STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. VIII. — ON THE REACTIONS OF INFUSORIA TO CARBONIC AND OTHER ACIDS, WITH ESPECIAL REFERENCE TO THE CAUSES OF THE GATHERINGS SPONTANEOUSLY FORMED.

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IT is well known that when certain infusoria are left undisturbed they do not remain scattered, but gather in more or less dense groups. Thus, if they are mounted on a slide in a thin layer of water, soon dense aggregations will be formed in certain areas, while the remainder of the slide will be nearly deserted. One of the first investigators to describe this phenomenon was Pfeffer.[1] He observed its occurrence in Glaucoma scintillans, and less markedly in Colpidium colpoda, Stylonychia mytilus, and Paramecium. Pfeffer was inclined to believe that these aggregations were due, partly at least, to a contact stimulus, resulting from a striking of the organisms against small solid bodies, and especially against each other.

In the first of this series of studies,[2] this phenomenon in the case of Paramecium was subjected to a thorough examination. It was demonstrated that while the contact stimulus plays a certain part in the production of these aggregations, the chief factor involved is a reaction to carbon dioxide. The Paramecia tend to gather into regions where the water is impregnated with this substance. Since the animals themselves produce carbon dioxide in their respiratory processes, any spot where a few have gathered (owing to the contact stimulus or for any other reason), becomes a centre for the production and diffusion of this substance. Therefore other Paramecia collect here ; more carbon dioxide is produced ; more Paramecia collect, and in time a dense aggregation is formed. It was farther shown that this

[1] PFEFFER: Untersuchungen aus dem Botanischen Institut, Tübingen, 1888, ii, p. 618.

[2] JENNINGS: Journal of physiology, 1897, xxi, pp. 258–322.

effect of carbon dioxide is due to the fact that it forms in water an acid solution ("carbonic acid") — the Paramecia collecting in the same way in any substance having a weakly acid reaction.

As the other infusoria which form similar aggregations of course likewise produce carbon dioxide in their respiratory processes, it seems very probable, as was pointed out in the paper just referred to, that the same factors are at work here as in the case of Paramecium; that the spontaneous aggregations formed are due to the tendency of the organisms to collect in carbonic acid. This probability has been set forth also by later investigators, as for example in the recent paper of Rothert.[1] But no one has hitherto undertaken to determine by experiment in how far this may be true.

This is the problem which the study here presented has attempted to solve for a certain number of infusoria (sixteen species). The primary question to be answered is therefore as follows: — Are the spontaneous aggregations formed by certain species of infusoria due to their gathering in carbon dioxide excreted by themselves? The investigation involved a test of the reactions of the organisms studied both to carbon dioxide and to acids in general, and at the same time brought out a number of points as to the method in which the reactions of the organisms are produced; these secondary matters are likewise set forth briefly in the following paper.

Methods.

The method of experimentation most used was that described in the first and second[2] papers in this series of studies. The organisms were studied in a thin layer of water, by mounting them on a slide covered with a large cover glass supported near its ends by slender glass rods. Their reactions were tested by introducing with a capillary pipette a drop of the substance in question beneath the cover glass, or in some cases by allowing it to diffuse inward from the side of the cover glass. In the case of gases, as carbon dioxide, it was found very convenient to proceed as follows. The gas is introduced into a large rubber bulb, such as is used with syringes or atomizers. To this is attached by the rubber tube a glass tube drawn out to a fine point. By inserting the point beneath the cover glass and pressing the bulb, a bubble of gas is introduced into the preparation. Where

[1] Rothert: Flora, 1901, lxxxviii, p. 402.

[2] Jennings: This journal, 1899, ii, pp. 311–341.

still different methods were used, these are mentioned in the account of results.

In attempting to determine the reactions of organisms to carbon dioxide, it is of course absolutely necessary that there should be no considerable quantity of this substance already present in the water. And since the organisms are continually producing carbon dioxide in appreciable quantities, some method of getting rid of the gas is a practical requirement of the highest importance. The simplest method is to aerate the water thoroughly immediately before each test. This may be done as follows. Place a few drops of the fluid containing the organisms, — as much as will be placed on the slide at once, — in a watch glass; then with a clean pipette inject it repeatedly over the surface of the watch glass, force bubbles into it, and mix it thoroughly with the air. Then place on the slide, cover, and perform the tests at once. Repeat the aeration before every test, as it requires only a very short time for water crowded with organisms to become impregnated with carbon dioxide. Of course it is not reasonable to expect organisms to gather in carbon dioxide when the water in which they are found already contains this substance in the optimum concentration. This precaution is equally necessary in testing other acids, as it is the common factor in all acids to which the effects of the carbon dioxide are due.

