# STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. I. REACTIONS TO CHEMICAL, OSMOTIC AND MECHANICAL STIMULI IN THE CILIATE INFUSORIA.

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(*From the Physiological Laboratory of the University of Jena.*)

CONTENTS.



## 1. Introduction.

The work, an account of which is given in the following pages, consists of a study, as exact as possible, of the life activities of a single unicellular organism. So far as I know, we do not possess an even approximately complete account of such activities as may be referred to reactions to stimuli for any single cell or any single organism. We possess indeed many excellent researches on various forms of reaction in various organisms. But we are not yet able to form a mental picture of the continual interplay of such activities in the

ordinary life of any cell; the interaction of the various stimuli; the modifications in reaction due to the simultaneous presence of two or more stimuli; or the changes in reaction due to varying life conditions or physiological states of the organism. Certainly a beginning has been made in unravelling this complicated network of activities, but the studies which have been made are scattered over a wide field;—they are extensive rather than intensive.

Perhaps it may be said that we possess of no unicellular organism so full an account of the life activities as of the common infusorian *Paramœcium aurelia.* The ease with which it may be obtained in great abundance and preserved in healthy condition for indefinite periods has made it a favourite subject for investigation of the life phenomena of free cells. The general anatomy and functions of the parts of this organism are treated fully in the text-books and manuals. The nuclear phenomena in conjugation and division have been made the subject of elaborate studies by distinguished investigators. Turning to more purely physiological matters, the digestive processes of Paramœcium have been studied by Greenwood and Saunders and others; the effects of a lack of oxygen, and of an atmosphere of carbon dioxide by Loeb and Hardesty; the effect of various chemical reagents by Schürmayer, Bokorny and others. The reaction to the electric current has been described by Verworn and by Ludloff: geotaxis by Jensen; thermotaxis by Mendelssohn. Chemotactic and tonotactic movements have been the subject of scattering notices by various authors[1]. The prospect for gaining a fairly full general view of the life activities of this organism is perhaps as favourable as for any that can be named: it was therefore selected for further study.

Of the above-mentioned reactions, not all have been studied in any sense fully. Electrotaxis, geotaxis and thermotaxis may be considered as having been placed on a solid basis by the systematic investigations of Verworn and Ludloff; Jensen, and Mendelssohn. The other reactions mentioned—chemotaxis and tonotaxis—are known only from scattered and partly incorrect notices in the works of various authors. It is the purpose of the present paper to present a systematic account of these and certain other reactions.

In discussing motions which occur as responses to stimuli, two sets of terms are used by different authors, one set having the termination *-taxis* or *-tactic,* the other ending in *-tropism* or *-tropic.* I shall use throughout for the reaction movements of the free infusorian Para-

[1] For references see end of Paper.

mœcium the terminations *-tactic* and *-taxis*, speaking thus of chemotaxis, electrotaxis and the like, instead of chemotropism, electrotropism, etc., often used in the same sense. *Positive* taxis signifies a tendency to move *toward* a source of stimulus; *negative* taxis a tendency to move away from such a source.

The reactions to be described are somewhat complicated by the interplay of different stimuli, so that it is difficult to give an account of the phenomena due to any one stimulus without at the same time treating of activities due to different causes. The difficulty in presentation thus caused will perhaps be best avoided by the following procedure. I will first give an account of the phenomena observed under certain simple conditions. The complex of phenomena presented in this "introductory experiment" will then be analysed into its simple components,—that is, into activities due to simple stimuli, and each of these simple reactions will then be treated at length, with details of experiments and other proof of the correctness of the analysis. The Paramœcia used in the experiments were procured in the customary manner. A handful of hay or grass is placed in a jar and covered with hydrant water. In a few weeks the solution of decaying vegetable matter swarms with Paramœcia. In such a jar they may be kept for indefinite periods in almost unlimited numbers.

### *Introductory Experiment.*

The phenomena which we wish to study will be best brought before us by the following simple procedure. A large number of Paramœcia are removed from the culture jar by means of a pipette, and placed, together with a small bit of decaying vegetable material or a mass of bacterial zooglea from the same jar, on a glass slide. The drop of water containing Paramœcia is then covered with a cover-glass resting upon glass rollers or other convenient supports. The preparation thus made is shown in Fig. 1.

At first the Paramœcia are distributed uniformly throughout the preparation, as shown in Fig. 1. In a few minutes however we observe a noticeable gathering of the infusoria about the bit of decaying material. This increases in number and density: the Paramœcia press close against the decaying material, and soon all the infusoria in the preparation (with individual exceptions) are gathered in a dense mass about it. The preparation now presents the appearance shown in Fig. 2. As many as possible of the animals press their anterior ends

against the decaying substance: the rest crowd in behind, trying to get as close as possible.

After some minutes we notice that the dense mass of Paramœcia about the decaying material is becoming looser. The Paramœcia begin to separate; they no longer remain closely pressed against the bit of material, but swim hither and thither. However, they do not swim away freely, but remain confined to a small round area in the immediate region of the decaying mass. Very slowly the area becomes larger, but remains clearly defined, with a distinct boundary which the Paramœcia do not pass. Most of the infusoria are gathered at the boundary of the area; some few swim back and forth within it, while

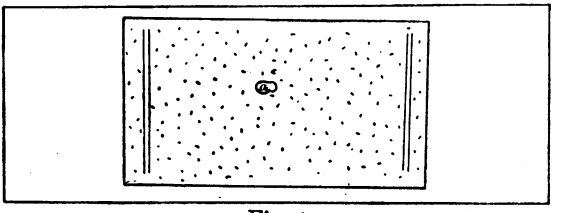



Fig. 1

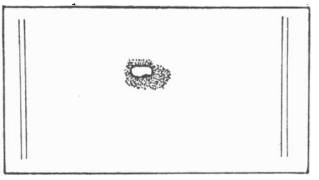


Fig. 2

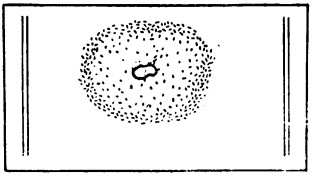


Fig. 3

Fig. 1. Paramœcia which have just been covered with a cover-glass. *a* Bit of decaying vegetable matter.

Fig. 2. Same preparation shown in Fig. 1, ten minutes later.

Fig. 3. The same preparation shown in Figs. 1 and 2, a half hour after the condition shown in Fig. 2.

scarcely a single specimen will be found outside the area. A swimming individual starting to pass the outer limit stops suddenly, as if prevented by an invisible barrier, and remains or turns back. The limits grow on all sides slowly and steadily, the area thus retaining its regular form. A stage in this process is represented in Fig. 3. The area continues to increase in size, and after some time it reaches the edges of the cover-glass. The Paramœcia then form a narrow band about the periphery of the preparation.

If the above preparation has been made in such a way that a constant electric current can be passed, by the use of unpolarisable

electrodes, through the water containing the Paramœcia, certain very peculiar phenomena may be observed in the reactions of the infusoria to the current. If the electric circuit is closed at the beginning of the experiment, when the Paramœcia are distributed throughout the preparation (Fig. 1), we obtain the typical reaction described by Verworn (1889 (*a*)). The Paramœcia all swim with one accord to the cathode side of the preparation. If the current is reversed the Paramœcia swim to the opposite side. Opening the circuit, the Paramœcia distribute themselves again uniformly throughout the preparation.

If the electric circuit is again closed after the Paramœcia have gathered close about the bit of decaying vegetable material (Fig. 2), no visible effect is produced. The Paramœcia remain gathered about the decaying mass and show no response to the current. My attention was called to this striking phenomenon by Professor Verworn, and it formed the starting point for my investigations.

The circuit may now be opened and the normal development of the preparation observed farther. If the circuit is again closed at the stage shown in Fig. 3, we obtain a still different result. The Paramœcia start in the direction of the cathode, but at that boundary of the circular area which lies next to the cathode they stop. If the current is continued all the Paramœcia gather in a mass at the cathode side of the area, seeming to make vain efforts to cross the invisible boundary. The area has now entirely disappeared, since the Paramœcia are all collected at one spot on its cathode side. If now we reverse the current, the Paramœcia start in the opposite direction, but are again stopped by the invisible barrier, which lies at exactly the spot which previously formed the limit of the area on this side. Opening the circuit, the Paramœcia again distribute themselves in the area, chiefly about its margin, presenting anew exactly the appearance shown in Fig. 3.

What now is the meaning of this peculiar drama? Why do the Paramœcia gather about the bit of decaying vegetable material, placing themselves in contact with its surface? Why do they not respond to the electric current under these conditions? Why do they soon begin to leave the mass, which they first crowded upon with so much ardour? Why do they then remain in a clearly defined growing area, gathered at its boundaries, but not overpassing its invisible limits even when urged by the electric current?

We will consider first the question, Why do the Paramœcia gather about the bit of decaying vegetable material? The conjecture which lies nearest is, that we have here a case of

## 2. Chemotaxis.

Before entering upon a series of experiments to determine the truth of the above conjecture, let us review briefly what is known of chemotactic phenomena in Paramœcium. Verworn (1889, p. 108) and Loeb und Hardesty state that Paramœcium is positively chemotactic toward oxygen. Pfeffer, Verworn, and Massart (1889, p. 558) state that they do not find the Infusoria Ciliata to be positively chemotactic toward other chemical agents. Loeb und Hardesty state that Paramœcia are *negatively* chemotactic toward carbon dioxide, and Loeb und Budget that they are negative toward acids and alkalies. Massart (1889, p. 558) on the other hand says that in accordance with his own researches and those of Pfeffer, the Ciliata are "absolument insensible" to chemical substances. He explains their tendency to move away from solutions of chemical substances as due to *tonotaxis*,— that is, it is the result of differences in osmotic pressure, and has nothing to do with the chemical quality of the solutions. We might infer from the above summary that the only facts regarding chemotaxis that can be stated with any degree of certainty are (1) that Paramœcium is positively chemotactic toward oxygen; (2) that it is probably negatively chemotactic toward $CO_2$.

It now remains to test the matter by experiment. For this purpose results were obtained by two methods. The first is Pfeffer's method of the introduction of capillary tubes containing the substance in question beneath the cover-glass of a preparation, or into a vessel, containing Paramœcia. If the animals are positively chemotactic to the substance in the tube, they gather into its open end. Many experiments were tried in this way, but a second method was found to give results incomparably quicker, more definite, and more reliable. The Paramœcia are placed on a glass slide and covered by a supported cover-glass as described in the "Introductory Experiment." A pipette is drawn out at the end into a capillary tube of sufficient fineness to pass between the slide and cover into the water containing the Paramœcia. The latter swim at first hither and thither in every direction, so that no portion of the preparation is left free. Now a drop of the fluid to be tested is taken up with the capillary pipette and introduced beneath the cover-glass into the preparation (Fig. 4). The drop fills the space between slide and cover, and spreads out into a round disk, so that it is in contact only by the very thin edge of the

disk with the surrounding water; it therefore diffuses only very slowly, as may be seen by introducing a drop of some coloured fluid. The

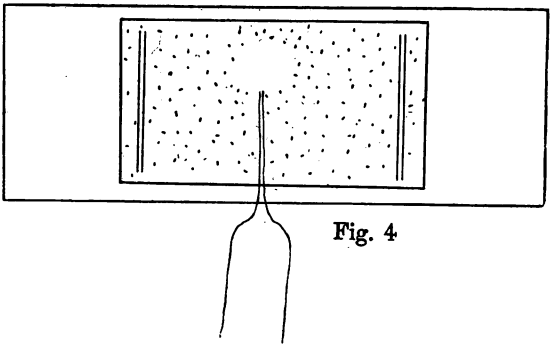


Fig. 4

Method of testing the behaviour of Paramœcium toward chemical substances.

Paramœcia in their random hither and thither swimming of course begin at once to come in contact with the edge of the drop, whereupon their conduct toward the substance of which the drop is composed may be observed. When I speak later of a drop of fluid being introduced beneath the cover-glass it will be understood that it was done in the way just described.

The first question to be tested is, Are the Paramœcia positively chemotactic toward the decaying vegetable material of the culture jar? Since Paramœcia taken from the culture jar are already bathed on all sides by fluid from this decaying vegetable material, they cannot be used at once for a decisive test of the question; before a test can be made they must be brought into water containing none of this fluid. To accomplish this, I have used a method similar to one described by Ludloff (p. 529) as a convenient way of bringing many Paramœcia into a small amount of water. A glass tube $1\frac{1}{2}$ cm. in diameter and 80 cm. in length, closed at one end, was filled about half full of fluid from the culture jar, containing large numbers of Paramœcia. The remaining half of the tube was filled with hydrant water, and the tube allowed to stand in an upright position. The Paramœcia, being negatively geotactic, rise to the upper end of the tube into the hydrant water. They are then poured into another vessel, the tube emptied and thoroughly cleaned, and the Paramœcia restored to the lower half of the tube. Hydrant water is again added in the upper half and the Paramœcia rise into it as before. This may be repeated as often as thought necessary, till the Paramœcia are washed entirely free from the fluid of the culture jar.

A quantity of this pure hydrant water containing Paramœcia is then placed on the slide and covered in the ordinary way. The Paramœcia scatter uniformly throughout the preparation. Some fluid is now drained from the decaying vegetable material of the culture jar and filtered. A drop of this filtered fluid is then introduced beneath the centre of the cover-glass. The Paramœcia begin at once to gather in the drop. In a few minutes there is a marked collection of individuals in this region, and after a short time all the Paramœcia in the preparation, with individual exceptions, are gathered in the small circular area which marks the spot where the drop was introduced (Fig. 5), the rest of the slide being quite empty.

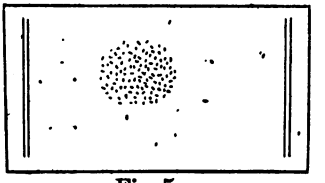


Fig. 5

Similar results were gained by introducing capillary tubes containing filtered fluid drained from the decaying vegetable material of the jar into vessels of hydrant water containing many Paramœcia. A typical experiment of this sort is as follows. Paramœcia in hydrant water are placed in a shallow watch-glass. Into this glass are then introduced pieces of capillary tubing, some containing filtered fluid from the culture jar, the others hydrant water. These are arranged in pairs, each pair consisting of two tubes of the same length and diameter, lying side by side, one containing the fluid from the jar, the other hydrant water. In about three-quarters of an hour the ends of the tubes containing fluid from the culture jar are crowded with Paramœcia, while the others are empty.

Paramœcium aurelia in hydrant water thus tends to gather into areas or tubes containing water from a culture jar of decaying vegetable material. Apparently the infusoria are positively chemotactic toward such material. In order to avoid misconception, I may state at once that this conclusion is shown by the remainder of the investigation to be false, and the results gained by the above experiments are to be explained in an entirely different manner, as will be set forth in due time.

Experiments were now tried with many other substances, using both the above methods. The latter method—introduction of capillary tubes into a vessel containing many Paramœcia—is not at all reliable, so that I gradually ceased to use this, and my results depend almost entirely upon experiments with the other method. Certain reasons why the capillary tube method is unreliable will be given later.

With Paramœcia in hydrant water a filtered extract of meat was tested. This was prepared by boiling a bit of lean beef in water and filtering off the fluid. The Paramœcia gather into a drop of this exactly as into a drop of the fluid from the culture jar. Meat extract prepared by soaking lean meat in cold water acts in the same way, as does also Liebig's meat extract.

The infusoria were also positive toward *rancid oils*, such as rancid olive-oil and rancid cod-liver oil. Paramœcia gather quickly into a drop of water which has been well shaken up with such oils and introduced beneath the cover-glass of the Paramœcium preparation.

With many other substances no such gathering occurred. Cane sugar, salt, a pepsin solution, putrid meat juice, putrefying gelatine, saliva, egg albumen, all gave negative results: the Paramœcia were either indifferent or repelled by these substances.

The same results were gained with all the above substances if the Paramœcia tested were left in the water from the culture jar instead of being brought into hydrant water.

