STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. — VI. ON THE REACTIONS OF CHILOMONAS TO ORGANIC ACIDS.

By H. S. JENNINGS.

IN the January number of this Journal appeared two papers dealing with the reactions of infusoria.[1] Both the papers treat, among other matters, somewhat fully of the reactions of Chilomonas, but from a different standpoint in each case. Garrey's paper considers the subject chiefly from a chemical standpoint, giving an account of the chemical substances in solutions of which Chilomonas forms aggregations, and of other substances which the animals leave vacant, and determining the exact factors in the solutions to which these results are due. Garrey did not, however, work out the mechanism of the reactions, — that is, he did not determine the exact movements of the animals when stimulated. My own paper, on the other hand, dealt with the matter from the side of the organism, and was concerned especially with the mechanism of the reactions, showing the exact movements of the animals under stimulation. I did not, however, treat of the special chemical substances in which aggregations are formed, or which are left vacant, by the organisms. The direct application of the reaction mechanism which I have described to the phenomena of aggregation, etc., described by Garrey has, therefore, not been made. As the conception presented by Garrey of the nature of the activities of the organism in these reactions is different from that to which I was led by a study of the reaction mechanism, it seems important to make this application. I am convinced that there is no contradiction between the observations of Garrey on this point and my own observations, but that on the contrary the two supplement each other neatly and lead to the same conclusions.

The organism concerned is the small Flagellate *Chilomonas*. Brief descriptions of the organism are given in both the papers above-

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[1] (1) The effect of ions upon the aggregation of flagellated infusoria, by Walter E. Garrey: This journal, iii, no. vi, pp. 291–315. (2) Studies, etc. V. On the movements and motor reflexes of the Flagellata and Ciliata, by H. S. Jennings: This journal, iii, no. vi, pp. 239–260.

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mentioned; one point which becomes of much importance for an understanding of the essential nature of the reactions was not specifically mentioned in either paper, however. This is the well-known fact that Chilomonas is an unsymmetrical animal, the so-called "upper," or larger lip lying at the dorso-dextral angle of the body, as figured by Bütschli, in Fig. 9 b of Plate XLV, in his great work on the Protozoa, — so that the organism cannot be divided either dorso-ventrally or dextro-sinistrally into similar halves, and it is impossible to speak of " symmetrical points on the surface of the body," as is done in bilaterally symmetrical organisms.

Garrey finds that certain chemicals " cause the organism to become restless, very swift shooting movements being caused." As a result of these movements the organisms soon leave the area of operation of the chemical causing the reactions. This phenomenon Garrey calls " chemokinesis." Expressed in the more precise terms of the " motor reflex " described in my own paper (*loc. cit.*), this is as follows. These chemicals cause in a marked degree the motor reflex of Chilomonas; this reflex consists of the following activities. The animal darts *backward*, turns sideways *toward the smaller lip*, then swims forward in the new path thus determined by the position of the smaller lip. This being repeated, the animal is in time brought out of the region of the agency causing the motor reaction. The whole phenomenon is evidently precisely analogous to the conduct of Paramecium in leaving vacant a drop of some chemical to which it is " negatively chemotactic " (or chemokinetic). The only difference between the two cases seems to be that Chilomonas forms a ring around the outside of such a drop, while Paramecium does not. This is due to the fact that Chilomonas is normally much less active than Paramecium, and usually comes to rest as soon as a motor stimulus is lacking; it therefore comes to rest as soon as it gains the outside of the drop of the chemical which acts as a stimulus. Paramecium, on the other hand, is a strong swimmer, and once in motion, it continues to move until stopped by another stimulus; it therefore forms no collection about such a drop. — The above is given as merely supplementary to Garrey's account, and not as in any sense a correction of it: I apprehend that there would be no disagreement on these points.

