration causes insusceptibility to the light and to gravity.

§ 6. The attaching of *Chlamydomonas* to the glass side (thigmotaxis) occurs, as the ends of the cilia stick to the glass. The thigmotactical phenomena only take place in solutions, which are not distinctly alkaline. In solutions which only contain acid, at any rate little salt, the phenomenon often occurs, when the amount of acid is nearly as large that the motility is very slight and also insusceptibility to light and to gravity acts.

The individuals of *Chlamydomonas* can also mutually stick together with the cilia. This phenomenon can act so generally in a preparation, that it becomes also visible macroscopically.

§ 7. Here are discussed the substances on which *Chlamydomonas* reacts chemotactically. It is possibe to produce in the dark under cover-glass with *Chlamydomonas* an oxygen-line. This is not yet considered to be a proof, that *Chlamydomonas* really reacts to the oxygen of the air.

Here is discussed how with the experiment under cover-glass also the carbonic acid concentration must change. By means of *Spirillum species* it could be shown, that under the influence of carbonic acid as a gas a removal of the oxygen-line, formed by *Spirillum* was caused; with nitrogen, oxygen and air, this was not possible.

Chapter III.

§ 1. It can easily be observed, whether the organisms are very movable or only little movable. By defining limiting concentrations, it was possible to calculate the critical concentration, where theoretically the transition of very movable to little movable took place. The motility was judged by means of the reaction to gravity.

§ 2. It appeared to be necessary to let the experiments take place in little tubes with the same diameter. The influence of the temperature was of small importance. The experiments were done, exposed to one-sided diffuse day light; an attempt, to experiment in the dark, failed, since very irregular results were attained by it.

§ 3. Here is discussed that the algae freed from the culture-fluid were brought into the electrolyte solutions. They were washed with distilled water for a short time. The susceptibility to electrolyte solutions was not changed by this. For every experiment was used about the same number of organisms.

§ 4. In connection with the great influence of the H-ions and the OH-ions, it was desirable to have the solutions change regularly not only with regard to the salt concentration but also with regard to the amount of H-ions and of the OH-ions. The solutions were used in series. In every series the H-ion concentration was constant. The different series varied, as regards the hydrion concentration. By means of the series limiting concentrations were fixed and from these critical concentrations were calculated. The electrolytes are for the greater part split up into ions; it is a matter of course to attribute the influence of the electrolytes to the ions. In neutral salts the anions and the cations are always present in the same proportion. In salt solutions we always observe the influence of equal quantities of anions and cations collectively.

The product of the number of H-ions and OH-ions is constant. By this it is possible to examine separately the influence of the H-ions and of the OH-ions. In consequence of this we can find in a series with constant salt concentration, but with regularly increasing H-ion concentration, two critical concentrations, while in a series with a constant H-ion concentration one critical concentration is found.

§ 5. The critical concentrations and the limiting concentrations are denoted in a rectangular coördinate system; on the ordinate-axis is stated the acid or base concentration and on the abscissa-axis the salt concentration.

§ 6. Salt solutions with regularly changing H-ion concentrations were obtained bij adding small quantities of acid or base. By this we must observe special measures of precaution.

With the acetate solutions, the fluids were made acid with acetic acid. In this case we obtain „buffer solutions", which offers special advantages.

Chapter IV.

§ 1. Here is discussed, that the H-ions and the OH-ions have a great influence on the motility of *Chlamydomonas*. To show this, we used mixtures of sodium acetate with acetic acid and with potassium hydroxyd, in which the amount of acetate was constant, whilst the H-ion concentration changed regularly.

§ 2. The experiments under influence of acid and alkaline solutions of K_2SO_4, KCl and KNO_3 were done sixfold, bij which the results were much more reliable. It is denoted how the critical concentration is calculated. By the use of a new culture the immediately preceding experiments were repeated to see whether the organisms of the new culture were as sensible to electrolytes as those of the old

culture. Generally the differences were insignificant. For KCl was once observed an important change of the sensibility. Here is discussed that the changed sensibility might be caused under the influence of a culture-fluid of a somewhat different composition.

§ 3. There is discussed that the influence of base and salt according to the course of the curve for K_2SO_4 in most alkaline solutions is about additive, while the influence of mixtures of acid and salt cannot be additive.

Acid and salt counteract one another's influence. This also appears from the figure of sodium acetate in the most acid solutions.

A similar conduct with regard to mixtures of salt and base and of salt and acid, was stated by Hardy with the dissolving of colloïdal globulins, which were flocculated by small quantities of electrolytes.

§ 4. Here is discussed that the middle part of the curves often has a capricious out look, by the presence of maxima and minima. In general five maxima can be distinguished. Through this part of the curves an axis of symmetry can be drawn. This axis is often not horizontal. The possibility exists that the symmetry is caused, as we have to do with the action of ions.

The principal direction of the middle part of the curves is influenced perhaps by the valence of the ions.

The presence of the tops cannot immediately be explained.

§ 5. Here is shown, that the plasmcolloïds can only be compared to colloïds, which can behave as acid and base but further contain properties of the suspenoïds. For a comparison the globulins ask for our attention.

§ 6. By the figures is proved that the isoëlectric point (according to Michaëlis) of the plasmcolloïds can be expected in weakly alkaline solutions. The place of this point could not be fixed directly.

§ 7. Here is discussed, that there are yet two other phenomena, which might give information about the place of the isoëlectric point, namely the sticking to the glass and the mutual sticking together of the algae with their cilia. The first phenomenon, however, took place in solutions, which were more acid than those, in which the plasmcolloïds, according to the course of the curves are isoëlectric. This is attributed to the negative charge of the glass with regard to alkaline, neutral and very weakly acid solutions.

In which solutions the mutual sticking together took place, was not noted down. Besides the fact that in the sticking to the glass the influence of the charge of the boundary surface glass water plays a part, a removal of the phenomena to more acid solutions can be expected in all cases in which the motility of the algae is very great. Under the influence of the light or of gravity the algae come continually with the cilia into contact with the glass, by which loss or taking up of electrical charge can take place.

We point to the fact that the acid optimum of *Chlamydomonas* with chemotactical experiments is perhaps an acid optimum only under the influence of the glass.

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