The positive results gained with rancid oils suggested that the active attractive principle was some member of the fatty acid series. I determined therefore to test various members of this series. During the short delay necessary for getting samples of the acids desired, I directed my attention to certain other phenomena, the study of which placed the whole problem in a new light. As previously stated, Massart (1889, p. 558) maintains that the apparently negative chemotaxis of the ciliate infusoria is not really chemotaxis at all, but is due merely to differences in osmotic pressure between the fluid the animals are already in and that of the fluid introduced; the motions due to this stimulus he calls tonotaxis. Desiring to test this matter for Paramœcium, in the case of substances apparently negatively chemotactic, I undertook first to determine the weakest concentration to which the Paramœcia are negative, in the case of some strong chemical. For this purpose *copper sulphate* was selected: the saturated solution was diluted until experiment proved that it was not quickly fatal to Paramœcia introduced directly into it, and tests were made with this. The strength thus used was $\frac{1}{30}$ % of the saturated solution; a drop of this was introduced beneath the cover-glass of a Paramœcium preparation (cp. Fig. 4).

The Paramœcia were, contrary to all expectation, distinctly positively tactic to the copper sulphate. They formed a dense collection about the margin of the drop, but did not enter into its centre, being

apparently negatively tactic to the stronger solution within. The infusoria seemed not to be injured at all by the fluid forming the margin of the drop, but were much excited, swimming hither and thither with great rapidity, but not leaving the drop of copper sulphate.

In view of this surprising result with copper sulphate a number of other strong chemicals were tested, some with positive, others with negative results. Without going at this point into details, I will give here a table showing in general the results gained with the chemicals tested. Positive taxis is indicated by the sign (+), negative taxis by the sign (−). The Paramœcia were tested in the fluid from the culture jar. Solutions of the chemicals made up both with distilled water and with water from the culture jar were tried: the same results were gained by both methods.

| Substance | Weak sol. | Strong sol. | Substance | Weak sol. | Strong sol. |
|---|---|---|---|---|---|
| Copper sulphate | + | − | Sodium chloride | − | − |
| Sulphuric acid | + | − | Sodium carbonate | − | − |
| Hydrochloric acid | + | − | Sodium bicarbonate | − | − |
| Acetic acid | + | − | Potassium hydroxide | − | − |
| Nitric acid | + | − | Sodium hydroxide | − | − |
| Tannic acid | + | − | Potassium bromate | − | − |
| Mercuric chloride | + | − | | | |

From the table it appears (1) that all acids tested were positive (that is, attractive) in a weak solution; negative in a strong solution: (2) that all alkalies tested were negative in whatever strength used: (3) that some salts act positively, others negatively. Those salts which are attractive in a weak solution give an acid reaction ($CuSO_4$ and $HgCl_2$); salts giving an alkaline reaction are negative, as are also certain neutral salts.

I now undertook to determine the *weakest* solution of certain of these chemicals, to which the Paramœcia show sensibility. The first substance thus tested was sulphuric acid. The concentrated solution was mixed with distilled water and the solutions thus gained were used in testing the Paramœcia, the latter being in water from the culture jar.

The Paramœcia seemed to show a perfectly astounding sensitiveness to the dilute $H_2SO_4$. Solutions of $\frac{1}{1000}$ %, $\frac{1}{2000}$ %, $\frac{1}{4000}$ %, $\frac{1}{8000}$ %, $\frac{1}{16000}$ %, were successively used; in every case the Paramœcia showed strong positive taxis, gathering quickly into the introduced drop. Finally, as a control, a drop of pure distilled water was introduced in

the ordinary way. The Paramœcia showed strong positive taxis to the distilled water. They gathered into the drop, forming a dense collection, exactly as shown in Fig. 5.

This result set the entire problem in a new light, and compelled a re-interpretation of all previous observations. To say that the Paramœcia are positively chemotactic toward distilled water is meaningless; we must reverse the statement and say that Paramœcia are negatively chemotactic toward the fluid of the culture jar in which they live.

We have seen above that the infusoria are negative to all alkalies and positive to acids. The fluid of the culture jar in which the Paramœcia live shows with litmus paper a distinctly alkaline reaction. Thus however remarkable it may seem that Paramœcium should be negatively chemotactic to the fluid in which it lives and thrives, the reaction stands in entire agreement with the behaviour of the animal toward other reagents.

The conclusion is further reinforced by the exact method in which the Paramœcia react to the various substances tested. Certain features are common to all the experiments. Suppose a slide and cover preparation has been made, in which the Paramœcia are distributed uniformly, and a drop of some substance is introduced as in Fig. 4. The behaviour of the Paramœcia at even a slight distance only from the drop is not thereby influenced in the least: the drop has no effect at an appreciable distance from its evident margin. But all the Paramœcia continue to swim hither and thither at random, and in a remarkably short time almost every individual under the cover-glass will have come in contact with some part of the drop. There is however no swimming in straight radial lines to the drop as a centre. It is only as the Paramœcia come by chance in contact with the drop that their conduct toward the substance of which it is composed can be observed.

With this preliminary general statement, we may now study the conduct of the Paramœcia on coming in contact with (1) a drop of distilled water, (2) a drop of weak acid, (3) a drop of alkali. The Paramœcia in every case are in the natural water from the culture jar, having an alkaline reaction.

Suppose therefore that a drop of distilled water is introduced. A Paramœcium in its random swimming comes against the margin of the drop. Just what happens here varies with individuals; some make a sudden pause, often jerking back a fraction of their own length, then

passing into the drop; others show not the slightest hesitation of any sort at the boundary. In any case the animal continues its course with undiminished rapidity until it comes to the opposite boundary of the drop, where it would naturally pass out into the culture fluid again. Here it leaps back perceptibly,—the effective stroke of the cilia being reversed in direction,—turns, and swims in another direction. Again meeting the boundary it draws back, turns again, and swims till it again strikes the boundary. It thus continues to swim about in the drop as if caught in a trap, the changes of motion indicating always negative chemotaxis to the surrounding fluid. Fig. 6 represents the course of a single Paramœcium entering a drop of distilled water and remaining as above described. In a short time the drop thus becomes crowded with individuals, and the preparation shows exactly the appearance represented in Fig. 5.

If in place of distilled water we introduce a drop of $\frac{1}{100}$ % $H_2SO_4$ the conduct of the Paramœcia is somewhat different. On swimming by chance against the edge of the drop the animal suddenly reverses its cilia and swims a long distance straight backward, often ten times its own length, or much more. It then either starts off in another

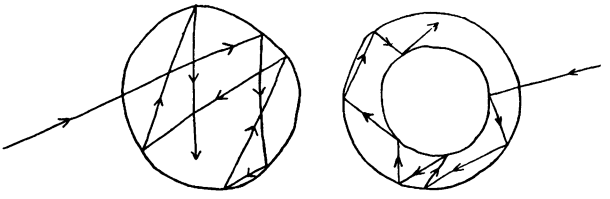


Fig. 6 Fig. 7

Fig. 6. Course of a Paramœcium in a drop of distilled water.
Fig. 7. Course of a Paramœcium about a drop of $\frac{1}{100}$ % sulphuric acid.

direction, in which case of course it leaves the drop entirely, or it swims straight forward again to the margin of the drop. Here the reversal of the cilia occurs again, but the animal generally does not swim so far backward as before. This may be repeated a number of times, until finally the Paramœcium does not reverse its cilia and swim backward on striking the drop, but merely turns away and swims in another direction. Here however it comes to another invisible boundary at a slight distance outside of the evident margin of the drop of acid, from which it turns again toward the interior,—turns from this as at first only to meet the outer boundary again, and thus it continues to swim in a ring about the drop of acid, repelled by both the inner and

outer boundary of the ring. The course of a Paramœcium in such a case is shown in Fig. 7. As the surrounding medium is alkaline while the drop itself is acid, it appears that the ring to which the Paramœcia are confined must be nearly or quite neutral in reaction. The outer boundary indicates negative chemotaxis toward alkaline fluid: the inner boundary indicates negative chemotaxis toward (strong) acid. Such a ring becomes in a few minutes densely crowded with Paramœcia; Fig. 8 shows a preparation into which was introduced five minutes before a drop of $\frac{1}{100}$ % $H_2SO_4$. If a very weak solution of $H_2SO_4$ is used —about $\frac{1}{800}$ %—the Paramœcia act toward it exactly as toward distilled water. If a very strong solution of acid is used, the results above described are modified in one respect. Many of the Paramœcia, swimming too violently against the inner boundary of the ring are there killed by the strong acid before they can swim away, so that the inner boundary is indicated by a zone of dead Paramœcia.

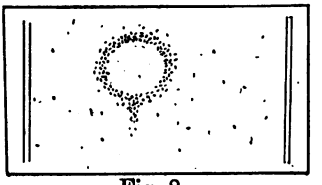


Fig. 8

Paramœcia gathered in a close ring about a drop of $\frac{1}{100}$ % $H_2SO_4$.

Essentially similar results are gained by the use of proper degrees of concentration of any of the other acids mentioned, or with the acid salts, copper sulphate or mercuric chloride.

If after the Paramœcia have gathered in the drop of weak acid or of distilled water the constant electric current is passed through the preparation, the same results are gained as described on p. 262, for the group formed in the "introductory experiment." The Paramœcia swim to the cathode side *of the drop*, but no further; nor can they be forced over the boundaries of the drop without a very strong and long-continued current. If the substance is a drop of stronger acid, so that the Paramœcia are confined to a ring around the outside of it, being negative to the centre (Fig. 8), when the electric circuit is closed the Paramœcia do not swim across the drop, but follow around its circumference, from one side to the other.

Finally, if we introduce into a preparation a drop of some alkali —for example, a $\frac{1}{200}$ % solution of KOH, in place of the distilled water or the acid, an entirely different result is gained. The Paramœcia on coming in contact with the margin of the drop, reverse their cilia, though much less violently than in the case of a strong acid, and jerk back, perhaps a fraction of their own length. At the same time they turn and swim in another direction. The drop thus remains entirely

empty: there is no collection either within it or at its margins. The Paramœcia are not injured by it as they are by an equally powerful acid, because they form no gathering about its margin and therefore do not venture too deeply into it for safety.

The same results are gained by the use of other alkalies or salts having an alkaline reaction. Fig. 9 shows the effect of the introduction, 10 minutes before, of a $\frac{1}{120}$ % solution of $Na_2CO_3$.

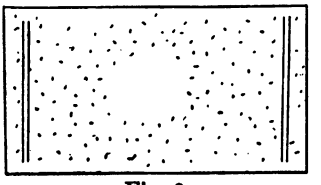


Fig. 9

Preparation into which was introduced 10 minutes before, a drop of $\frac{1}{120}$ % $Na_2CO_3$.

In hydrant water the results are the same and possibly even more pronounced than in the water from the culture jar, as the Paramœcia are even more strongly negative to the hydrant water. This is demonstrated by testing Paramœcia in culture water with hydrant water; they are negatively chemotactic to it. The converse experiment—the introduction of a drop of water from the culture-jar into a preparation of Paramœcia in hydrant water—gives the opposite results; the Paramœcia in hydrant water are positively chemotactic to the fluid from the culture jar. The hydrant water in Jena is strongly impregnated with calcium carbonate, and gives a distinctly alkaline reaction with litmus paper, so that negative chemotaxis toward it is what might be expected.

I must mention here a peculiar variation in the results gained, which at first caused confusion in my own observations, and would doubtless do the same in the case of anyone repeating these experiments. The Paramœcia in the culture jars are not distributed uniformly in the water, but are gathered in dense collections,—on the decaying vegetable matter just below the surface of the water, as well as forming a broad white line on the walls of the vessel a few millimeters below the water surface. If for making the tests Paramœcia are taken by means of a pipette directly from one of these collections, and brought in the same pipette under the cover-glass, results entirely different from those above detailed will be gained on the introduction of a drop of the various chemical reagents. The Paramœcia show themselves under these circumstances distinctly and strongly *negative* to every substance introduced, including distilled water and acids. If however the Paramœcia on being removed from the culture jar, are brought first into a watch-glass, and are there thoroughly stirred up and brought into contact with the air, by the use of the pipette, and the tests are then made with slide and cover preparations of these Paramœcia, the results gained

are those above described. The negative results gained when the Paramœcia are not thus stirred up are due to the presence in the water of a volatile substance to which they are much more strongly positive than to any of the solutions introduced. This is shown by their beginning at once to gather into groups in different parts of the preparation, avoiding throughout the introduced drop. To determine what this volatile attractive substance is, is one of the chief objects of this portion of the paper.

We may summarize our results up to this point as follows: The Paramœcia live in a fluid having an alkaline reaction. They are negatively chemotactic to this fluid and to all other alkaline fluids. As a result of this negative chemotaxis, they gather into a drop of distilled water introduced into the fluid in which they live. The Paramœcia are also negative to strong acids, but gather quickly into a weakly acid fluid introduced into the alkaline water in which they live. Whether this is because the Paramœcia are really positively chemotactic to the acids or only gather in a region where the acid neutralizes the alkali of the surrounding fluid is not yet determined.

The next question for determination is therefore whether the Paramœcia are really positively chemotactic toward acids, or whether all the phenomena observed can be explained by the negative chemotaxis toward alkalies. In order to test this question the Paramœcia must first be brought into an entirely neutral medium,—that is, into distilled water.

The infusoria are easily brought into distilled water by the use of the glass tube, taking advantage of the negative geotaxis of the animals, as described on p. 264; distilled water is simply substituted for hydrant water in the procedure there described. The Paramœcia are not appreciably harmed by the transfer and live in the distilled water for weeks, seemingly as lively as ever. In the first one or two days after the transfer is made the animals are excessively sensitive to all chemicals, showing marked negative chemotaxis to almost all substances tested, and positive chemotaxis to almost none. But after three or four days or a week in distilled water the conduct becomes quite normal, except that they are much more sensitive to chemical agents than under normal circumstances.

Slide and cover preparations were made as before, with the Paramœcia in distilled water, and the same substances were re-tested under these circumstances. This is a piece of work requiring great pains and much time. The sensitiveness of the Paramœcia to chemical reagents

is so increased that solutions of acids which formerly acted positively now have a distinct negative effect. In order to be certain whether positive chemotaxis toward a given substance occurs at all or not, many experiments are necessary to discover the precise degree of concentration which acts positively. This is further complicated by a peculiar fact. After the Paramœcia have remained some days in distilled water, they become positively tactic to distilled water in which Paramœcia have *not* lived. The explanation of this fact is probably that the distilled water containing many Paramœcia becomes slightly contaminated by matter excreted by the animals. I have tested such water carefully with litmus paper, but was unable to discover any evidence of its having gained an alkaline reaction. However, the fact renders it inadmissible to mix the reagents with ordinary distilled water, since of course if the concentration of the substance to be tested is lowered to such an extent that it becomes entirely ineffective, the *positive* reaction due to the simple distilled water is gained, leading to false conclusions.

It was therefore necessary to mix the reagents with the *same* water in which the Paramœcia to be tested are swimming. The only certain method is to have the Paramœcia, in distilled water, in a vessel of some size; then to remove from this same vessel with a pipette water (containing Paramœcia, of course) for mixing with the reagents. It is even necessary to keep the vessel thoroughly stirred up, as otherwise the Paramœcia in one part of it may become positively or negatively tactic toward water from a different part of the same dish,—a peculiar fact of which the explanation will be given later.

However, all these precautions being observed, the following results are gained. Toward all the substances given in the list on page 283 to which the Paramœcia in the water from the culture jar were negatively chemotactic, the Paramœcia in distilled water are likewise negatively chemotactic. In the latter case the negative chemotaxis is more powerful, inasmuch as solutions which are so diluted as to be entirely ineffective in the former case, are strongly negative in effect if the Paramœcia are in distilled water.

Toward substances with regard to which the Paramœcia in the water from the culture jar are positive, the Paramœcia in distilled water are likewise positive. But in the latter case the positive chemotaxis is much *weaker* than with Paramœcia in water from the culture jar. The positive chemotaxis occurs, when the Paramœcia are in distilled water, only with very weak solutions, and all stronger solutions of the same

substance act negatively. Moreover, the collections of Paramœcia formed are scarcely so dense and often do not last so long as in the previous experiments.

The greatly increased sensitiveness in distilled water, indicated by the changed chemotaxis, is shown also in another way. Chemical solutions which had almost no injurious effect on Paramœcia under natural conditions, are quickly fatal to Paramœcia in distilled water.