This or an equally efficacious method of aeration is an absolute necessity, if clear cut and constant results are to be obtained with carbon dioxide or acid solutions in general. This cannot be too much insisted on. Sometimes definite reactions will be obtained without aerating the water, in case it happens not to be already impregnated with carbon dioxide, but a little later the same organisms may give negative results.

A second precaution worthy of mention is the necessity of having the water containing the organisms relatively free from débris: — filamentous bacteria, and the like. Most of the infusoria are markedly thigmotactic, tending to come to rest upon coming in contact with small solid bodies. If a preparation contains a network of fine bacterial filaments, frequently the infusoria will not gather in the acid at all, but remain at rest on the filaments, while if the filaments are removed, as by straining through coarse cloth, marked positive reaction is at once obtained.

No attempt was made to determine quantitatively the exact strength of solution to which the organisms react. The purpose of the work was to determine whether the organisms do or do not give at any concentration a certain reaction to the substances in question. This was accomplished by beginning with a concentration so slight that the organisms did not react to it at all, and gradually increasing the strength till the solution is destructive. Somewhere between these limits will be found the characteristic reaction of the organisms. The value of quantitative determinations of the exact concentrations of acids to which the organisms react is largely illusory, in the majority of cases, as this varies with the amount of carbon dioxide present in the water, — a factor not under exact control. It varies also apparently with organisms from different cultures, and with the thickness of the layer of water in which the infusoria are confined. In experimenting with carbon dioxide especially, it is impracticable to attempt the use of solutions of known strengths; the introduction of a bubble of gas into the preparation gives all concentrations, from saturation next to the bubble to zero at some distance from it.

In the following account of the work, the organisms will be taken up in the order suggested by the nature of the results obtained.

A. Organisms which collect in Solutions of Carbonic and other Acids.

Chilomonas paramecium. — This small flagellate is perhaps the commonest and most abundant member of the group to which it belongs. It is therefore the most accessible form for experimentation on the Flagellata, and it will probably usually be employed when work on this group is undertaken. It is therefore important that the fundamental facts as to its reactions should be well established. An extensive piece of work has already been done by Garrey[1] on the reactions of this organism to chemicals, especially to acids. To our great regret we were compelled to come to results essentially different in some respects from those set forth by Garrey. It is unfortunate that there should be such disagreement, as this is likely to result in leaving the subject doubtful in the minds of other investigators. We believe however that we are able to point out exactly the factor to which the differing results are due, and to show that Garrey's results would probably not have differed from our own if this factor had been taken sufficiently into consideration. This factor is the normal pres-

[1] Garrey: This journal, 1900, iii, pp. 291–315.

ence of an acid, — a solution of carbon dioxide excreted by the organisms, — in the fluid in which Chilomonas occurs.

Reaction to carbon dioxide. — Water containing Chilomonas is aerated in the manner above directed, and any bacterial filaments are removed by straining through coarse cloth. It is then placed on a slide, covered, and a bubble of carbon dioxide introduced. At first there is no gathering of the organisms, but soon they begin to collect about the bubble of gas, and gradually a dense ring is formed. Fig. 1 gives the general appearance of the progress of the experiment; it was taken from an actual preparation.

This experiment we have repeated many times, always (when the conditions were properly fulfilled) with the same results. The ex-

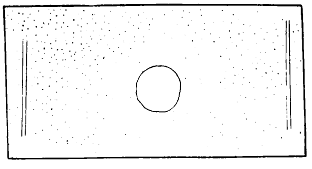

FIGURE 1, *a*.

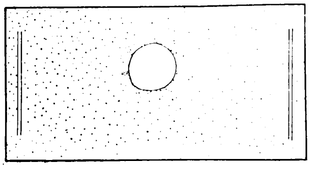

FIGURE 1, *b*.