In certain organic acids (acetic, butyric, lactic) and their salts Garrey finds that Chilomonas forms dense aggregations, — as Paramecium does in weak solutions of acids of any kind. The special

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problem here is, how do the organisms gather in the region of the organic acid ? Garrey states that " a study of the mechanics by which the organism is oriented, or by which it is prevented from moving from the ring into the stronger acid of the clear area or the weaker acid surrounding the ring, proved fruitless" in his case, so that the question as to the mechanics of the phenomenon remains an open one. In what follows, I shall set forth observations which I believe give a clear answer to this question.

In studying the reactions to organic acids (acetic and butyric were chiefly used), I have employed two methods. In one of them an apparatus similar to that described by Garrey[1] was used. This method, in which the reactions are examined in a chamber 1 to 1½ mm. deep, is well suited for showing the grosser phenomena of aggregation, since it is thus possible to work with a very large number of organisms, and the aggregations formed are dense. On the other hand, it is, of course, impossible to study the exact behavior of creatures but 40 μ long in a layer a millimetre deep. The second method was that used by me in studying the reactions of Paramecium: it is described and figured in the second of my Studies,[2] p. 314. In this method the animals are mounted in a very thin layer of water under a supported cover-glass, and a drop of the substance to be tested is introduced with a capillary pipette into the preparation. The aggregations of the animals are then produced in the same manner as by the first method, but of course contain comparatively few individuals, so that the gross appearance is not striking. But the thickness of the layer of water can be diminished to any desired extent by decreasing the size of the supporting glass rods, so that the individuals can be watched even with a high power lens; thus the exact behavior of the animals may be noted.

Using this second method, when a drop of weak acetic or butyric acid is introduced, the swimming Chilomonads behave as follows. Those which swim against the edge of the drop enter it without reaction. If they continue to swim across it, when they come to the opposite boundary, where they would, if unchecked, pass again to the outer medium, the change in the solution at this point produces the characteristic reflex, by which the animals are turned toward the smaller lip, and thus must pass back again into the drop. This may continue, so that the animal remains in the drop. Others enter in

[1] GARREY : *loc. cit.*, p. 294, Fig. 3
[2] JENNINGS : This journal, 1899, ii, pp. 311–341.

the same way and are retained in the same way, so that the drop in time contains large numbers of individuals. Frequently an individual on entering the drop simply settles down against the slide or cover-glass, so that no motor reaction or orientation of any sort is necessary to keep it in the drop. The reason why a number of Chilomonads collect in the drop of acetic acid is then as follows. When the swimming individual comes from the outside against the drop of acid, the change in passing from the outer medium into the drop is not of such a nature as to cause a motor reflex, while *the opposite change, from the acid to the outer medium, is of such a nature as to produce the motor reflex.*[1] The further question, of why one sort of change should cause a motor reflex, while the opposite does not, is of course here, as in other motor reflexes, unanswerable; to determine what agencies will cause a motor reflex is always a matter for experiment.

As to the matter of orientation, it is, of course, evident that when a Chilomonas enters the drop, it will, as a rule, be so oriented that its anterior end is directed approximately toward the centre of diffusion of the chemical, otherwise it would not enter the drop at all. If the drop in diffusing reaches a number of resting Chilomonads, these start to swim; those which swim toward the centre of the drop are not stopped, while those which swim in other directions, coming quickly to the edge of the drop, give the motor reflex, and are therefore turned back into the drop. At a certain time then, under these circumstances, if the drop is large a considerable number of Chilomonads may be seen swimming with common orientation toward the middle region of the drop, since motion in any other direction is quickly stopped by the production of the motor reflex. Under ordinary circumstances, however, neither the individuals in the drop nor those around it will all show at a given instant a common orientation. Each organism is in a certain sense oriented whenever the motor reflex occurs, in so far as it cannot swim farther in the original direction, but may swim in some other direction. But the motor reflex does not usually occur in a large number of individuals at the same time, and it is only when this occurs that a common orientation of a large number of individuals can be expected. In spite of this usual lack of common orientation, the Chilomonads do in time form marked aggregations in the drops of acid, in the manner I have described.

[1] This is perhaps the most important point for understanding how the collections are formed: if it is not clearly apprehended the remainder of the account cannot be understood.