We may conclude from the foregoing series of experiments that Paramœcium is, (1) positively chemotactic toward substances having a weak acid reaction; (2) negatively chemotactic toward alkalies and some salts. The results given in the beginning (page 266) for certain complex substances readily take their place under this view. Rancid oils have of course an acid reaction, and the same is true of extract of meat; hence the positive chemotaxis toward these substances. The apparently positive chemotaxis of Paramœcia in hydrant water to fluid from the decaying vegetable material in the culture jar is seen to be only an expression of the fact that they are less strongly negative to the water of the culture jar than to the hydrant water. Paramœcia in distilled water are strongly negative both to water from the culture jar and to hydrant water, these both having an alkaline reaction.

*Relation of Chemotaxis to Tonotaxis, and the actual rôle played by Tonotaxis.*

There remains undecided, however, the relation of the phenomena described to Massart's tonotaxis. Probably no one would invoke the aid of tonotaxis to explain what I have described above as *positive* chemotaxis. But is not what I have heretofore spoken of as negative chemotaxis perhaps of the nature of Massart's tonotaxis? Tonotaxis signifies a motion of free organisms as a reaction to a stimulus due to a change in the osmotic pressure of the surrounding fluid.

It supposes that when an organism is living normally in a fluid of a certain osmotic pressure an increase in this osmotic pressure will cause fluid to pass out of the organism, a decrease in this osmotic pressure will cause fluid to pass into the organism, and that the passage of fluid in either case is a stimulus to the organism causing it to move.

The question, therefore, which we wish to decide is, whether the negative taxis of Paramœcium in coming into contact with solutions of

various substances is due to the osmotic pressures of the solutions or to their chemical characters. Obviously the way of testing this is to determine how far the reactions of the Paramœcia vary with variations in the osmotic pressure, and how far they vary with variations in the chemical nature of the surrounding fluid.

In working this out the method I have followed is to find the weakest solution of any substance $A$, to which the Paramœcia show a recognizable negative taxis, to determine the same point for a second substance $B$, and then to compare the osmotic pressures of the two solutions. If the two solutions have the same osmotic pressures, *i.e.* are isotonic, the evidence is in favour of the theory that the taxis is the result of the osmotic pressure, otherwise not.

In estimating the percentage of a substance $B$ which is isotonic to a given percentage of a substance $A$, I make use of the formula

$$\text{p.c. of } A \frac{(\text{molecular weight of } B)\ (i \text{ of } A)}{(\text{molecular weight of } A)\ (i \text{ of } B)} = \text{p.c. of } B,$$

and of the molecular weights and values for $i$ given in the following Table. The values for $i$ I have taken from the works of de Vries, Hamburger, Köppe and Arrhenius. The variations from the true value which there may be in some instances are too slight to affect the results obtained in this investigation.

TABLE I. *Substances tested for Tonotaxis.*

| Substance | Mol. wt. | Value of $i$. | Substance | Mol. wt. | Value of $i$. |
|---|---|---|---|---|---|
| Potassium hydroxide | 56 | 1·92 | Ethyl alcohol | 46 | 1·00 |
| Sodium hydroxide | 40 | 1·92 | Chloral hydrate | 147·5 | 1·09 |
| Sulphuric acid | 98 | 2·12 | Cane sugar | 342 | 1·00 |
| Hydrochloric acid | 36·5 | 1·94 | Dextrose | 180 | 1·00 |
| Sodium chloride | 58·5 | 1·66 | Mannite | 182 | 1·00 |
| Sodium carbonate | 106 | 2·20 | Glycerine | 92 | 1·00 |
| Potassium bromate | 167 | [1·61] | Urea | 60 | 1·00 |
| Copper sulphate | 249 | 1·16 | | | |

The experiments were carried on by means of the slide and cover preparations and introduced drop (cp. Fig. 4). The solutions were made by dissolving the proper proportions of the substances by weight in distilled water: thus a 2 % solution would signify a solution made by adding 2 g. of the substance to be tested to 98 cc. of water. Throughout the experiments I have used a solution of ordinary salt (Na Cl) as a standard with which to compare other substances. This

substance was chosen as a standard for several reasons. It has a decidedly negative action wherever present in effective quantities. But this negative action is not violent, so that the solutions become ineffective without being excessively diluted; this seemed to indicate that the action might be purely osmotic in nature, making it a favourable substance for comparison with others. Of course this adoption of a standard is purely a matter of convenience; the general results will be the same whatever the standard used.

The experiments are much more difficult in the execution, if accurate results are to be obtained, than might appear at first. The plan is as follows. Paramœcia in the water of the culture jar or in hydrant water are positively tactic toward distilled water, so that if a drop of distilled water is introduced with the capillary pipette, it is quickly filled with Paramœcia. If, however, some substance to which the Paramœcia are negatively tactic is dissolved in the distilled water the drop remains empty on being introduced into the preparation. Exactly that concentration must now be sought out to which the Paramœcia are nearly or quite indifferent: that is, such a concentration that they enter the drop on coming in contact with it, but do not form a collection within it. If a higher concentration is used they will not enter the drop, while if a lower concentration is employed they act toward it as toward a drop of distilled water. This indifference point can be determined with a close approximation to accuracy. But it is not possible to determine this point for any standard substance, as, say, Na Cl, once for all, because of the great variations in reaction under varying circumstances. Among the many circumstances to be taken into consideration are the following. First, the Paramœcia in the culture fluid are not always positive toward distilled water, so that this point must be carefully tested beforehand. Of course if the experiments are carried on with Paramœcia that are negative to distilled water it is impossible to determine the point where the negative action of the substance in solution ceases. Secondly, having tested the Paramœcia and found them positive to distilled water—they may soon become negative to it if they are not prevented by occasionally stirring them up thoroughly. Thus the later members of a series of experiments will show entirely different results from the first ones, and the entire series is rendered worthless through a neglect of this precaution. Thirdly, Paramœcia at different times and under different circumstances show an entirely different degree of sensibility to the same solution. These remarkable variations will all find a satisfactory explanation at a later

point in the investigation. Fourthly, the reaction depends to a certain extent on the thickness of the layer of water between the slide and cover-glass. If the space between the slide and cover is great, the introduced solution diffuses more quickly and the reaction of the Paramœcia is therefore not so pronounced. Hence the results of experiments carried on with the cover-glass resting on supports of varying thickness are not comparable.

All these precautions are satisfied by the following method of procedure. Paramœcia are removed from the culture jar with a large pipette, placed in a small beaker and thoroughly stirred up and brought into contact with the air. Thereupon, as before stated, they become positive to distilled water. This stirring must be repeated often to prevent their becoming again negative. A preparation is then made by bringing the Paramœcia upon a slide with a long cover-glass (22 mm. by 40 mm.), supported near its ends by two pieces of capillary tubing of equal and uniform diameter. A number of beakers are arranged, one containing a solution of Na Cl of about the strength that will probably (from previous trials) be found to be nearly neutral; the second a solution, isotonic with the Na Cl solution, of the other substance to be tested; a third containing distilled water. A drop of the Na Cl solution is taken up with the capillary pipette and introduced beneath the cover-glass, but near one end, forming there a small circle 5—8 mm. in diameter. A drop of the second solution to be tested is introduced in the same manner near the *other* end. The two drops are thus under precisely similar conditions, and the conduct of the Paramœcia toward them can be observed without fear of disturbance of the results by varying outer conditions. The tests must however be made *at once* after the Paramœcia are brought on to the slide, otherwise clear results will not be gained.

It will be understood from the above description of the method of experimentation, that if in one case the lower limit to which the animals are sensitive is given for an NaCl solution as 0·10 %, in another as 0·033 %, there is no contradiction involved, and the conclusions to be drawn are not thereby influenced, since the other substance for comparison was tested in each case under exactly the same conditions as the NaCl.

Where substances to which the Paramœcia are *positively* chemotactic (to a weak solution) are found in the table (acids), it is of course to be understood that we are dealing here with the weakest solution to which the Paramœcia are *negative:* to a still weaker solution they are positive.

The results for the different substances are given in Table II. In this table the fluid in which the Paramœcia were tested is given, that is, whether the Paramœcia in this experiment were in water from the culture jar, hydrant water, or distilled water. This should of course make no difference to the results gained, as the two substances (NaCl for comparison and the substance under discussion) were tested under the same conditions. The concentration of NaCl to which the Paramœcia are in this case exactly indifferent is stated, and the concentration of an *isotonic* solution of the substance in question; that is, a solution which has the same osmotic pressure as the above NaCl solution. If the cause of the negative taxis of the Paramœcia lies in the osmotic pressure, we ought to find experimentally also that this is the weakest solution to which the Paramœcia are negative. The minimum to which the Paramœcia are negative, as actually found by experiment, is then given, and this is followed by a statement of the ratio between the *calculated* concentration and this actual concentration observed. To be favourable to the tonotaxis theory, this ratio should of course be 1 or in the neighbourhood of 1.

TABLE II.

*Bases.*

1. Potassium hydroxide. Paramœcia in hydrant water.
   Indifferent to 0·10 % NaCl, isotonic with 0·083 % KOH.
   Actual minimum observed for KOH, 0·005 %.
   Ratio of calculated minimum to actual minimum, 16 to 1.
2. Sodium hydroxide. Paramœcia in culture water.
   Indifferent to 0·066 % NaCl, isotonic with 0·039 % NaOH.
   Actual minimum, 0·0025 % NaOH. Ratio, 16 to 1.

*Acids.*

3. Sulphuric acid. Paramœcia in culture water.
   Indifferent to 0·05 % NaCl, isotonic with 0·065 % $H_2SO_4$.
   Actual minimum, 0·00125 % $H_2SO_4$. Ratio 52 to 1.
4. Sulphuric acid. Paramœcia in distilled water.
   Indifferent to 0·033 % NaCl, isotonic with 0·042 % $H_2SO_4$.
   Actual minimum, 0·00125 %. Ratio 33 to 1.
5. Hydrochloric acid. Paramœcia in culture water.
   Indifferent to 0·05 % NaCl, isotonic with 0·026 HCl.
   Actual minimum, 0·00125 %. Ratio 20 to 1.

*Salts.*

6. Sodium carbonate. Paramœcia in hydrant water.
   Indifferent to 0·066 % NaCl, isotonic with 0·090 % $Na_2CO_3$.
   Actual minimum, 0·004 %. Ratio 22 to 1.
7. Sodium carbonate. Paramœcia in distilled water.
   Indifferent to 0·033 % NaCl, isotonic with 0·045 % $Na_2CO_3$.
   Actual minimum, 0·00208 %. Ratio 22 to 1.
8. Potassium bromate. Paramœcia in distilled water.
   Indifferent to 0·066 % NaCl, isotonic with 0·19 % $KBrO_3$.
   Actual minimum, 0·0208 %. Ratio 9 to 1.
9. Copper sulphate ($CuSO_4 + 5H_2O$). Paramœcia in culture water.
   Indifferent to 0·05 % NaCl, isotonic with 0·304 % $CuSO_4$.
   Actual minimum, 0·00125 % $CuSO_4$. Ratio 243 to 1.

*Organic Compounds.*

10. Ethyl alcohol. Paramœcia in culture water.
    Indifferent to 0·10 % NaCl, isotonic with 0·13 % alcohol.
    Actual minimum, 1 % alcohol. Ratio 1 to 8.
11. Chloral hydrate. Paramœcia in culture water.
    Indifferent to 0·05 % NaCl, isotonic with 0·19 % $CCl_3COH$.
    Actual minimum 0·066 % $CCl_3COH$. Ratio 3 to 1.

From this table it is evident that the negative taxis of Paramœcium to these substances bears no relation to the osmotic pressure of the solutions. Taking NaCl as unity, the ratio of the strength of solution reckoned on the basis that the osmotic pressure is the active agent in the stimulus, to the strength of solution actually found varies all the way from 243 to $\frac{1}{8}$. There can be no question therefore that we are dealing here with negative chemotaxis and not with tonotaxis. A considerable number of other substances were tested with sufficient precision to determine that their negative taxis was much stronger than could be reckoned on the tonotaxis theory,—though not with such exactness that they could be taken into the above table.

The conclusion that we are dealing with negative chemotaxis and not with tonotaxis is rendered exceedingly probable by certain other considerations. The Paramœcia live in water which contains a considerable quantity of various salts, especially $CaCO_3$, so that a solution in distilled water of, for example, $\frac{1}{800}$ % copper sulphate is probably much less dense than the water the Paramœcia are already in. The disinclination of the Paramœcia to enter such a solution might possibly be considered negative tonotaxis to a less dense solution than that to

which the animals are accustomed; tonotaxis of this sort is not uncommon, according to Massart. But this view is of course rendered inadmissible by the fact that the Paramœcia *do* enter the distilled water, which is still less dense than the $\frac{1}{800}$ % $CuSO_4$. I have thought it best however to make a thorough test of the matter from the other point of view, in order to set the question entirely at rest. Whether we consider the solutions used as denser or less dense than the water in which the Paramœcia are found, it is equally impossible to hold the reactions to be due to tonotaxis.

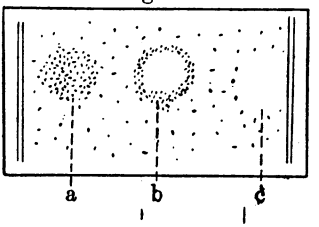
But the impossibility of considering the above reactions to be due to the osmotic pressure of the solutions is rendered, not more certain, but more striking, when certain other substances are drawn into the circle of experimentation. By the aid of these we are able to see precisely the relations of chemotaxis to tonotaxis, and to observe the effects of tonotaxis acting alone. Toward a number of organic compounds—cane sugar, dextrose, mannite, glycerine, and urea—the Paramœcia are chemically entirely indifferent, so that with them tonotaxis may be studied in its simplicity. This will be best shown by detailing certain experiments.

(1) An ordinary slide and cover preparation is made, with Paramœcia in water from the culture jar. They are tested and found to be positive to distilled water. Now with the capillary pipette a drop of a 2 % solution of cane sugar in distilled water is introduced. The Paramœcia act toward it exactly as toward a drop of distilled water. On coming to the edge they either hesitate an instant, or cross at once, swimming *into* the drop, but stopping on coming to the opposite side, exactly as described on p. 268 for distilled water. Thus in a short time most of the Paramœcia in the preparation are gathered into the drop of 2 % sugar solution.

A 2 % solution of cane sugar has the same osmotic pressure as a $\frac{1}{5}$ % solution of NaCl. Introducing a drop of $\frac{1}{5}$ % NaCl in distilled water into the preparation, the Paramœcia are strongly negative. Successive trials are made with weaker solutions of NaCl, until it is found that the Paramœcia become barely indifferent to a $\frac{1}{20}$ % solution of NaCl. This therefore works more powerfully than a solution of sugar having four times its osmotic pressure. The same Paramœcia were tested with $CuSO_4$, and it was found that while the margins of the drop exercise a positive attraction to the Paramœcia, they are negative to the inside of a drop of $\frac{1}{800}$ % $CuSO_4$ in distilled water. They are thus more strongly negative to a solution of copper sulphate than to

a solution of cane sugar having about 1000 times the same osmotic pressure. Fig. 10 shows a preparation to which a drop of a 2 % cane sugar solution at *a*, a drop of $\frac{1}{800}$ % $CuSO_4$ at *b*, and a drop of $\frac{1}{20}$ % NaCl at *c* had been added five minutes earlier.

Fig. 10




The apparently positive taxis to the sugar solution is due merely to the fact that the solution is made up in distilled water, as is shown by the following experiments:

(2) Paramœcia in distilled water. Introduced a drop of 2 % sugar solution in distilled water. The Paramœcia are entirely indifferent to it; they swim in or out of the drop, exactly as in any other part of the preparation.

(3) Paramœcia in water from the culture jar. A 2 % solution of cane sugar was made up also in the culture water, and a drop of this introduced into the preparation. The Paramœcia are entirely indifferent, as in the previous experiment.

(4) Paramœcia in culture water. Under one end of the cover-glass a drop of 2 % sugar solution in distilled water is added; under the other a drop of pure distilled water. The Paramœcia behave in exactly the same manner to both,—forming a gathering in one as in the other.

To a 2 % cane sugar solution therefore the Paramœcia are entirely indifferent, behaving toward it as they would to a drop of the pure fluid in which the solution is made. The sugar solution may be made negative by mixing with it a slight quantity of some substance to which the animals are negative, as NaCl, or positive by adding to it a trace of acetic acid. In all these respects it is exactly like ordinary water.