FIGURE 1. — Reaction of Chilomonas to a bubble of CO_2. *a*, Preparation immediately after the introduction of the bubble, before the organisms have collected. *b*, The same preparation a few minutes later, showing the dense collection of infusoria about the bubble.

periments succeed equally well in the apparatus used by Garrey, and figured on page 294 of his paper. With a long capillary pipette a bubble of carbon dioxide can be introduced into the chamber beneath the cover glass. The flagellates at once gather about it in a dense ring, while they do not thus gather about bubbles of air similarly introduced.

Chilomonas thus gathers about bubbles of carbon dioxide in dense collections, just as Paramecium does. The conditions above referred to as necessary of fufilment are (1) that the carbon dioxide should be properly removed from the water just before making the experiments; (2) that the bacterial filaments and other débris in the water should be largely removed.

The justification of the first condition is at once seen. It is idle to test the organisms with carbon dioxide when they are already immersed in a solution of that substance. It is undoubtedly to a neglect of this precaution, which is nowhere so much as referred to by Garrey, that the negative results of this investigator are due.

The necessity for the removal of the débris is evident on examining the behavior of the organisms. Chilomonas is very strongly thigmotactic; if when swimming through the water it comes in contact with a bacterial filament or bit of débris of any sort, it at once attaches itself by one of its two flagella, and comes to rest. Thus, in a preparation containing such filaments, all the individuals will soon be found quietly attached, which of course prevents their collecting anywhere.

Reaction to other acids. — To what factor is the collecting in the solution of carbon dioxide due? Is it, as in the case of Paramecium, due to the acid qualities of this solution (to the H ions, according to the dissociation theory)? To answer this question, tests were made

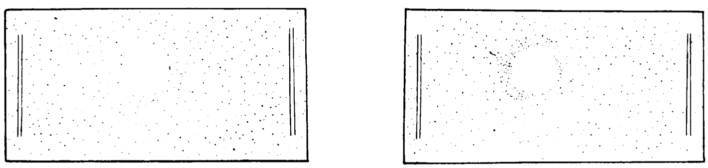

FIGURE 2, *a*. FIGURE 2, *b*.

FIGURE 2. — Reaction of Chilomonas to a drop of $\frac{1}{50}$% HCl. *a*, Preparation immediately after the introduction of the drop (no organisms either within or gathered about the drop). *b*, The same preparation a few minutes later.

with other acids, and the organisms were found to collect in weak solutions of these exactly as in the carbon dioxide solution. These results were clear cut and unmistakable; they were obtained with hydrochloric, nitric, sulphuric, acetic, formic, butyric, propionic, citric and oxalic acids.

As a control, the organisms were tested with distilled water; no gathering was formed about or in it at any time, but the organisms remained quite neutral in their behavior toward it.

The details of the phenomena vary with the strength of the acid solution used. With a stronger solution (say $\frac{1}{50}$ per cent HCl), the animals gather in a dense ring about the margin of the drop, leaving vacant the area within, containing the stronger acid (Fig. 2). With a weaker solution the organisms gather into the interior of the drop, leaving no part of it vacant (Fig. 3).

No characteristic difference was to be observed between their behavior to inorganic acids, such as HCl and HNO_3, and that toward organic acids, such as acetic and butyric, save that of course different concentrations were required to produce the same result. In Garrey's work, the results obtained with inorganic acids differed from those obtained with certain organic acids. Garrey studied the organisms in a much thicker layer of water, and introduced the acid through a tube-like opening at one side of the preparation. He observed that a dense gathering was formed about hydrochloric acid, but explained this as follows: When the strong acid reaches the organisms they begin at once to swim violently. This soon takes them outside of the area of acid, leaving it clear. On reaching the outer boundary, they stop, since there is no further cause for movement, thus forming a dense aggregation just outside the drop. "That in the zone surrounding the area there is a dense gathering (in other

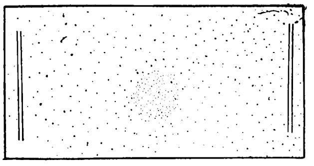

FIGURE 3.

FIGURE 3. — Collection of Chilomonas within a drop of $\frac{1}{100}\%$ HCl.

words that there is a ring formation) is in my opinion due to the fact that those individuals which were in the clear area are now gathered in the space immediately surrounding it" (*loc. cit.*, p. 296). The result is thus in a sense accidental, and would occur with any chemical which had the property of setting the organisms in violent movement. In Garrey's experiments no gathering was ever formed in the centre of a drop of inorganic acid, no matter how weak it was.