If we substitute an 8 % solution of cane sugar for the 2 % solution, the phenomena remain almost entirely unchanged. If the 8 % sugar solution is made in distilled water and the Paramœcia are in culture water, they collect in the drop of sugar solution as in distilled water. If the sugar solution is prepared in the same water as that in which the Paramœcia are swimming, they are quite indifferent to it, swimming in and out of the drop without hesitation. If the animals are observed *in* the drop of 8 % sugar solution, however, their conduct is seen to be slightly modified. They seem excited, and swim more hurriedly and irregularly than in the pure distilled water. At the same time they may be seen to shrink a little, owing to the withdrawal of water from

the protoplasm through the osmotic effects of the sugar solution. These effects are not marked, however.

If the density of the sugar solution is increased to 20% the Paramœcia behave as follows. On coming to the edge of the drop they make no sign of stimulation, but pass directly in, the motion becoming however a little slower. This is doubtless a mechanical result of the thickness of the sugar solution. Many specimens continue to swim straight ahead very slowly, but before the animal has passed half-way across a drop 6 mm. in diameter it has shrunk to a flat plate, owing to the plasmolysing effects of the dense sugar solution. The animal stops and dies. Other individuals shortly after passing the boundary hesitate, swing the anterior end back and forth, then pass on till they are plasmolysed. Others again after this moment of hesitation *turn* and swim in another direction; some of these thus pass out of the drop and so escape destruction. Still others, after swimming quietly some distance into the drop, stop, then *reverse* the cilia and swim straight backward, often a sufficient distance to carry them out of the drop. But the large majority of all those individuals which, in their random course, swim against the edge of the drop, pass in and are killed by the plasmolysing effects of the sugar solution. In a short time the spot where the drop of sugar solution was introduced is indicated by a large collection of dead Paramœcia.

A drop of 20% sugar solution is thus incomparably more dangerous to the Paramœcia than a drop of some strong alkali. If a drop of 2% KOH is introduced under the cover-glass, scarcely a Paramœcium is injured by it, except such as were overwhelmed by the drop as it was introduced beneath the cover-glass. In the case of the alkali the negative chemotaxis of the Paramœcia warns them long before the danger point is reached, so that they swim in another direction, while the sugar solution does not act as a stimulus until plasmolysis has begun and the animal is in too deep to be extricated.

The same results are gained by a study of the conduct of Paramœcia toward dextrose, mannite, glycerine, and urea. Toward solutions of all these substances the infusoria seem entirely indifferent. Solutions of strength insufficient to plasmolyse the animals call forth no reaction whatever. If a drop of very strong solution of any of these is introduced, the Paramœcia enter without hesitation, then show signs of agitation, perhaps reverse the cilia, and finally die of plasmolysis.

The precise conduct of the infusoria toward strong osmotic fluids was studied with especial care in the case of a 10% solution of glycerine.

After swimming without hesitation some distance into the drop the plasmolytic action begins. The animal reverses its cilia, swimming straight backward some distance, but generally not far enough to carry it out of the drop. The cilia then begin to strike in the original direction again: the animal therefore darts forward. Quickly the cilia are reversed again and the animal swims backward, a somewhat less distance than after the first reversal; then forward a short distance, then backward, the distance covered at each reversal of the cilia becoming less and less. At the same time the animal begins to revolve rapidly on its long axis. Finally the impulses to forward and backward motion seem to become equal; the animal remains in place, revolving rapidly on its long axis, and in a few seconds is dead, the body having shrunken to a flat plate.

Thus while changes in osmotic pressure *do* act as a stimulus for Paramœcium, the stimulus is so weak and slow in action that it does not suffice to save the animals from destruction by plasmolysis. The Paramœcia apparently do not respond until a decided and outwardly evident shrinkage of the cell body has occurred. Tonotaxis seems therefore to play no important part in the life activities of the animal.

The conclusions tentatively expressed earlier in regard to chemotaxis may therefore be now reaffirmed with absolute confidence. Paramœcium aurelia is (1) positively chemotactic toward solutions having a weak acid reaction, (2) negatively chemotactic toward solutions having an alkaline reaction, (3) negatively chemotactic toward *strong* acid solutions, (4) negatively chemotactic toward some neutral salts and some organic compounds, (5) chemically indifferent toward certain organic compounds.

It may be well to give here a list of the substances toward which I have determined definitely the manner of reaction, as they are scattered in the above discussion, and some substances tested have not been heretofore mentioned.

Paramœcium aurelia was shown to be positively chemotactic toward weak solutions of the following substances:—meat extract, rancid olive oil, rancid cod-liver oil, mercuric chloride, copper sulphate, sulphuric acid, hydrochloric acid, nitric acid, acetic acid, tannic acid. All these substances have an acid reaction.

Toward the following, Paramœcium showed itself negatively chemotactic: (*a*) strong solutions of all the substances in the foregoing list; (*b*) *all* effective solutions of potassium hydroxide, sodium hydroxide,

sodium carbonate, sodium bicarbonate, sodium chloride, ammonium chloride, potassium bromate, lead acetate, ethyl alcohol and chloral hydrate.

To the substances in the following list Paramœcium is indifferent, so far as the chemical nature of the substances is concerned: cane sugar, dextrose, mannite, glycerine, and urea.

To the list of substances to which Paramœcium is *positive* should be added oxygen, according to the observations of Verworn (1889) and Loeb and Hardesty. According to Israel and Klingmann (p. 327), Paramœcium (aurelia?) is positively chemotactic to water in which metallic copper has lain for a time. The authors do not state definitely whether the "copper water" used in these particular experiments was prepared with distilled water or with the same water as that in which the Paramœcia lived. In the former case the positive taxis observed would probably be due to the distilled water alone.

Regarding the negative chemotaxis of Paramœcium toward $CO_2$, as reported by Loeb and Hardesty, I shall have something to say in the ensuing part of this paper. It has been shown above that the statement of Loeb and Budget that Paramœcium is negative to acids and alkalies is correct for stronger solutions; these authors themselves state that if weaker solutions are used somewhat different reactions are obtained, though what these different reactions are they do not say.

### *Determination of the Chemical Substance causing the Collections of Paramœcia under normal conditions.*

We are now prepared to investigate the further problem: What is the attractive substance in response to which the Paramœcia gather about the bit of decaying vegetable material in the centre of the slide, as shown in Fig. 2?

As will be recalled, the evidence seemed at first to point to the view that the Paramœcia were positively chemotactic to soluble substances produced by the decaying vegetable material. Later (p. 274) this was shown to be incorrect. The incorrectness of this view may be demonstrated in a most striking manner by a simple alteration in the experiment. Let a bit of *filter paper* or a minute piece of *linen fibre* be substituted for the decaying vegetable material; the Paramœcia gather in a dense collection about it, exactly as about the bit of decaying matter. The experiment may be performed in water

from the culture jar in hydrant water or in distilled water; the results are the same.

This experiment raises the question whether the entire foregoing investigation of chemotaxis has not been a movement in a false direction so far as explaining this phenomenon is concerned. If the Paramœcia gather in the same manner about any bit of substance having no active chemical properties whatever does not that prove that chemical action has nothing to do with the phenomenon?

The implication contained in this question is an exceedingly plausible one. However, it may be easily shown to be false. Let the Paramœcia gather about the bit of filter paper, as in Fig. 2. After a time the dense gathering becomes looser; the Paramœcia spread out into a larger clearly defined area, as described in the "Introductory Experiment," and shown in Fig. 3. As will be noticed, the arrangement is much like that shown for the chemotaxis of Paramœcium about a drop of acid substance, in Fig. 8. Now let a fresh preparation of Paramœcia be made, without the bit of filter paper. The Paramœcia are in this scattered uniformly, as shown in Fig. 1.

Next we introduce the point of the capillary pipette beneath the cover-glass of the preparation in which the Paramœcia have formed a group about the bit of filter paper (as in Fig. 3), and remove therewith a drop of water from *within* the area to which the Paramœcia are confined, close to the filter paper. This drop is injected beneath the cover-glass of the second preparation, in which the Paramœcia are uniformly distributed. As soon as a Paramœcium, swimming at random in this second preparation, comes to the drop of fluid injected, it swims directly *in* without hesitation, but on coming to the opposite side of the drop, it stops at once and turns back—exactly as illustrated in Fig. 6, p. 269. It thus remains in the drop, swimming about in the liveliest manner, but never leaving it. Successive Paramœcia are thus quickly collected, and in five to ten minutes all the Paramœcia in the preparation are gathered in the small area where the drop was introduced, exactly as shown in Fig. 5. If a drop of water from some *other* part of the first preparation, outside the area of Paramœcia, is injected into the second preparation, no effect is produced.

Evidently there *is* some substance in the collection of Paramœcia about the bit of filter paper, to which the infusoria are strongly positively chemotactic. Furthermore, it is evident that this substance cannot have been produced by the bit of filter paper or linen fibre. It must then have been produced by the Paramœcia themselves.

This becomes apparent also when the mechanism of the collecting together of the Paramœcia about the bit of filter paper is carefully observed. They swim at first hither and thither in every direction throughout the preparation. By chance a few individuals come in contact with the bit of paper or linen fibre. Here they stop (cp. below, p. 298). The small assemblage of Paramœcia thus formed begins to excrete some attractive substance, for the region becomes at once a centre of great attraction for the remainder of the Paramœcia. Moreover, it is not the bit of solid material which is the centre of attraction, but rather the group of Paramœcia, as such. This is proved by the following facts. (1) The Paramœcia will gather in a dense mass about a bit of paper so small that the outer Paramœcia in the mass cannot get within ten times their own length of the paper. (2) In some cases the assemblage begins to take place by chance on one side of the paper. Thereupon all the Paramœcia are attracted to this side: the mass grows in this direction until finally it becomes entirely separated from the bit of paper, which is left quite alone. The latter may then be removed with a needle, while the dense gathering of Paramœcia remains as before. As this is an important matter, an experiment may be given in detail.

9.8 a.m. Preparation *D* made, on a slide, but without a cover-glass. In the centre of the slide an irregular piece of filter paper, with one side smoothly cut, the others torn.

9.24. A noticeable gathering about the bit of paper.

9.44. Almost all the Paramœcia are gathered about the bit of paper. A very large number along the cut edge; many of these cannot get within three Paramœcium-lengths of the paper, yet they crowd in as close as they can.

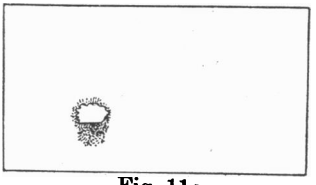


Fig. 11

10.10. The thickest mass of Paramœcia is now at the cut edge of the paper; the outer Paramœcia are at least ten times their own length from it (Fig. 11).

10.20. The Paramœcia have all left the torn edges of the bit of paper, to gather on the cut edge in a single dense mass as large as the entire paper.

10.51. The mass of Paramœcia are now completely separated by a narrow space from the piece of paper; all are pressing toward a centre lying within the group of Paramœcia.

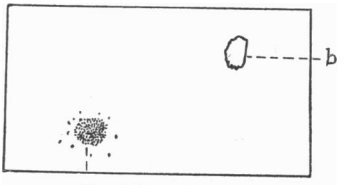



Fig. 12

*a*, Paramœcia; *b*, bit of paper.

10.53. The bit of paper was removed with the needle from the region of the mass of Paramœcia to the other side of the slide, but the mass of Paramœcia still remains in place (Fig. 12).

The mass of Paramœcia thus entirely separated from the bit of paper was observed for more than half-an-hour longer. It did not loosen and separate into such an area as is shown in fig. 3, because of the absence, in this case, of a cover-glass. (Why this should cause a difference will be explained later.)

It is evident therefore that the Paramœcia are attractive to each other, and the experiment previously described, in which a drop of water from the group of Paramœcia proved attractive to the Paramœcia of another preparation, shows that this must be due to some substance excreted by the Paramœcia and passing into solution in the water. Furthermore, the same substance in somewhat greater concentration produces a negative reaction, since the Paramœcia after a time avoid the centre of the area and gather chiefly about its margin, as shown in Fig. 3.

From what we have learned concerning the phenomena of chemotaxis in general in Paramœcium, we should expect this substance to be an *acid* of some sort. When we inquire what acids may be excreted by Paramœcia, there seem to be two possibilities. The first is that the substance may be the $CO_2$ excreted in the respiratory process of the animals. The second, that some acid is excreted in connection with the digestive functions. Greenwood and Saunders have shown that an acid is produced in the food vacuoles of infusoria, and have demonstrated that this acid is not $CO_2$, since it dissolves granules of calcium phosphate. According to the observations of Greenwood and Saunders the acid disappears before the food vacuoles are ejected. However, the possibility suggests itself that a certain amount of this acid persists and is discharged with the waste matter into the surrounding water. Our first approach to a determination of the attractive substance must be by testing whether it can likewise be shown not to be $CO_2$.

This test was made by introducing granules of finely powdered chalk ($CaCO_3$) into the centre of one of these groups of Paramœcia and observing the effects. The exact form, size and position of a number of irregular granules of the minutest size were noted with the microscope, then the preparation placed in the moist chamber for a number of hours. At the end of this time the granules of calcium carbonate had not suffered the slightest change. On the other hand, a similar trial with the weakest solution of HCl to which the animals

showed any sensitiveness produced a marked corrosion of such granules in a much shorter time. The evidence gained in this way is therefore against the presence of any acid stronger than $CO_2$. What is now desired is a delicate test for $CO_2$.

At the suggestion of Professor Verworn I used for this purpose a solution of *rosol*. This produces with water a beautiful red solution, which is decolorized by carbon dioxide—as well as by other acids, of course. A weak solution of rosol is not injurious to the Paramœcia, so that a large number of the infusoria were brought directly into the solution, and a preparation was made with this. A bit of filter paper was placed in the centre, and the Paramœcia formed a dense gathering about it, as usual.

Shortly after the collection has become densest the solution begins to become colourless about the bit of paper. The group of Paramœcia begins to loosen and separate, and the colourless space to become larger. The Paramœcia remain confined to a definitely limited area (as shown in Fig. 3) which continually but slowly grows larger; the colourless area is exactly identical in size and limits with this area to which the Paramœcia are confined and holds exact pace with it in its increase in size. If the preparation be now viewed with a dark background the Paramœcia are seen distinctly as white dots gathered in a definitely limited area, the greater number of Paramœcia about the outer boundary of this space. If the same preparation be viewed with a white background the Paramœcia disappear, and one sees only the clear red colour of the greater part of the slide contrasted with the entirely colourless area in the centre, of exactly the form and size of the area previously seen to be marked by the Paramœcia. The reaction is a most striking, precise and satisfactory one, and, taken in connection with the lack of reaction towards $CaCO_3$, clearly indicates the presence of $CO_2$ in the area.

There remains but to test $CO_2$ directly. Pure carbon dioxide was made by the action of HCl upon $CaCO_3$. The gas was collected over water and washed carefully by shaking it up with a solution of sodium carbonate. The tests were made in exactly the same manner as for other chemical substances. A preparation was made in which the animals were scattered uniformly beneath the cover-glass; then a bubble of carbon dioxide was introduced with the capillary pipette. Immediately before or after, a bubble of air was brought into another part of the same preparation as a control.

The result of the experiment is not doubtful for a moment. The

Paramœcia are strongly positively chemotactic to the carbon dioxide. They gather at once in a dense collection about the bubble of $CO_2$, pressing against the very walls of the bubble and crowding each other to get as near as possible. Meanwhile the bubble of air is left entirely alone.

I give herewith three figures from my record of experiments, illustrating the chemotaxis towards $CO_2$. The three figures are from a preparation of Paramœcia in distilled water. A bubble of air (*a*) was introduced near the left end of the cover-glass, a bubble of $CO_2$

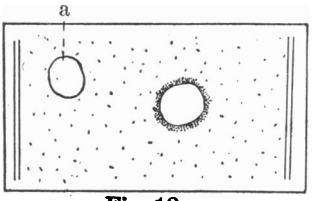



Fig. 13

Chemotaxis of Paramœcia toward $CO_2$. Two minutes after the introduction of the bubble of $CO_2$.