In our own work, the gatherings which occurred in drops of inorganic acids, were clearly not explicable in this manner. (1) When the drop was first introduced (Fig. 2 *a*) there were no individuals either within the drop or forming a ring about it. Later a dense ring was slowly formed (*b*). As there had been no individuals within the drop, of course the ring was not formed by individuals moving out of the drop and then stopping. Moreover, the process of ring formation is clearly evident to observation; it is due to the swimming of organisms into the ring from outside. (2) If a weak solution of acid is used, it is at first empty, but later becomes completely filled with organisms (Fig. 3). This of course could not possibly take place in the way assumed by Garrey. Collections of this sort were observed in the case of all inorganic acids studied. They were not formed when distilled water alone was used. (3) The gatherings about bubbles of carbon dioxide of course could not occur in the manner described by Garrey, as the bubble contained no individuals to move out and form a ring.

In the case of the organic acids, the phenomena observed by us were precisely parallel to those occurring in inorganic acids. It is possible that the gatherings formed last longer in the case of some organic acids, though to us this seemed not usually very marked.

The results obtained by Garrey with organic acids varied much in different cases. With oxalic, formic, citric, succinic and valerianic acids the phenomena were the same as with inorganic acids; *i. e.*, a clear area was formed, sometimes with a ring of organisms surrounding it (if the acid was strong); this ring formation Garrey explained as in the case of inorganic acids.

" Malic, tartaric and mandelic acids produce a clear area, often with a ring about it. In the formation of the ring, the phenomena were so inconstant that I was unable to say that it was or was not due to a migration of the organisms from without to it " (*loc. cit.*, p. 307).

Finally, with acetic, butyric and lactic acids, a clear area surrounded by a dense ring was formed, and Garrey was able to assure himself that the ring formation was due to a migration of the organisms from the outside.

Thus Garrey's results with acids can be placed in three categories. (1) Some showed a ring formation, which in the author's opinion was due merely to the driving of the organisms out of the area in which the acid was found. (2) Some gave such inconstant results that the author was not certain what he should conclude about them. (3) Some showed a ring formation of such density and clearness that it was evident that the organisms came from outside of the acid area.

Now this inconstancy and uncertainty in the results with acids is exactly what is obtained when the carbon dioxide is not removed from the water by thorough aeration before each experiment. The culture water contains varying amounts of carbon dioxide and in some cases a part of it is accidentally driven off in the manipulations preparatory to the experiments, in other cases not. The presence of carbon dioxide means also the presence in the water of whatever it is that gives acids their characteristic qualities. Hence in such a fluid the organisms are already in an acid solution and naturally do not react with any precision when an acid is introduced, while in cases where the carbon dioxide is partly or entirely driven off, distinct reactions are obtained; the results thus become inconstant and uncertain. After careful removal of the carbon dioxide before every experiment, the results with all acids are, according to our observations, essentially the same, — *i. e.*, a ring or group is formed by immigration of the organisms from the outside. It was this same neglect to remove the carbon dioxide from the water that led Garrey to deny the results with Paramecium, though these are demonstrable with ease.

In view of the contrasted results obtained by Garrey on the one hand and by ourselves on the other, it is much to be desired that some third person should reinvestigate the reactions of Chilomonas, testing the various methods used, observing the precautions set forth, and perhaps taking counsel by correspondence or conversation with both sides, that there may be no omission which might seem to vitiate the work. It remains of course possible that Chilomonas from different cultures reacts differently, though we have used dozens of different cultures and have observed no such difference.

Our own results on Chilomonas may now be summarized. This organism reacts to carbonic and other acids just as Paramecium does, forming dense collections in localized areas, where carbon dioxide is present. The spontaneous aggregations sometimes formed by Chilomonas may therefore be due to their collection in carbon dioxide excreted by themselves.

Cyclidium glaucoma. — This ciliate infusorian likewise gathers in carbon dioxide and in solutions of acids in general. The collections thus formed are dense and lasting. Cyclidium was not observed to form spontaneous collections, though this may occur.

Colpidium colpoda. — This is one of the infusoria which was described by Pfeffer as collecting spontaneously into groups. It reacts to solutions of carbon dioxide and other acid solutions, just as Paramecium and Chilomonas do, gathering in dense aggregations about a bubble of CO_2, or in a drop of weak acid. It is therefore probable that the spontaneous groups are due to carbon dioxide. If Paramecium and Colpidium are mounted together, they will gather spontaneously into groups, each group containing both kinds of infusoria, the boundary of the groups being practically the same for each. The cause of the grouping is thus evidently the same in the two cases.

B. Organisms which form Spontaneous Gatherings, but do not collect in Solutions of Carbonic or other Acids.

Oxytricha aeruginosa. — This organism, when mounted on a slide, forms spontaneous groups which are similar in every respect to those formed by Paramecium. The method of reaction to a stimulus in Oxytricha is by backing, and turning to the aboral (or right) side, — the side which is not notched. If the organisms are at first scattered uniformly throughout the preparation, they will soon be found to be forming groups in one or more regions. If the individuals within the groups are observed, they are found to be swimming hither and thither in all directions. But when one comes to the outer boundary of the group, it at once swims backward a short distance, turns toward the aboral side, and then starts forward again. As this happens every time the boundary of the group is reached, the animal remains within it. Individuals outside, whose course carries them by chance into the areas where a group is forming, do not react at all as they enter the area. But after swimming across, they do react as above described upon coming to the outer boundary of the area. Hence every Oxytricha that enters a group remains within it, and after a time a dense aggregation is formed. The groups thus produced increase in area, spreading out regularly, but maintaining a definite boundary.

The phenomena seem thus in every way identical with those observed in the case of Paramecium (see the first and second of these studies). It might therefore be reasonably expected that the cause would be found to be the same. But experiment shows that this is not the case; Oxytricha aeruginosa does not collect in regions where carbon dioxide is present, nor in other acid solutions. If a bubble of carbon dioxide is introduced into the preparation, the Oxytrichas do not gather about it, but on the contrary give their " motor reaction " when they come into its neighborhood, — reversing the direction of movement, and turning toward the aboral side. They thus leave the space about the carbon dioxide empty. Toward drops of acid solutions of all sorts they react in the same manner.

If Oxytricha and Paramecium are present in the same culture, or if the two are mixed together and experimented upon in the usual way, the results are as follows. The Paramecia collect about the bubble of carbon dioxide, or in the drop of acid, at once; the Oxytrichas do not. Thus a separation of the two kinds of infusoria is soon brought about.

If Oxytricha and Paramecium are mounted together and the slide is allowed to stand for a time, both kinds of infusoria will form spontaneous groups, but the two groups are quite separate. The Paramecia gather in one region, the Oxytrichas in another. Individuals of either kind may pass directly across the groups formed by the other, or swim in and out of the area where the other group occurs. The groups are thus clearly due to different causes in the two cases.

Oxytricha therefore forms spontaneous gatherings similar to those of Paramecium, but not due to the same cause. It seems evident that Oxytricha must excrete some other substance, not an acid, which acts upon it in the same way that the excreted carbon dioxide acts upon Paramecium. The nature of this substance remains to be discovered.

Loxocephalus granulosus. — In the case of this organism the facts are closely parallel to those described for Oxytricha aeruginosa. It forms spontaneous gatherings, but does not collect about bubbles of carbon dioxide nor in acid solutions in general. Mounted on the same slide with Paramecium, the two organisms form separate groups in different regions of the preparation. Clearly, Loxocephalus, like Oxytricha, excretes some substance which brings about the collections, but this substance is not carbon dioxide.

In preparations containing both Paramecium and Loxocephalus, the following may be observed as to the relations of the two organisms. Loxocephalus swims in and out of the groups of Paramecia, paying no attention to the limits so strictly observed by the Paramecia. In the same way Paramecium swims indifferently in and out of the groups of Loxocephali, when the latter groups are first forming. But after a group of Loxocephali has become well established and contains very large numbers of individuals, a Paramecium passing accidentally into the group usually remains there. Thus after a time a considerable number of Paramecia may be mingled with the Loxocephali. The Paramecia swim about freely within the group, but turn back on coming to an outer limit. It is to be noted that this outer limit is not the same as that which turns back the Loxocephali, but lies a little outside of it, so that the area in which the Paramecia are confined is larger than that which limits the Loxocephali, inclosing the latter.