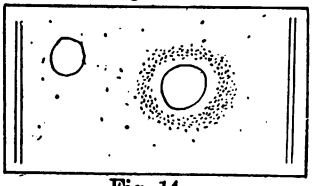


Fig. 14

Two minutes after Fig. 13.

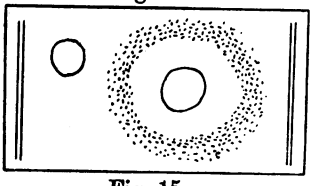


Fig. 15

Same preparation 18 mins. later than Fig. 14.

near the right end. Figure 13 shows the preparation two minutes after the bubbles were introduced. Many Paramœcia are crowded close about the bubble of $CO_2$.

The condition two minutes later is shown in Fig. 14. The gathering of Paramœcia about the $CO_2$ has become larger, but the animals are not crowded so close to the bubble; there is a distinct space between it and the dense ring of Paramœcia. They are evidently *negative* toward a too concentrated solution of $CO_2$.

Fig. 15 shows the condition 18 minutes later. The ring is broad and not so dense as before; the inner boundary is much farther from the surface of the bubble than in Fig. 14. The Paramœcia in the

ring are exceedingly active, generally swimming around the bubble in a circular path.

A large number of such experiments were performed and always with the same result. The experiments may be performed with the Paramœcia in distilled water, in culture water, or in hydrant water: the results are the same. We must conclude therefore that Paramœcium aurelia is positively chemotactic toward a solution of $CO_2$ of a certain strength; negatively chemotactic toward a stronger solution. The positive chemotaxis toward $CO_2$ seems much more powerful than towards other substances, since Paramœcia which had been brought only the day before into distilled water, and showed almost no recognizable positive chemotaxis toward other substances, were at once strongly attracted by a bubble of $CO_2$.

The positive chemotaxis toward $CO_2$ may be easily shown in another manner. If a bubble of ordinary breath is blown beneath the cover-glass, with a capillary tube, the Paramœcia are distinctly positively chemotactic to it, though not so powerfully so as towards pure $CO_2$. The experiment will of course not succeed unless the breath is blown through the tube sufficiently beforehand to be sure that all ordinary air is removed from it before the experiment is made.

As will be noticed, the phenomena observed with $CO_2$ are exactly the same as those observed with the substance excreted by the Paramœcia, and which we had concluded on other grounds was probably $CO_2$. A drop of water can be taken from within the ring, in the one case as in the other, and this drop proves strongly attractive to the Paramœcia of a fresh preparation. If the electric current be passed through the preparation shown in Fig. 14, the Paramœcia all gather on the cathode side of the ring, but go no farther toward the cathode, exactly as was described in the case of the preparation shown in Fig. 3. There can be no question that we are dealing with carbon dioxide in both cases.

Armed with this knowledge, we can now explain a number of exceedingly puzzling phenomena which have already been mentioned in this paper or elsewhere.

### *Explanation of previously observed phenomena by means of the reaction toward* $CO_2$.

We may first summarize briefly the essential features of the solution of our original problem—the course of events in the "Introductory Experiment" (p. 260). A certain number of Paramœcia strike

by chance against the bit of decaying vegetable material, and remain there. The water in this region then becomes more strongly charged with $CO_2$ than elsewhere, owing to its excretion here by this collection of Paramœcia. This region then becomes a centre of attraction for other Paramœcia, owing to their positive chemotaxis toward $CO_2$; so all collect here. The production of $CO_2$ is thereby so increased about the bit of vegetable material that the solution in the water here becomes too strong: the Paramœcia become negative to the $CO_2$ and leave the centre of the group. They go only to that point where the concentration of $CO_2$ is that to which they are positive, however, and here they remain, on the margin of the area. Individual differences exist in the sensitiveness to $CO_2$, so some Paramœcia will be found in the centre of the area after most are gathered at its margins. The continued growth of the area is due to the continued excretion of $CO_2$ by the Paramœcia. If the constant electric current is passed through the preparation, the Paramœcia react to the electric stimulus normally till this reaction comes in conflict with the chemotactic reaction, which occurs at the cathode side of the area; the chemotactic proving generally the stronger, the Paramœcia do not leave the area.

A phenomena related to the above was described and figured by Jensen. When Paramœcia are brought upon a slide, beneath a cover-glass, they soon begin to avoid the outer edges of the water, not swimming within a certain distance of the margin. The free margin thus left becomes broader and broader till the Paramœcia are confined to a comparatively small central space. Jensen explained this as probably due to the greater concentration of the salts in the water about the edge, due to the evaporation here. It seems much more probably due to the discharge of the $CO_2$ which is being continually produced by the Paramœcia, into the air about the edge of the cover-glass, leaving this region much poorer in $CO_2$ than the inner part. The Paramœcia begin to gather on this account into the central region—whereupon the difference in the amount of $CO_2$ between the outer and inner regions becomes greater than ever, tending still more to accentuate the gathering of the Paramœcia in the central region. That this is the true explanation is rendered especially probable by the fact, at least when a large cover-glass is used, that the collection does not always take place under the middle region of the cover-glass, but may occur in almost any part, the precise place where the assemblage occurs being determined by chance factors. Such a "chance factor" is a bit

of filter paper or linen fibre. A slight roughening of the surface of the glass, or the presence of the merest trace of some attractive substance may determine the place of gathering in the same manner. If there is no such determining factor, the Paramœcia simply avoid the outer edges and gradually gather in the centre, as described by Jensen. The water from one of these central collections of Paramœcia is attractive, on being introduced into a fresh preparation. This is of course inexplicable on the theory that the gathering together of the Paramœcia is due to the concentration of salts about the margin of the preparation. If after such a central group has been formed, the electric current is passed through the preparation, the Paramœcia respond only in so far as the electrotaxis does not come in conflict with chemotaxis. This explains why, as is often noticed, the Paramœcia of a preparation may at first respond typically to the electric current, while later the Paramœcia of the same preparation respond but incompletely or scarcely at all.

In close relation to this stands the cause of the fact mentioned on page 277, that in testing various solutions with Paramœcia on the slide, it is necessary to make the tests at once after the Paramœcia are brought into the preparation, or the results are unreliable. After remaining some time the Paramœcia will have gathered into a group in some part of the slide, and will there have produced a certain amount of $CO_2$. Being more strongly positive to this than to any substance which can be introduced, they may now show negative taxis toward the solution tested, when previously they would have reacted positively to the same solution.

A related phenomenon is the following. On page 271 it was stated that if Paramœcia are removed from a dense collection in the culture jar by means of a pipette and brought at once beneath the cover-glass of a preparation, they show themselves negatively chemotactic to all substances introduced, of whatever nature. In such a case the Paramœcia do not remain even a short time uniformly distributed beneath the cover-glass, but will be found gathered in some particular region of the slide a few seconds after they are introduced.

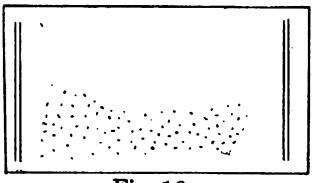


Fig. 16

Fig. 16 shows a preparation made in this manner; the Paramœcia are gathered in a long irregular strip near one side. The explanation is as follows. In the dense collections of Paramœcia in the culture jar

the water contains in solution a large amount of $CO_2$ excreted by the Paramœcia. These collections are so small that in removing Paramœcia from them with a pipette water from outside the collection, containing little $CO_2$, is drawn into the pipette also. The whole is now brought at once under the cover-glass without a thorough mixture having taken place; the Paramœcia betake themselves at once to that part of the water containing more $CO_2$, leaving the rest of the slide empty. Now, if some substance is introduced beneath the slide, to which the Paramœcia are generally positively chemotactic, they do not respond positively, being more attracted by the $CO_2$. If however the water from the culture jar is thoroughly stirred up with the pipette and brought in contact with the air before being placed on the slide, the $CO_2$ is driven off, and the conduct of the Paramœcia is not therefore modified by its presence; they give the normal positive reaction to the solutions introduced.

On page 286 it was stated in describing an experiment, that the Paramœcia, having gathered in a dense mass, did not later loosen and separate, on account of the absence, in this experiment, of a cover-glass. The reason for this is easily seen when one considers the effect of a cover-glass in preventing the escape of carbon dioxide which is produced beneath it. If no cover-glass is present, the $CO_2$ continually escapes, so that the water solution does not increase in strength beyond that concentration which is attractive to the Paramœcia; therefore they continue to crowd together in order to reach the point where the $CO_2$ is densest. If the $CO_2$ in the thin layer of water is prevented from escaping by the cover-glass above it, the solution becomes more and more concentrated till it reaches a strength to which the Paramœcia are negatively chemotactic; hence they separate, remaining chiefly about the margins of the $CO_2$ area. It is probable that under natural conditions the $CO_2$ hardly ever reaches such a concentration as to be repellant, owing to its continual diffusion and escape. Thus while the positive chemotaxis of Paramœcium toward $CO_2$ plays a very large part in the everyday life of the infusorian, it is probable that the negative chemotaxis toward a denser solution comes hardly ever into operation.

Certain phenomena described by Pfeffer (p. 618) in other infusoria than Paramœcium probably receive their explanation also by the attraction of the infusoria toward the carbon dioxide excreted in a dense group. Pfeffer observed that when numbers of *Glaucoma scintillans* are brought upon a slide, together with some small neutral solid

bodies, such as bits of filter paper, the infusoria quickly gather into dense groups about the solid bodies. Such large and dense gatherings were formed and there was such variation as to which particular bit of the solid should form the centre of a group, that Pfeffer felt that the phenomenon could not be explained by the contact stimulus exercised by the solid alone, and was inclined to believe that it was partly due to a contact stimulus resulting from the striking together of the infusoria in such a collection. Similar collections in a less pronounced degree were observed by Pfeffer in *Colpidium colpoda*, and still less marked in *Stylonychia mytilus* and in *Paramœcium aurelia* itself. As we know that the collections are due in the case of Paramœcium to the attraction toward the $CO_2$ excreted in a dense group, the conjecture is exceedingly plausible that the same cause accounts for the phenomena in the other cases.

If a quantity of water containing many Paramœcia is brought into an open watch-glass or other small vessel, the infusoria are at first distributed uniformly. Very soon, however, the Paramœcia begin to avoid the upper surface and gather toward the bottom. Soon a distinct boundary is evident between the water containing Paramœcia and that containing none. The Paramœcia continue to draw together toward the bottom until at last a small definitely bounded space in the bottom of the watch-glass alone contains Paramœcia. The individuals may be seen to swim straight forward within this space, until, coming to the invisible boundary, they are turned back. This is evidently a phenomenon of the same nature as those above described. The $CO_2$ produced by the Paramœcia is given off at the surface of the water: therefore the Paramœcia draw away from the surface. They continue to draw together until a balance is struck between the amount of $CO_2$ produced and that given off; they remain confined to the space in which the $CO_2$ reaches that concentration to which they are positive. A drop of water taken from this gathering in the bottom of the watch-glass proves attractive when introduced into a slide of fresh Paramœcia. If two unpolarisable electrodes are dipped into the water on opposite sides of a watch-glass containing such a group of Paramœcia in the bottom, and the circuit is closed, the Paramœcia may be induced by the electric current to swim from one side of the small bottom group to the other, but only with the greatest difficulty can they be forced across the boundary of the group into the surrounding water. This furnishes the explanation of the fact often noticed that in order to demonstrate clearly the phenomena of electrotaxis in a watch-glass of

Paramœcia, either the circuit must be closed soon after the infusoria are brought into the glass, or the water must be thoroughly stirred up occasionally.

If in place of a concave watch-glass a vessel is used having a broad flat bottom, the Paramœcia gather in one or more well-defined groups in some particular part of the dish. They do not scatter all over the bottom, as must be the case if the collecting together were due to simple concentration of the salts in the upper layers of the water, owing to evaporation. Exactly *where* the Paramœcia shall gather depends on chance circumstances; a slight variation in the warmth of the two sides; the presence of a small solid object in one spot, or a roughening on the surface of the glass. Anything which leads in the beginning to a collection of a few individuals in a certain region, results later in the gathering of all (or most) of the Paramœcia in the vessel in this place, owing to the greater concentration of $CO_2$ in this region. The original occasion for the collection may then be removed, but the collection itself remains.

On p. 276 it was stated that if Paramœcia which are to be used in experimentation are placed in a beaker, and are found by testing to be *positive* toward distilled water, they may later become *negative* to it unless well stirred up at short intervals—thus causing the series of experiments to give inconstant and worthless results. This is explained by the observations just described. If left untouched for some time, the Paramœcia gather together in some part of the vessel and excrete $CO_2$, to which they are strongly positive. If now, some of these Paramœcia, in this water strongly charged with $CO_2$, are brought upon the slide and tested with distilled water, they avoid the latter, on account of their *greater* positive chemotaxis toward $CO_2$. If the carbon dioxide is driven out of the water by stirring it and bringing it into contact with the air, the Paramœcia gather in the distilled water. In the same way the chemotaxis may vary toward other substances.

The explanation of the fact noted on p. 273, that Paramœcia may become positively or negatively chemotactic to water from a different part of the same dish as that in which they are swimming, lies along the same line. If Paramœcia taken from a part of the dish where the water contains much excreted $CO_2$ are tested with water from a part poor in $CO_2$, the Paramœcia are negative; in the converse experiment they are positive.

The place where the Paramœcia will form a permanent gathering in a shallow vessel may sometimes be determined artificially by the use

of the electric current. As is well known, when a constant electric current is passed by means of non-polarisable electrodes through a vessel of water containing Paramœcia, the latter gather *behind* the cathode. The explanation why they gather behind the electrode rather than in front of it or upon it was given in principle by Ludloff, though he did not apply the principle to this particular problem. He showed that the movement toward the cathode was due, not to any attraction of the cathode, but to the effect of the current in orienting the animal with its anterior end toward the cathode. The animal then swims in that direction, but there is no reason why it should stop on arriving at the cathode. On coming to the latter, the animal is as it were left to itself, and can swim where it pleases, the slight current components behind the electrode being ineffective so far as the Paramœcia are concerned. If the animals return into the space between the two electrodes, they are at once brought back as far as the cathode, then left to themselves again. Thus there is naturally produced finally an assemblage of Paramœcia behind the cathode. This may be very well demonstrated by performing the experiment in a long trough, with the cathode in the middle of the trough, the anode at one end, and all the Paramœcia at first between the two electrodes. The Paramœcia are soon all brought by the current into that half of the trough lying behind the cathode, where they swim about at random, paying no further attention to the electric current. If the experiment be performed with the cathode close to the wall of the vessel, so that only a small space is left behind the electrode, the Paramœcia gather in a dense mass in this small space. If a rather weak current is used and this is long continued, the Paramœcia may settle themselves against the wall of the vessel, and remain there after the electric circuit is opened or the electrodes removed, in consequence of their positive chemotaxis toward the $CO_2$ produced in the dense assemblage. This experiment does not always succeed, as the electric current often produces such excitement in the Paramœcia that on being released from its action they swim in every direction from the spot where they were collected. This is especially likely to happen if the gathering has lasted but a short time, so that little $CO_2$ has been produced. Moreover, if the gathering of Paramœcia so formed lies near the upper surface of the water, the $CO_2$ may be dissipated as fast as it is produced, so that the Paramœcia do not remain after the circuit is opened.

Jensen (p. 438) has described the conduct of Paramœcia when left in an upright open tube of water. They rise to the upper end of the

tube, on account of their negative geotaxis. But after a time they draw back from the surface, leaving first the small elevated columns of water about the margin of the concave upper surface, later sinking even lower, and presenting a clearly defined upper boundary, which they do not pass. (See the Figs. II. III. and IV. of Jensen.) This phenomenon may likewise be explained by the escape of carbon dioxide from the upper layers of the water. The Paramœcia sink and gather together until their own production of carbon dioxide suffices to supply the loss due to the escape of the gas from the upper surface of the water, remaining at the point where the charge of $CO_2$ is that to which they are most attracted.

It was stated on page 265 that the method of testing the chemotaxis of Paramœcium toward various substances by introducing capillary tubes containing the solution to be tested into a vessel containing many Paramœcia and leaving them there for some time is unreliable. One reason for this is as follows. After some time the Paramœcia may occasionally be found to have gathered into tubes containing substances to which they are entirely indifferent, as for example, water of the same sort as that contained in the rest of the vessel. This is evidently due to the following. Certain Paramœcia stray by chance into the tube and there of course produce $CO_2$, which is prevented from diffusing. Others accidentally entering the mouth of the tube are attracted by this $CO_2$ and likewise remain. Thus the production of carbon dioxide increases till the gas perhaps begins finally to diffuse about the mouth of the tube. Thereupon this becomes the centre of attraction for other Paramœcia, till at last all the animals in the dish are gathered in an assemblage about the mouth of the tube or within it. We may say then that the fact, taken by itself, that Paramœcia gather in a certain region is no evidence that the region previously contained substances attractive to the animals.

In general, it will be found that very many of the phenomena presented by the behaviour of Paramœcia in fluids are explicable on taking into consideration the following factors; (1) the production of $CO_2$ by the Paramœcia: (2) the positive chemotaxis of Paramœcium toward $CO_2$ in a certain concentration: (3) the negative chemotaxis of Paramœcium toward a higher concentration of $CO_2$ (much less important than the foregoing); (4) the diffusion and escape of $CO_2$ in the water. In all experimental studies on reaction to stimuli in Paramœcium, close attention to these factors is an absolute necessity, if reliable results are to be obtained.

The explanation of these phenomena as due to the positive chemotaxis of the organisms toward carbon dioxide is of course a reversal of the reasoning commonly used, in which explanation is sought by assuming that organisms are always repelled by $CO_2$. Such a reversal will doubtless be met with criticism and opposition on first thought.

### 3. Thigmotaxis.

It has been shown above (p. 287 *et seq.*) that the chief cause of the gathering of Paramœcia around a piece of paper or linen is the production of $CO_2$ by the Paramœcia themselves and their positive chemotaxis toward this substance. We must now investigate the exact part played by the solid body.

Dewitz showed that a tendency to cling to and move along solids plays an important part in the life activities of the spermatozoa of the cockroach. A similar fact was made known by Massart (1888 and 1889 *a*) for the spermatozoa of the frog. Pfeffer (p. 618) observed collections of *Glaucoma* and other infusoria about bits of filter paper or other neutral solids, and referred these collections to the stimulus of contact. Verworn (1889, p. 90) introduced for this directive stimulus due to the action of contact the name Thigmotropism; in accordance with the terminology used throughout this paper I shall employ the word thigmotaxis. Verworn (*l.c.*) pointed out in a general way that positive thigmotaxis plays a part in the movements of diatoms, oscillaria, and hypotrichous infusoria, and that negative thigmotaxis was to be recognized in the sudden backward movements of Flagellates and Ciliates on striking solid objects, as well as in the retraction of the pseudopodia of rhizopods on being subjected to a mechanical stimulus. Loeb (1890) studied the stimulus produced by mechanical contact in a number of higher animals, and gave to the phenomena which he observed the name *stereotropism.*

Any small object of character similar to filter paper acts equally well as the centre of a gathering of Paramœcia. A piece of any other paper, a small bit of linen or other cloth fibre, a bit of sponge, etc., all have the same effect. Any of these substances may previously be boiled in the same fluid in which Paramœcia are found, in order to remove all traces of oxygen, carbon-dioxide or any other soluble substance which might be contained within it; yet the effects are the same. The experiment may be performed with the Paramœcia in hydrant water, in water from the culture jar, or in distilled water, without altering the result.

There can be no question therefore that the bit of paper or other body acts merely in virtue of its being a solid of a certain physical structure, and not in consequence of any chemical action, in determining where a collection of Paramœcia shall take place.

The beginning of a collection about such a body, possessing as it does no influence at a distance, is made possible by the continuous motion of the Paramœcia. If a preparation of Paramœcia on a slide, containing in one spot a small bit of filter paper is closely observed, the Paramœcia are seen at first to swim hither and thither in every direction, apparently without directive tendency of any sort. They swim now in a curved path, now in a straight one, sometimes about the margin, or again cutting across the middle. Soon a single individual strikes in its headlong course the bit of paper. It stops at once, often starts backward a slight distance, and whirls on its short axis two or three times, then settles against the bit of paper, and remains. Quickly another and another strike in the same way and remain. Now the excretion of $CO_2$ by the animals gathered together begins to take effect; the region becomes a strong centre of attraction, and in ten to fifteen minutes, and often less, the paper is surrounded by a dense swarm of Paramœcia, containing a large majority of all those in the preparation. Of course only the beginning of the collection is to be considered due primarily to the reaction to solids; the remainder of the phenomenon comes under the head of chemotaxis.

The habit which the Paramœcia have of swimming swiftly hither and thither, often changing the direction of the course, is evidently essential to the occurrence of the thigmotactic effects. In the preceding part of the paper it was shown to play likewise an important part in chemotaxis. The continual and rapid change of position of the body of the animals subjects them to many more stimuli than would otherwise fall to their lot, and brings every individual in a preparation in a comparatively short time into the region of influence of any stimulus the direct action of which is confined to only a limited area.

Thigmotaxis alone is not capable of producing the dense assemblages of Paramœcia above described. If a quantity of water containing Paramœcia is placed in a flat-bottomed open dish, and a bit of filter paper or other similar solid introduced, the Paramœcia commonly form no collection about it. If such a solid is watched with the microscope it will be found that the behaviour of a Paramœcium on striking it by chance is at first exactly as toward the bit of paper under a cover-

glass. The Paramœcium applies itself to the paper and remains. But the $CO_2$ continually escapes, so the place does not form a chemotactic centre, and in a short time the Paramœcium swims away. There is much chance in this matter, however: if other causes combine to make this particular region a centre for the Paramœcia, then sufficient $CO_2$ is produced to supply the escape, and the Paramœcia *do* gather on the bit of paper. For example, this may be the case if the solid body lies in the bottom of a convex watch-glass (cp. p. 294).

The Paramœcia do not gather with equal avidity about solids of all sorts; the body must be of a certain type of physical structure to excite easily the thigmotactic reaction. *Fibrous* bodies, such as frayed filter paper, are especially effective. Materials composed of light particles have a similar effect; thus Paramœcia quickly swarm upon a mass of powdered carmine when it is introduced beneath the cover-glass. Starch grains act in the same way. Also if saliva be mixed with the water, the Paramœcia swarm about the remains of the dead cells contained in it, though to filtered saliva they are negatively chemotactic. Surfaces covered with a layer of sticky mucus-like material are likewise very effective; a bit of glass which has been dipped in saliva or white of egg is soon surrounded by the infusoria. This reaction is not due to positive chemotaxis, since if the Paramœcia are tested with albumen or saliva alone, they are found to be indifferent or negatively chemotactic. It is not however a matter of mechanical fastening to the surface by being caught in the sticky substance. The Paramœcia are entirely free in their motions, shifting their positions, leaving and returning and acting in all respects like the individuals gathered about fibrous or powdery bodies.

To smooth hard bodies the Paramœcia do not respond unless chemotaxis is combined with thigmotaxis. Perfectly clean glass, stone, wood, and the like rarely become the seat of such swarms as above described.

The tendency to gather about and settle upon solids is strikingly increased by the presence in the surrounding water of some substance to which the Paramœcia are positively chemotactic. This is especially true when the water contains the proper amount of $CO_2$. For example, as just stated the animals do not commonly tend to gather on smooth hard substances, such as the glass tubes generally used in my experiments for supporting the cover-glass. Suppose, however, that in the experiment with $CO_2$ shown in Figs. 13 to 15 (p. 289) the bubble of gas is situated close to one of the glass rollers used in supporting the cover. As the ring of Paramœcia spreads out, it will at perhaps the

stage shown in Fig. 14 come in contact with the glass roller. Thereupon the Paramœcia in that part of the ring settle upon it. As all the Paramœcia in the ring swim around the bubble in a circular path, soon almost all will have come in contact with the glass roller and may settle upon it, till there is nothing left of the ring of Paramœcia except the spot where it was tangent to the glass roller. As the $CO_2$ continues to spread the circular area becomes larger, so that the glass roller cuts the circle in two points instead of being tangent to it. The Paramœcia follow the circumference of the circle as it moves along the glass rod, the original simple group of attached Paramœcia separating into two, which move farther and farther apart along the rod as the circle becomes larger. A stage in such a preparation is shown in Fig. 17.

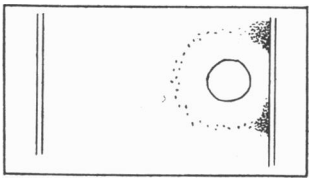


Fig. 17

Within the area formed by the excretion of $CO_2$ by the Paramœcia (such as shown in Fig. 3), it can be observed that, even if no large solid body is present about which the Paramœcia may gather, many of them will have their anterior ends applied to small particles in the water, or numbers of them are collected about roughened spots on the surface of the glass slide. The increased tendency to react to the contact stimulus in the presence of $CO_2$ is observable in many ways.

The phenomena of thigmotaxis may be exhibited toward bodies of extraordinarily minute size. This is shown by such observations as the following. A single carmine grain, of not more than $\frac{1}{5}$ the diameter of the short axis of a Paramœcium had become fastened by a minute fibre of some sort in such a way that it was movable, but could not be carried away from the region where it was anchored. Paramœcia were numerous in the preparation, and occasionally one would strike with its anterior end against the grain of carmine. Thereupon it would stop with its anterior end applied to the grain, and begin to swing in a circle about the latter, pushing it hither and thither, but not being able to loose it from its moorings. This continued for a minute or more, the energetic ciliary motion nicely adjusted in such a way as not to destroy the contact with the grain. After a minute or two the Paramœcium left the grain. Soon another individual struck it and the performance was repeated. Occasionally two or even three would gather about the grain at the same time, all swinging together in such nice adjustment that none were displaced.

Very often one notices on a slide containing many of the infusoria

numerous small groups of Paramœcia, consisting of half-a-dozen individuals, more or less, having their anterior ends applied together, and the whole group revolving and progressing like a colonial infusorian. I have examined carefully many such cases and have never failed to find at the centre of such a group a minute particle of some sort, against which the anterior ends of the Paramœcia were pressed. Such observations show an extreme sensitiveness to contact in the anterior ends of the Paramœcia.

In a similar way it will often be observed that a Paramœcium is carrying about, at its anterior end, a single minute grain of some substance. Under such circumstances the infusorian exhibits extremely characteristic motions (cp. p. 304).

*Position of the Animal and Motion of the Cilia in Thigmotaxis.*

In the case of individuals gathered about a larger solid body, such as a bit of paper or a piece of decaying plant tissue, the anterior end is commonly applied to the surface of the solid, as just described in the case of minute granules. This is not by any means always the case, however; almost any part of the resting individual may be applied to the solid. For example, in the case of fibrous bodies, the animal may often be seen to lie lengthwise along a fibre, touching it only on one side, while both ends are free (Fig. 18). In other cases a Paramœcium may lie crosswise to a fibre, or be touched in two different regions by different fibres.

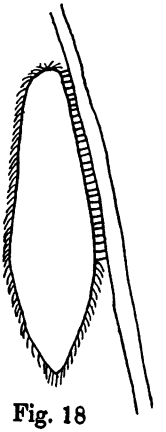


Fig. 18

There seems to exist a typical arrangement and motion of the cilia in individuals exhibiting thigmotaxis, but this arrangement is not invariable, and a great variety of motions may occur in thigmotactic individuals. It will be important however to gain a conception of what this typical condition is, both for its own sake, and because of its significance for an understanding of the peculiar interference of thigmotaxis and electrotaxis, to be described below.

For this purpose it is impossible to use the method employed by Ludloff for studying the motions of the cilia in electrotaxis,—the introduction of the animals into a thick gelatine solution. In such a solution they are not under normal conditions and do not respond to the contact stimulus, at least not in a pronounced and normal way. The motion of the cilia must therefore be observed under normal

conditions in ordinary water. Direct observation of the cilia in this way presents great difficulties: it can be used to a certain extent as a control for other methods, but the most satisfactory results may be obtained by observing the currents produced by the cilia, shown by the motion of particles in the surrounding water. As such particles the bacteria in the water in which the Paramœcia live may be employed. The clearest results however are gained by the well-known method of introducing powdered carmine into the water.

Using these methods we find the typical arrangement and motion of the Paramœcia in thigmotactic individuals to be as follows:

The cilia in that region which is applied to the solid body are entirely motionless. They are not pressed down against the body of the animal, but appear like minute straight stiff rods, attaching the Paramœcium to the object (Fig. 18). Where the latter is very small, as a grain of carmine, the few cilia touching it gain the appearance of stiff prehensile organs, used for holding the object. If the body is large and soft, as in the case of the decaying plant tissue commonly used as feeding grounds by the Paramœcia, the anterior end is imbedded for a certain distance in the soft material, and the cilia in this whole imbedded region are stiff and quiet.

In the oral groove the strong cilia are exceedingly active, all striking toward the mouth. Within the groove the cilia are therefore directed toward the rear, producing a strong backward current of the carmine particles. On the elevated edges of the groove the cilia strike inward, toward the middle of the groove, so as to drive particles into the current passing along the groove toward the mouth. Behind the mouth the strong current passing backward is stopped and turned outward, away from the animal. The result is the formation of a strong whirlpool on the oral side of the anterior half of the infusorian, as shown in Fig. 19. (Whether this strong motion of the oral cilia is to be considered in itself a reaction to the contact stimulus, or whether these cilia move in the same manner in the free swimming animal I have not determined.)

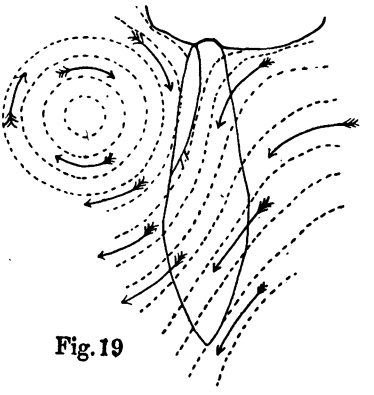


Fig. 19

Side view of Thigmotactic Paramœcium, showing currents of water.

Over the remainder of the body of the animal the cilia are comparatively quiet. They are not held stiffly immobile, as at the part in contact with the solid body, but may generally be seen to be quivering everywhere. Occasionally in one or the other part of the body the cilia are exercised energetically, changing the position of the animal, forcing it closer to the object or the like. In cases where several individuals are gathered about a single movable particle, as described earlier, the cilia covering the body (which may be distinguished as *locomotor* cilia as compared to the *oral* cilia in the groove), must of course be in active co-ordinated motion. The characteristic effect of the contact stimulus on the locomotor cilia is to inhibit their motion: they may however respond to a certain extent to other stimuli, while in this condition, as will be shown in detail for the electric stimulus.

As the locomotor cilia are thus comparatively still, they do not produce currents when the animal is quietly feeding; the entire set of characteristic currents under this condition is due to the whirlpool caused by the cilia of the oral groove. The water rushes in from all sides to join this whirlpool, so that the currents present an arrangement which is shown in side view in Fig. 19. From the anterior end and the aboral side the currents pass obliquely toward the mouth, and then to the rear, so that everywhere at the posterior end of the animal the currents are to the rear. This is modified in some cases, probably by the position of surrounding objects, in such a way that the currents at the posterior end form an arch, obliquely forward on the aboral side, obliquely backward on the oral side. Either of these arrangements is comprehensible as due to the presence of the whirlpool on the oral side of the anterior end. In the former case the water forming the currents which pass into the whirlpool from the aboral side comes more from the anterior end; in the latter case more transversely or partly from the posterior end,—the anterior region being blocked by the presence of a solid body.

When the solid body inducing the thigmotactic reaction is very small and movable, as in the case of a single carmine grain at the anterior end of the animal, the Paramœcium does not itself remain quiet, but moves in a peculiar manner. The creature moves partly forward, partly sideways, in such a way that it describes a circle, or rather, its two ends describe two concentric circles, the inner one being traced by the anterior end; the long axis of the infusorian always lying in a radius of the two circles. Reflection shows that

this motion is merely the resultant of the various directive impulses due to the motion of the cilia in the oral groove, as indicated in Fig. 19. The cilia beating strongly backward in the oral groove of course impel the animal forward. But as the active cilia are all on one side, there is also a tendency to move toward the opposite side. The resultant of these two motions at right angles to each other is a motion in the circumference of a circle. In fact, the animal moves forward in exactly the lines indicated by the arrows in Fig. 19, only of course in the opposite direction from the water currents. The ciliary motion is thus the same whether the thigmotactic animal is at rest or in motion: in the former case it is the water currents that move obliquely backward; in the latter case the animal moves on the same lines obliquely forward. The Paramœcium is, as it were, whirled about in its own whirlpool.