These phenomena are probably to be explained as follows. Loxocephalus is not affected by carbon dioxide, therefore does not gather in the groups formed by the Paramecia, but swims in and out of them indifferently. But it does excrete some other substance, not of an acid nature, into which it gathers; hence the spontaneous collections formed. To this substance Paramecium is indifferent, hence it swims indifferently in and out of the groups of Loxocephali, at first. But of course Loxocephalus produces carbon dioxide in its respiratory processes, hence after a group of these organisms has been formed for some time, the water becomes impregnated with carbon dioxide, as well as with the other (hypothetical) substance. Paramecia now passing into the group remain, owing to the carbon dioxide. The areas over which the carbon dioxide and the hypothetical substance are effective are not identical, that for the carbon dioxide being a little larger; hence the limit of the excursions of the Paramecia is outside that for the Loxocephali.

C. Organisms which do not collect in Carbonic or other Acids, and which were not observed to form Spontaneous Gatherings.

The following organisms were tested with carbon dioxide and with solutions of various acids in the same manner as those hitherto described. Every precaution was taken to remove the carbon dioxide from the water before making the tests, and the experiments were repeated under various conditions, with uniform results. None of these organisms gather about bubbles of carbon dioxide or in solutions of acids. They are as follows: Oxytricha fallax, Euplotes charon, Stylonychia pustulata, Colpoda cucullus, Spirostomum teres, Stentor cæruleus, Enchelys farcimen, Halteria grandinella, Didinium nasutum, Euglena viridis, and Heteromita globosa.

Some of these organisms, on coming in contact with a solution of carbon dioxide, at once give their characteristic "motor reaction," backing and turning toward a structurally defined side; thus they turn away from the area in question, leaving it empty. Others do not react at all to carbon dioxide, and to other acids only when very strong. Those that were indifferent were Oxytricha fallax, Stentor cæruleus, Didinium nasutum, Euglena viridis, and Heteromita globosa.

D. The Method by which the Gatherings are Brought About.

Throughout the work attention was given to the method by which the infusoria gather together. The point which was especially studied was the question of orientation. Do the organisms collect in the region where a certain chemical is present because they become oriented in the lines of the diffusing ions? Or are the collections brought about in the manner described for Paramecium, in the first and second of these studies?

The phenomena were carefully examined in all the infusoria in which collections were observed, — in Colpidium colpoda, Oxytricha aeruginosa, and Loxocephalus granulosus, and additional observations were made on Paramecium and Chilomonas. All the ciliates mentioned are of sufficient size so that their movements can be exactly observed with the Braus-Drüner stereoscopic binocular, and there can be no doubt as to the method in which the gatherings take place. They collect in essentially the same manner as has been shown in previous studies to be true for Paramecium. The organisms are at first swimming freely hither and thither. When the drop of acid is introduced, or collections are produced in other ways in certain regions, some of the individuals swim into the area in question merely through their usual movements. They do not change their course or react at all as they enter the area. But as they swim across it and reach the opposite side, where they would if unchecked pass out of the area into the surrounding water, each infusorian gives its characteristic "motor reaction." Oxytricha after moving backward turns toward its unnotched side, Loxocephalus to the aboral side, Colpidium toward its convex side, Paramecium toward the aboral side. The animal is thus prevented from leaving the area containing the chemical, but swims in another direction within this area. As it reacts in the same way every time it comes to the outer boundary of the area, it does not leave it at all. Other individuals enter in the same way, through their random movements, and remain through the same reactions, so that after a time the areas in question swarm with infusoria.

If the animals are allowed to come thoroughly to rest before introducing the chemical, usually no collection is formed within it. This shows the essential part played in the reaction by the random movements of the organisms.