Summing up, we may say that under the influence of the contact stimulus the cilia of three regions of the body are affected in three different ways. The cilia which are in contact with the solid body are held stiff and immobile, at right angles to the surface of the animal. The cilia of the oral groove have a strong motion directed toward the mouth. Over the remainder of the body the cilia are quiet or quiver ineffectively.

### *Interference of Thigmotaxis and Electrotaxis.*

As described in the "Introductory Experiment" (page 260), after the Paramœcia have gathered closely about the bit of decaying plant tissue or other solid, they no longer respond to the electric current. Before collecting about the bit of paper, the Paramœcia respond to a current from five or six or even a less number of chromic acid cells. After they have collected on the solid, the same current seems not to affect them. Increase the current to 10 cells; 20 cells; the Paramœcia still remain quietly against the bit of paper. When 30 cells are employed the current is so strong that sparks are obtained as the quicksilver key is closed, and it passes through a very thin layer of water containing the Paramœcia, so that the intensity must be great. Still the Paramœcia remain in place. Very slowly, however, the outermost Paramœcia in the group may now be seen to loosen themselves and swim to the cathode,—especially if the direction of the current is reversed several times in succession. But many maintain their position for a long time. With a weaker current—say of 10 cells—the Paramœcia retain their

position indefinitely, though under other circumstances they respond at once and strongly to such a current.

We know from the work of Ludloff that the effect of the constant electric current upon Paramœcium is due to its influence on the stroke of the cilia. At the side or end of the Paramœcium which is directed toward the anode the cilia are so influenced that the effective stroke is to the rear, and urges the animal forward. On the cathode side or end the influence is such as to make the effective stroke of the cilia forward, thus urging the animal itself backward. The result is that if the animal lies in a position which is oblique to the direction of the current, it is at once turned by the action of the cilia above described, until it lies with its long axis in the direction of the current and its anterior end to the cathode. The animal then swims to the cathode as a result of the greater effectiveness of the backward stroke of the cilia on the posterior (anode) half of the body, as compared with that of the forward stroke on the anterior (cathode) half. (For details see the paper of Ludloff.)

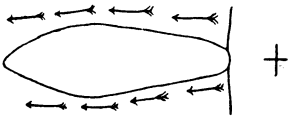
The key to the fact that thigmotactic Paramœcia do not respond to the electric current must be looked for therefore in some change in the way that the cilia react to the electric stimulus. I have studied this reaction in the same manner as above described for the study of the ciliary motion in thigmotaxis alone. Carmine grains were introduced into a preparation of Paramœcia in which the animals were gathered about some solid object, then the currents of water produced by the motion of the cilia when the constant electric current was passed through the preparation were observed. For applying the unpolarisable electrodes to the preparation, the cover-glass was supported at each end either by pieces of filter-paper, or by disks of clay, as described by Ludloff.

The necessary observations require a great deal of time and patience, as individuals under exactly the required conditions are not easy to find. The Paramœcium to be observed must of course lie in the characteristic thigmotactic position, resting against some solid body. Furthermore, it must be a solitary individual, at some distance from others, in order that the currents produced by its cilia may not be interfered with by currents from other Paramœcia. As the Paramœcia tend to congregate, this condition is hard to find fulfilled. Then the individual must lie either parallel with or transverse to the direction of the electric current, as obliquely lying individuals do not give clear results by the method of studying the currents. Having found such an

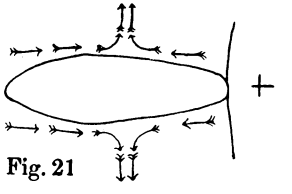
individual, it is next necessary to observe carefully the direction of the currents caused by the cilia before the electric circuit is closed, as well as to note which end or side is toward the future anode or cathode.

We will take up first the case where the Paramœcium lies with its long axis in the direction of the electric current, the anterior end to the *anode*, the posterior to the cathode. The anterior end of the animal is pressed against the piece of filter paper, and the currents of water are as shown in Fig. 19. As will be seen from this figure, in the commoner case the current is obliquely to the rear over the whole animal, and if the infusorian is seen in any but a side view this obliqueness disappears, the currents seeming to pass straight back. The position of the animal and direction of the currents may therefore be indicated by Fig. 20.

Fig. 20




Thigmotactic Paramœcium, showing direction of water currents with electric circuit *open*.




Same individual shown in Fig. 20, after closing the electric circuit.

Fig. 21

If now the electric circuit be closed, with cathode and anode in the position shown, the current of particles is quickly *reversed* on the posterior half of the animal, but continues in the same direction on the anterior half. The result is shown in Fig. 21.

If now the electric current is reversed, so that the cathode and anode change places, the direction of the stream of granules over the posterior half of the body is likewise reversed, passing again to the rear as at the beginning. The animal retains its position undisturbed, however; or sometimes it swings into an oblique position, so that the effects are no longer clear. But often the reversal may be repeated a large number of times, the animal remaining in place. I will copy from my record two typical cases of this sort.

(*a*) Paramœcium as shown in fig. 20. With the electric circuit *open* the stream of particles is to the rear. Circuit closed with the posterior end to the cathode; the stream of particles now passes *forward* on the posterior half

of the animal. Electric current reversed; stream of particles likewise reversed, passing again to the rear. In 30 seconds the electric current was reversed 13 times, accompanied in every case by a reversal of the stream of granules. The circuit was then opened and the animal was observed 45 seconds longer; no reversals occurred during this time.

(*b*) Paramœcium as in fig. 20. Electric current reversed 10 times in 20 seconds, accompanied each time by a reversal of the stream of granules. Then the circuit was open for 30 seconds; no reversal during this time.

If however instead of reversing the electric current we allow the circuit to remain closed, with anode and cathode as shown in Fig. 21, we find that the current of granules over the posterior half of the animal *does not continue* to pass forward, as in Fig. 21, but in a few seconds the currents begin to flow again in their original direction (Fig. 20). The electric circuit remaining continuously closed, in a few seconds there is another reversal of the current at the posterior end (Fig. 21), lasting but an instant, then a return to the original direction. Then another reversal; another return, and so it continues indefinitely. A few extracts from my notes, records of actual cases observed, will bring out the conditions and limitations of this phenomenon. The position of the animal was in every case as shown in Fig. 20. A "reversal" signifies a period when the cilia strike *forward* over the posterior half of the body.

(*c*) The animal was watched 40 seconds continuously, with *open* circuit; during this time the stream of granules was always to the rear. Circuit closed; current of particles at the posterior end reversed, then returned to the original direction. Circuit remained closed 25 seconds, during which time there were seven such reversals, at intervals of 2 to 5 seconds. The reversals became more frequent in the latter part of the period; the animal became restive, swung back and forth, till finally it swung entirely out of its position and was not observed farther.

(*d*) Six reversals of the current of granules in 15 seconds with closed circuit.

(*e*) With circuit *open*, no reversals in 45 seconds. Circuit closed; three reversals in 60 seconds.

(*f*) Circuit closed; six reversals in 18 seconds; circuit opened, no reversals in 12 seconds.

(*g*) Circuit closed for 60 seconds; nine reversals at nearly equal intervals. Each reversal commonly lasted but an instant; sometimes however 4 or 5 seconds.

(*h*) Circuit closed 30 seconds; 10 reversals.

(*i*) Circuit closed; no reversals for 15 seconds; then six at intervals of about three seconds; then no more for 30 seconds. Observations interrupted.

(*j*) Circuit open for 30 seconds; no reversals. Closed 90 seconds; 8 reversals. Circuit continues closed 60 seconds longer, but no more reversals occur.

(*k*) Circuit closed 28 seconds; 18 reversals in this period.

From the above it appears—

(1) That the current of granules, originally toward the posterior end in thigmotactic individuals, is reversed at the cathode end when the animal is subjected to the electric current with the cathode at the posterior end;

(2) That the reversal does not last, but the current produced by the cilia quickly returns to its original direction, *even when the electric circuit remains closed.*

(3) That the length of time which the reversal lasts varies from an instant to several seconds.

(4) That the interval of time between the reversals varies from a fraction of a second (case *k*) to 20 or more seconds (case *e*).

(5) That in some cases the electric circuit may remain closed a considerable time without causing any reversals (cases *i* and *j*).

(6) That sometimes the first reversal does not occur till some seconds after the circuit is closed (case *i*). This is often true.

I may add—

(7) That cases occur, though rarely, where the ordinary current used (10 cells) causes no reversals at all. In other cases, while no actual *reversals* of the currents of particles occur, sudden and repeated *stoppages* indicate the effect of the electric stimulus.

In Paramœcia occupying the positions shown in Fig. 20 no change was observed in the currents of granules over the anterior half of the animal when the electric circuit was closed.

If the Paramœcium lies in a position the reverse of that shown in Fig. 20—that is, if its anterior end is to the cathode, the posterior to the anode—the effect of the electric current on the stream of granules is not so noticeable as in the foregoing case. If the stream of granules over the posterior half of the animal (anode end) is already toward the rear when the circuit is open, closing the circuit simply increases the strength and rapidity of the current. In the few cases where the current of granules is obliquely forward at the posterior end of the animal when the circuit is open, closing the circuit with the anode at

the posterior end causes a reversal of this current—the granules passing to the rear. This case—the posterior end of the animal to the anode—is much more difficult to study than the opposite one, because the animals generally swing quickly into another position when the circuit is closed. At the anterior end (toward the cathode) commonly no change is visible. In a few cases, by direct observation of the cilia, they seemed to take a position such as to be directed a little more *forward*, as would be expected from Ludloff's results. In other cases however precisely the *reverse* change seemed to take place. But it is only natural that the results here should not be clear, when one considers the effect of the motion of the cilia over the posterior half of the body. The strong backward current of particles in this region produced by the closing of the circuit corresponds of course to a strong tendency to push the animal itself forward. As the anterior end is generally partly imbedded in the soft mass of decaying plant tissue, the forward push from behind tends naturally to bend the anterior cilia backward. Hence the variation in the observation, due to the varying ratio of the effects produced by the current itself (inclining the cilia forward, according to Ludloff) and the push from behind (inclining the cilia backward). The effect of the electric current on the posterior half of the body is clear; on the anterior half (by this method of study) it is not.

The third case which calls for discussion is that in which the animal lies transversely to the direction of the current. Suppose the current of particles is everywhere to the rear when the circuit is open. The circuit is now closed with anode and cathode on opposite sides of the animal. Immediately there is a reversal of the stream of granules on the cathode side, the granules here passing *forward*, while on the anode side it continues backward as before. The currents under this condition are shown in Fig. 22. If now the electric current is reversed, the streams of granules on both sides of the animal are reversed—passing backward on the previously cathode side (now turned toward the anode), and forward on the previously anode side (now turned to the cathode). The change in the direction of the motion of the cilia naturally produces some change in the position of the body of the infusorian, the animal swinging on its fixed anterior end as a pivot a certain distance towards the cathode. Therefore as the electric current is successively reversed, the animal oscillates back and forth.

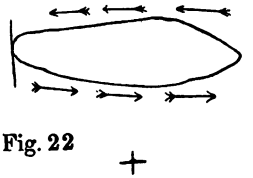



Fig. 22

Some examples may be given of the phenomena described.

(*l*) Paramœcium with the left side to the cathode; stream of particles on both sides to the rear when the circuit is open. Electric circuit closed; stream on left side now forward. Circuit open; stream on both sides again to the rear. Circuit closed three times in succession, each time producing a reversal on the cathode side.

(*m*) Animal transverse to the electric current, with the stream of granules on both sides to the rear. Circuit (30 cells) closed; stream of granules reversed on the cathode side, passing *forward*, and the animal swings toward the cathode. Electric current reversed; stream of granules on both sides reversed, and the animal swings in the opposite direction—toward the new cathode. Reversal repeated 25 times, always with the same result. The animal does not leave its place.

If the circuit remains closed after having caused a reversal of the at first backward stream of granules on the cathode side, in a few seconds the stream of granules returns to its original direction. The circuit remaining closed, the stream of particles is again reversed on the cathode side after a short interval. These oscillations continue, exactly as described in detail for the case in which the posterior end is directed to the cathode,—except that in the transverse position it is only one side which shows the repeated reversals—the stream of granules remaining always directed to the rear on the anode side.

In interpreting these results we must keep in mind the normal motion of the cilia in thigmotactic individuals. As described previously, in such animals the oral cilia strike strongly backward, while the locomotor cilia show merely an ineffective quivering. The reversal of the current on the cathode half of the body indicates thus that the locomotor cilia of this half are suddenly set in motion, such that they strike forward. It does not imply however a reversal of a previous motion of these cilia. The conflict between the two stimuli is therefore to be expressed as follows. The contact stimulus tends to keep the locomotor cilia at rest; the electric stimulus to set them into strong motion—directed forward at the cathode side or end; backward at the anode side or end. Whether the electric stimulus has an effect on the oral cilia also is not determined by these observations.

In summing up, we may say:

1. (*a*) On the anode end or side the effect of the constant electric current is to drive the stream of particles to the rear. The cilia are so affected as to make the backward stroke stronger.

(*b*) On the cathode end or side the effect of the constant electric current is to drive the stream of granules forward. The cilia are so affected that the forward stroke is made stronger.

For the anterior end these phenomena were not determinable by the method used, but the results were clear for the other parts of the body.

The results are thus (omitting from consideration the undetermined point just mentioned) a confirmation of Ludloff's results. I have thought it worth while to emphasize them, as they were gained by a different method from that used by Ludloff.

2. A *struggle* takes place between the contact stimulus and the stimulus of the constant electric current. The characteristic arrangement of the locomotor cilia in thigmotaxis is overcome by the electric current for an instant, then the contact stimulus resumes its sway; then the electric; and thus the oscillations continue. The two stimuli do not unite and give a resultant reaction; the cilia seem to react at a given time to one or the other alone. With the ordinary electric current that suffices easily to direct the free swimming Paramœcia to the cathode, the contact stimulus shows itself the stronger and the infusoria do not swim to the cathode, but remain in contact with the solid body. With a very strong current (30 small chromic acid cells newly filled), the action of the electric current is very powerful; the reversals in direction of the water currents and the accompanying changes in position of the Paramœcia become convulsive in character. One by one the Paramœcia loosen from their position and swim to the cathode. There are very great individual differences in regard to this; some few individuals retained their position on the bit of filter paper in spite of the strongest current I could produce with the battery at my disposal. But the great majority may be forced by such a current to finally leave the solid body and swim to the cathode.

I do not feel prepared at present to enter upon a discussion of the significance of this struggle between two stimuli. Probably the phenomena here described may find explanation from some simple point of view when the precise nature of reactions to stimuli in unicellular organisms is better understood, and especially if the essential nature of the effects of the electric stimulus become better known.

### 4. Part played by these Reactions in the Normal Life of Paramœcium.

Examination of the distribution and conduct of the Paramœcia in an ordinary culture jar indicates that three stimuli play the chief *rôles* in the daily activities of these infusoria. These are (1) negative geotaxis, (2) positive thigmotaxis, (3) positive chemotaxis toward $CO_2$.

The effect of *negative geotaxis* is at once evident in the presence of all the Paramœcia in the upper part of the water. The geotaxis of Paramœcium has been minutely studied by Jensen, so that no discussion is necessary here.

That *thigmotaxis* plays an important part in the daily life of the infusoria is shown by the fact that the large majority of them will generally be found clinging to some solid body. A large number are attached to the decaying plant tissue just below the surface of the water. As we have seen, there is no evidence that this material in itself exercises a chemical attraction for the Paramœcia, and they gather in exactly the same manner on entirely neutral substances. A second very large portion of the infusoria are gathered in a narrow band on the glass wall of the jar, not far below the upper surface of the water; here of course an active chemical action of the substratum is out of the question. The action of the cilia in thigmotactic individuals is, as we have seen, such as to tend to bring food into the mouth.