It seems difficult for many minds to believe that the dense gatherings observed can be produced in this way. That this is the real method by which the collections occur, can be very neatly demonstrated to the eye in the following manner. A number of Paramecia or other infusoria which collect in acids are mounted on a slide. Upon the upper surface of the cover glass a small circle about the size of the drop of acid usually introduced, is made in ink with the pen. By directing the attention to the area within the ring of ink, it will be seen that many infusoria (as many as ten per second or more, in an ordinary mount of Paramecium), cross the area every instant. It is therefore evident that if all of them could be stopped within the area, a dense group would soon be produced. With the capillary pipette a drop of acid is now introduced beneath the ring; the same number of infusoria now enter the area as before, but every one remains and a dense collection soon results.[1]

In the paper already cited, Garrey maintains that the flagellate Chilomonas collects in certain acids in a manner entirely different from that above set forth. He holds that the collections (in acetic acid, for example), are produced through an orientation of the organism in the lines of the diffusing ions. The reactions to other substances, drops of which are left empty by Chilomonas (as for example a solution of sodium chloride), take place in a way entirely different, according to Garrey. Here there is no orientation; the chemicals merely cause "swift shooting movements," by which the animal is carried out of the area, or prevented from entering it. The method of reaction exhibited in collecting in acetic acid is denominated by Garrey *chemotaxis*, while that shown in keeping out of or leaving a drop of sodium chloride he calls *chemokinesis*.

In the sixth of this series of studies, reasons drawn from a study of the movements of the individual Chilomonads have been given for rejecting this distinction in kind between the reaction in collecting and that in avoiding a region containing chemicals. Certainly no such distinction can be made in Paramecium, nor in the other ciliates above mentioned. Leaving out of account the direct observations on the movements of the individuals, there are certain experiments which amount almost to a demonstration that there is no such distinction in kind, — even in Chilomonas. They demonstrate at least that collections exactly similar to those produced through the supposed "chemo-

[1] This experiment was demonstrated on the screen by means of the stereopticon before the Society of Western Naturalists at the meeting in Chicago in December, 1900.

taxis" can be produced through the operation of the admitted "chemokinesis."

Acetic acid may be taken as a type of the substances toward which, according to Garrey, Chilomonas shows orientation, or "chemotaxis;" while sodium chloride is an example of the substances which cause no orientation, but merely "chemokinesis." If a drop of acetic acid of a proper concentration is introduced into a preparation of the in-

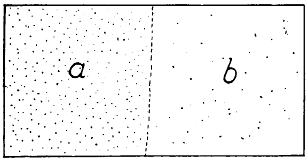

FIGURE 4.

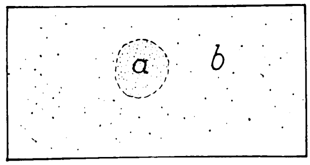

FIGURE 5.

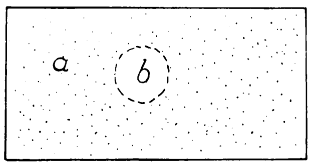

FIGURE 6.

FIGURES 4, 5, and 6. Diagrams showing how the grouping of the organisms depends on the relations of the two fluids to each other in space. *a* represents the area occupied by the fluid into which the infusoria may pass without giving the "motor reaction;" *b* the fluid into which it cannot pass without giving the "motor reaction." When *a* is water, *b* is a salt or alkaline solution; when *b* is water, *a* is an acid solution; in either case the grouping of the organisms (Paramecium or Chilomonas), is that shown in the figures.

fusoria, the latter soon collect in the drop ("chemotaxis"), while if a drop of sodium chloride solution is introduced, it remains empty ("chemokinesis").

But suppose we mount our infusoria in a weak solution of sodium chloride, and introduce a drop of water? The salt solution is admitted to produce no orientation, but merely "chemokinesis," yet in a short time the drop of water is filled with a dense group of infusoria,—just as was the acetic acid in the former case. Apparently the collection is formed in water just as quickly as in the acid, and the present authors have been able to detect no difference in the method of formation. It is at least demonstrated that collections can as well be formed without orientation as with it, and that if these infusoria possess the power of becoming oriented to diffusing ions, this power is a useless luxury.

According to our observations, the phenomena are identical in the two cases. The organisms swim about, in the solution in which they are mounted (water, or sodium chloride solution, respectively), and enter the drop (acetic acid, or water, respectively), without reaction. After having entered they give the usual "motor reaction" when they come to the outer boundary of the drop; hence they do not leave it, and the drop after a time swarms with the animals.

The following series of experiments is instructive and brings out clearly the facts as they appear to the present authors.