The *rôle* played by positive chemotaxis towards $CO_2$ in the conduct of Paramœcia under various circumstances has already been elucidated in a considerable number of examples (p. 290). In the culture jars the effect of this reaction is seen in the collecting together of the infusoria into dense assemblages. For example, the thigmotactic Paramœcia above described are not scattered here and there over the surface of the glass, but are gathered in a close band, not far below the upper surface of the water. The exact level at which this assemblage takes place is determined chiefly by the rapidity of the escape of $CO_2$ from the water surface. The Paramœcia tend of course to rise as high as possible, owing to their negative geotaxis. But close to the surface the carbon dioxide escapes so fast that the Paramœcia sink lower and remain at a level where the $CO_2$ produced by themselves reaches a concentration to which they are attracted. The same is true of the infusoria gathered on the decaying plant tissue. Moreover, even at the level where the gathering is formed, the Paramœcia are not collected in all parts in like

numbers. Here and there dense assemblages, where the animals are gathered literally into masses, are found; these are due to the crowding together of the Paramœcia, owing to their positive chemotaxis toward the $CO_2$ produced in such a gathering.

Other reactions seem to play a less important part in the life activities of Paramœcium. *Thermotaxis* is sometimes noticed in the collecting of the animals on the warmer side of the jar; in my cultures however this reaction seemed to come rather rarely into play. It is probable that in the case of Paramœcia subjected to great variations in temperature, as may sometimes occur under natural condition, thermotaxis plays an important *rôle*. *Electrotaxis* is of course unknown in the normal life of Paramœcium. Positive chemotaxis toward other substances than carbon dioxide seems to have no important place in the normal life activities of the animal, and negative chemotaxis is scarcely to be observed under natural conditions. The three reactions first discussed seem to be the chief determining factors in the life activities of Paramœcium.

## 5. General Discussion.

The present paper is to be considered as merely the first of a series the purpose of which is to contribute to the knowledge of the essential nature of reactions to stimuli in single-celled organisms, and especially to investigate the relations of the phenomena studied to morphogenesis. It is hoped that at the end of the investigation thus begun some general conclusions of value may be presented, but it does not enter into the plan of this first contribution to discuss at length the broad general problems which this field of work offers. Certain phenomena above described which have a definite bearing upon some of these general problems may however here be emphasized.

All the reactions studied in the foregoing investigation are expressed by some change in the motions of the cilia. Before therefore conclusions as to the nature of these reactions can be gained, the exact changes in the motion of the cilia, and the precise relation of these changes to the stimulus causing them must be determined. For Paramœcium the only reaction which may be said to have been studied in this manner is electrotaxis, as presented in the work of Ludloff. A similar method must be followed for other stimuli before satisfactory general conclusions can be gained. An investigation carried out on these lines would have such questions as the following to answer:— How do the cilia react to localized stimuli? For example, does the

same stimulus produce the same result whether applied to the anterior end, the posterior end, or the sides of the animal? Are these characteristic reactions for the different sorts of stimuli—as for different chemical reagents, and what are these reactions? If the stimulus is continued, do the reactions change? What differences are there between reactions to sudden and to gradual stimuli? Is there any single characteristic reaction to *all* very strong stimuli?

For determinations of these questions three methods may be used; observation of the motion of the animal's body, observation of the currents caused by the motion of the cilia, and direct observation of the motion of the cilia, under the influence of the proper stimuli. All these methods together would doubtless be necessary for accurate results in all points. In the foregoing investigation only the two first were used to any extent, and of course no attempt was made to answer all the above questions, yet some results were gained which would take their place in a systematic investigation of the sort above suggested.

For the contact stimulus, a characteristic position and motion of the cilia was demonstrated. According to the results gained in this field, we must distinguish in Paramœcium two different systems of cilia, whose reactions must be studied separately: these are the *oral* cilia, which lie in the oral groove, and the *locomotor* cilia, covering the rest of the body. The oral cilia were shown to have in thigmotaxis a definite method of action, resulting in well-characterized currents of water or motions of the animal's body. In the case of the locomotor cilia it is necessary to distinguish those which are in direct contact with the solid body, and those which are free. The former are held stiff, straight and immobile; the latter keep up an ineffective quivering. The ciliary motion when the animal was acted upon at the same time by a contact stimulus and the electric stimulus was also studied, and shown to consist in an alternate prevalence of the reaction due to each of the two stimuli taken separately. The two stimuli do not combine to produce a reaction which is the resultant of the two influences, but they alternate in controlling the motion of the locomotor cilia. The observations on the reaction to the contact stimulus show the exceedingly complex character of reactions, even in a unicellular organism. A single stimulus produces three different results in three different regions of the body. A renewed investigation both of electrotaxis alone and of its combination with thigmotaxis, with relation to the precise action of the *oral* cilia, as distinguished from the others, might bring out some new relations of value for our analysis. The division of the

body of the infusorian into two ends and an intermediate surface or "sides" is evidently entirely inadequate for an exact analysis of the reactions of the cilia; it is quite possible that other regions beside the oral groove will be found to take a separate position.

For chemical stimuli definite results were gained on certain of the questions above proposed. We will here leave out of consideration the question whether the Paramœcia gather about a source of stimulus or flee from it; such activities are too complex to give precise results as to the motion of the cilia. We will discuss here only such activities as indicate exactly the motion of the cilia causing them.

When the anterior end of an animal swimming forward comes in contact with a strong chemical stimulus, the reaction takes the form of a *reversal* of the effective motion of the cilia; the *forward* stroke becomes strongest, driving the animal *backward*. The strength and duration of the reaction bear a clear relation to the strength and quality of the stimulus. A weak stimulus causes only such a reversal as drives the animal backward but a fraction of its own length, while if the infusorian comes in contact in the same manner with a drop of strong acid it darts backward a long distance—twenty times its own length or more. It appears that this form of reaction is not characteristic for any particular kind of stimulus; a similar (though weak) darting back is observed when the infusorian strikes a mechanical hindrance. It is probable that any strong stimulus acting upon the anterior end of the animal causes the cilia to strike suddenly forward, thus forcing the animal backward.

The same effect is often produced if a sudden strong stimulus acts upon the animal from all directions at once,—that is, if the stimulus is not localized at all. Thus if the Paramœcia are suddenly dropped into a solution of some chemical which affects them strongly but does not at once kill them they begin at once to swim backward. The same is true if they are introduced suddenly into a chemically indifferent solution having a strong osmotic action, as into a solution of sugar or glycerine. Sudden introduction into water heated to 34° C. has the same effect.

For studying the nature of reactions due to a stimulus acting upon some definitely localized region of the body, other than the anterior end, other methods will have to be used, and the investigation will doubtless be accompanied with great difficulties. For the case of a stimulus acting upon one side, from which the Paramœcia turn away, as for alkalies and many other substances, three possible reactions suggest themselves. (1) The cilia on the side touched may have their effective backward stroke increased in power. This would turn the animal away from the

source of stimulus, but would at the same time urge the animal slightly forward. (2) The cilia on the side opposite the source of stimulus may have their effective stroke reversed, so as to strike most strongly forward. This would turn the animal away from the source of stimulus, and tend at the same time to drive it backward; it would therefore be apparently a more advantageous form of reaction than the foregoing, in the case of a strong and injurious stimulus. (3) There may be some combination of these two methods of reaction.

Similar considerations apply to the case of a one-sided stimulus to which the animals are positive. After these problems have been thoroughly worked out it will be possible to compare them with the reaction to the electric current described by Ludloff, and thus to gain some basis for a judgment in regard to a possible relation of the electric stimulus to the chemical effects of the constant current,—such as is suggested by Loeb and Budget.

Passing to a slightly different aspect of the subject, a peculiarity of the manner of reaction of Paramœcium to chemical substances may here be emphasized. The animal seems to respond only when there is a distinct and sudden change in the chemical nature of the medium. If a drop of some attractive substance is introduced into the centre of a preparation containing Paramœcia, they do not swim in straight radial lines to the drop as a centre. On the contrary, the course of the individual is apparently not modified in the least until an animal strikes in its random course the margin of the diffusing drop; then the characteristic reaction takes place. Furthermore, the collection of Paramœcia in a region containing an attractive substance gives the impression of being due rather to a negative taxis to the surrounding fluid, after having entered the attractive solution. The Paramœcium often swims into the drop of attractive substance without showing any indication of stimulation, but as it attempts to pass out on the other side a strong negative stimulus occurs; the cilia are wholly or partly reversed, and the animal remains in the drop.

Turning now to questions of a different character, we may ask what is the bearing of the fact that Paramœcium is negatively chemotactic to the water in which it lives? As I have shown, the water containing decaying vegetable material, in which the infusoria live and thrive, has an alkaline reaction, and the general rule that Paramœcia are negatively chemotactic to alkaline fluids suffers no exception in this case. The negative chemotaxis toward this fluid is shown by the avidity with which Paramœcia immersed in it gather into a drop of neutral fluid,

such as distilled water or sugar solution, as well as by the fact that Paramœcia already in a neutral solution avoid without exception a drop of water from the culture jar. What relation has this to the subject of *acclimatization* or adaptation to the environment?

It seems to me that we are driven to the simple conclusion that negative or positive taxis is not a test of adaptation. It cannot be doubted that the Paramœcia are thoroughly acclimatized to the solution in which they live. Here they thrive and multiply immensely. Yet they are strongly negatively chemotactic to this same solution.

A related problem is presented by the strong positive chemotaxis of Paramœcium toward carbon dioxide. This reaction plays a most important part in the life of the organism; in fact I believe that it is not too much to say that it is the chief directive influence affecting Paramœcium. Yet we know that carbon dioxide is decidedly injurious to most animal protoplasm, and Loeb and Hardesty have shown that it is a positive poison for this same infusorian Paramœcium,—acting in virtue of its own chemical properties and not merely through its taking the place of the necessary oxygen.

Doubtless other protozoa will be found that are attracted by $CO_2$. This is rendered especially probable for certain infusoria by the observations of Pfeffer, already referred to. Pfeffer (p. 618) observed such collections of individuals as are described in this paper for Paramœcium, in the case of *Glaucoma scintillans*, *Colpidium colpoda*, *Stylonychia mytilus*, and in Paramœcium itself. As I have tried to show on p. 294, the collections in all these cases are probably due to the same cause as in Paramœcium—positive chemotaxis toward the excreted carbon dioxide.

We must not forget however that Paramœcium is repelled by a concentration of $CO_2$ that is strong enough to be injurious. This would seem to make it probable that such solutions of $CO_2$ as are attractive, are so because they are beneficial. That carbon dioxide should serve any beneficial purpose in the animal cell seems however on general grounds highly improbable, and this improbability is increased by the fact that the $CO_2$ by which the Paramœcia are attracted is excreted by the Paramœcia themselves. It seems exceedingly paradoxical that an organism should excrete a substance to which it is strongly attracted. Yet this is undoubtedly the case. The paradox seems almost to rise to an absurdity however if it is held that the animals are attracted to this excreted substance because they *need* it; in that case why should it have been excreted?

It is perhaps comprehensible that an organism should sometimes be attracted by an injurious substance with which it normally never comes in contact. One might hold in such a case that the reaction toward this substance has never been determined by natural selection or otherwise; the organism might therefore react to this in the same way as toward some other substance having a somewhat similar action, but uninjurious, with which the animal *has* previously come into relation. Such a reaction might perhaps be called a mistake on the part of the organism, due to the similarity of two substances, one beneficial, the other injurious. In other words, the protoplasm has never become so differentiated as to give a different reaction with these two substances. It is of course a well-known fact, brought out in Pfeffer's researches, that organisms *are* often attracted by substances with which in a state of nature they never come in contact, these substances having a chemical similarity to certain others which form a customary means of attraction. Thus, it is known that potassium chloride is strongly attractive to certain bacteria and doubtless plays an important part in their life activities. These same bacteria may be likewise attracted by the closely related substance rubidium chloride, though under normal circumstances they undoubtedly never come in contact with this.

But any such explanation is of course entirely excluded in the case of carbon dioxide—a substance with which the Paramœcia are continuously in contact, in one way or another, especially as the reaction due to it plays such an important part in the life activities of the animal. The possibility occurs to one that the positive chemotaxis toward $CO_2$ may have some relation to the organisms upon which Paramœcium feeds—the bacteria of decaying vegetable material. There seems however to be nothing known to suggest this possibility. The evident result of the positive chemotaxis of Paramœcium toward $CO_2$ is the gathering together of the infusoria into dense collections, but what purpose is served by these collections does not appear.

The attraction of Paramœcia toward self-excreted $CO_2$ seems to throw light on a general question proposed by Pfeffer; namely, whether it is necessary, in order that a substance should act as an attractive stimulus, that it should be taken into the organism. As the $CO_2$ in the water was first produced within the Paramœcia, and acts as a stimulus after it has been given off to the outside, it appears that the stimulus must be an external one; Pfeffer's question, in accordance with this, is to be answered in the negative.

## 6. Summary.

1. Paramœcium aurelia is strongly positively chemotactic to a solution of *carbon dioxide* in water.

Carbon dioxide is excreted by the Paramœcia in quantities sufficient to be detected with proper reagents. By this excreted $CO_2$ the infusoria are attracted; hence they tend to gather into dense groups. This positive chemotaxis to the carbon dioxide excreted by themselves plays a very large part in the normal activities of Paramœcia.

2. Paramœcium is also positively chemotactic toward all weak acids and all solutions having an acid reaction, so far as tested.

Toward *strong* solutions of all acids, including $CO_2$, the infusoria are *negatively* chemotactic.

3. Paramœcium aurelia is *negatively* chemotactic to the fluid (water containing decaying vegetable matter) in which it lives and thrives. This fluid has an *alkaline* reaction. Paramœcium is likewise negatively chemotactic, so far as tested, to *all* solutions having an alkaline reaction. It is also negatively chemotactic to certain neutral salts and organic compounds.

4. Toward certain organic substances, as sugars, glycerine, urea, Paramœcium is entirely *indifferent*, so far as chemical properties are concerned.

5. Paramœcia which are collected in a drop of a solution to which they are positively chemotactic, show a modified reaction to the constant electric current. They swim toward the cathode till the boundary of the drop of solution to which they are positively chemotactic is reached, but cannot be forced past this boundary into the surrounding water, except by a very powerful and long-continued electric current.

6. *Tonotaxis*, the directive stimulus due to a change of osmotic pressure, acts only within wide limits, so that for most solutions it does not come into action at all, the chemical qualities of the solution determining the conduct of the Paramœcia long before the limit at which tonotaxis becomes effective is reached. While great variation in osmotic pressure does act as a stimulus, this stimulus is not of sufficient strength and definiteness to prevent the infusoria from swimming into solutions of such osmotic power that they are quickly killed. Tonotaxis therefore plays no important part in the life activities of Paramœcium.

7. Paramœcium responds in a characteristic way to the stimulus of

*contact* with solid bodies. This reaction consists of the following factors; (1) the Paramœcium places itself against the solid body (positive thigmotaxis), the cilia which are in actual contact with it being held straight, stiff and immovable; (2) the oral cilia have a strong and characteristic motion; (3) the locomotor cilia are nearly at rest, merely quivering ineffectively.

8. If through a preparation containing Paramœcia exhibiting the thigmotactic reaction a constant electric current is passed, the Paramœcia do not swim to the cathode, as under other circumstances, but remain in contact with the solid body.

Exact observation shows that under such circumstances the two stimuli alternate in controlling the locomotor cilia. On the cathode side or end the cilia are so affected by the electric current that the effective stroke is forward (as described by Ludloff), thus driving the water currents in the opposite direction from that which they have under the thigmotactic stimulus alone. But this reversal lasts but an instant; then the thigmotactic stimulus resumes its sway and the water currents again pass backward. The electric circuit remaining closed, another reversal takes place in a few seconds, then another return to the original direction, and this alternation of reactions may be repeated many times.

9. The reactions which play the chief part in the normal life of Paramœcium are *negative geotaxis*, *positive thigmotaxis*, and *positive chemotaxis toward carbon dioxide*.

The foregoing work was done in the Physiological Institute of the University of Jena, during the winter semester of 1896–97. It gives me pleasure to acknowledge here my great obligation to Prof. M. Verworn for much valued counsel and assistance throughout the work. I wish to express my thanks also to the Director of the Institute, Prof. Biedermann, for placing at my disposal in the most obliging manner the space and resources of the laboratory.

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