Mix a part of the infusoria (Paramecium or Chilomonas) with a weak solution of sodium chloride, not strong enough to injure them, mix others with a weak, non-injurious solution of acetic acid, and leave others in water. Now make mounts with the fluids in various relations to each other:

(1). Make a preparation (Fig. 4) in such a way that half the fluid on the slide is water (*a*) containing infusoria, while the other half is salt solution (*b*) containing infusoria. After a short time most of the infusoria will be in the half containing water alone.

(1a). Make a similar preparation (Fig. 4), save that one half (*a*) is acetic acid containing infusoria, the other half (*b*) water containing infusoria. In this case after a time most of the organisms will be found in the acid.

(2). Make a preparation (Fig. 5) in such a way that the salt solution surrounds the drop of water, the water (*a*) being introduced as a drop into the salt solution (*b*). After a time the drop (*a*) of water contains a dense swarm of the organisms.

(2a). Make a preparation as in the last, save that water (Fig. 5, *b*) surrounds the acid (*a*), which is introduced as a drop into the water. In this case there is likewise a dense aggregation formed in the drop *a* (of acid).

(3). Make a preparation (Fig. 6) such that the water (*a*) surrounds the salt solution (*b*),— the latter being introduced as a drop into the water. After a short time the drop *b* (of salt solution) is empty.

(3a). Make a similar preparation, in which the acid solution (Fig. 6, *a*) surrounds the drop of water (*b*). Soon the drop *b* (of water) is left empty.

With the same pair of substances we get, therefore, either a dense aggregation (or what has been sometimes called "positive chemotaxis"), or a certain definite area left vacant ("negative chemotaxis"), depending upon the relation in space of the two fluids to each other. And this result may be obtained whether we use as our chemical one like acetic acid, to which it has been maintained that the infusoria show positive "chemotaxis" proper, or whether we employ a salt to which they are held to react only by "chemokinesis."

Thus with either pair of fluids, whether we do or do not get a dense aggregation of infusoria depends "on the configuration of the two fluids" — on the relation of the two fluids to each other in space. General statements embodying these relations may be made as follows. If we distinguish as *b* that fluid into which the infusorian cannot pass without causing the "motor reaction," as *a* that into which it can pass without causing the reaction, then

If *b* surrounds *a* (Fig. 5), a dense aggregation is formed in *a* ("positive chemotaxis").

If *a* surrounds *b* (Fig. 6), the small area *b* is left empty ("negative chemotaxis").

If *a* and *b* occupy equal areas (Fig. 4), after a time most of the organisms will be found in *a*. (This last case is not so strongly realized in a minute organism like Chilomonas as in a larger creature, such as Paramecium, because the distances to be passed over are so great that a weak swimmer like Chilomonas will not soon reach the area *a*, and may come to rest in large numbers in *b* without reaching *a* at all. But in any case, a considerable majority will be found in *a*.)

If *a* is water, *b* may be a solution of an alkali or of a great variety of neutral salts; in the case of Paramecium, almost any neutral salt If *b* is water, *a* may be any acid. In either case the resulting phenomena will be essentially the same.

Summary.

1. In order to test the reactions of infusoria to acids, it is necessary to remove with great care from the water containing the organisms the carbon dioxide produced by the organisms in their respiratory processes.

2. Colpidium colpoda, Cyclidium glaucoma, and Chilomonas paramecium collect in solutions of carbonic and other acids, just as Paramecium does. The spontaneous collections formed by these organisms may therefore be due to their excretion of carbon dioxide.

3. Loxocephalus granulosus and Oxytricha aeruginosa form spontaneous collections similar to those of Paramecium, but do not gather in carbonic or other acids. The spontaneous collections in these cases must therefore be due to other causes.

4. The following infusoria do not collect in carbonic or other acids,

nor were they observed to form spontaneous gatherings: Oxytricha fallax, Euplotes charon, Stylonychia pustulata, Colpoda cucullus, Spirostomum teres, Stentor cæruleus, Enchelys farcimen, Halteria grandinella, Didinium nasutum, Euglena viridis, Heteromita globosa.

5. The collections, according to our observations, take place in the manner described in previous numbers of this series of studies for Paramecium. In cases where this has been disputed, it is shown that collections essentially similar to those produced by what has been considered "chemotaxis" proper are likewise produced by what is admittedly "chemokinesis."