In the preparations 1 to 1½ mm. thick, containing, of course, an immensely greater number of individuals coming under the influence of the stimulus at once, the Chilomonads about the diffusing solution do show a common orientation, according to Garrey : " It is easy to see that the organisms within this area are oriented with anterior ends (those bearing flagella) directed toward the ring, and that they are swimming in strictly radial lines, the lines of diffusion, toward the diffusing drop." [1] This is Garrey's ground for assuming that the aggregations are not brought about through a motor reaction such as I have described ; he states that, on the contrary, these aggregations are due to " true chemotropism." As to the nature of chemotropism, he quotes with approval Professor Loeb's generalization, " The essence of chemotropic orientation would then consist in the animals placing themselves in such a position that symmetrical points on the surface of the body are cut by the diffusion lines at the same angle."

Now, in interpreting Garrey's statement as to the orientation of Chilomonas, it is necessary to recall two points not mentioned by Garrey. The first is that Chilomonas is unsymmetrical, so that no " symmetrical points on the surface of the body" are distinguishable, and second, that the animal swims in spirals of some width, and not in *straight* radial lines. The statement as to their orientation implies then only that their anterior ends are directed in general toward the centre of diffusion, — the lack of symmetry and the spiral path making any more precise statement impossible.

In the cells 1 to 1½ millimetres deep that Garrey used the number of organisms in a given area is enormously greater than in the preparations thus far discussed, and they are not confined to a thin layer, so that it is comparatively difficult to observe individuals. Now when the diffusing acid reaches a large number of organisms, they at once start to swim in various directions. Those which swim away from the centre of diffusion quickly come to the outer boundary [2] of the acid and are turned through the motor reaction back into the acid area ; only those which swim approximately toward the centre of diffusion (therefore in radial lines) continue on their course unhindered. Hence shortly after the beginning of the reaction a large number of animals

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[1] *Loc. cit.*, p. 297.

[2] By "outer boundary" I mean, of course, in every case that point where the decrease in the concentration of the chemical is sufficient to be perceptible to the organism, — *i. e.*, sufficient to cause a reaction. This "boundary" is, of course, not a sharp line ; it varies for different organisms.

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are seen swimming toward the centre of diffusion, none in the oppo-
site direction, while a certain number are swimming in scattered
directions. Now, of course, when out of a confused movement of
a large number of particles there suddenly arises a movement of a
considerable proportion of the particles *in a definite direction*, the
attention is immediately attracted to the definite movement, and it is
only by a strong effort that the eye can be brought to attend to the
individuals that are swimming irregularly in all directions. It would
be necessary for me to state that my observations did not agree with
those of Garrey, if by his statement that " the organisms swim toward
the centre of diffusion along radial lines " he meant that all or ap-
proximately all the organisms swim in this manner. Certainly no one
would make such a statement for the aggregations which occur when
a drop of the acid is introduced into a preparation of the animals in a
thin layer of water, where the movements of the individuals can be
observed. Garrey does not state that *all* the organisms swim in
radial lines, nor does he give any idea of the proportion that does
so. Indeed, he gives distinctly the impression that this precise
common orientation is not, as a rule, very marked (which would
agree with my observations) when he says that " this migration [along
radially disposed lines toward the centre of the diffusing drop] was
most markedly evident in *two or three* [1] experiments in which the
organisms were gathered very densely about débris situated some
distance from the mouth of the diffusion tube. As soon as they came
under the influence of the diffusing acid the organisms left the débris
and fairly swarmed into the acid." [2] This observation, of course, ac-
cords perfectly with the account I have given of the way orientation
takes place. Coming under the influence of the stimulus, the large
number of organisms about the débris at once begin to swim (owing
to Garrey's " chemokinesis," or my own " reaction to a weak stimu-
lus ").[3] But only those which swim toward the centre of diffusion can
continue their course; others come quickly against the boundary of
the fluid and are stopped and turned (through the motor reflex) till
they too finally come into a direction approximately toward the
centre. Thus in a moment after the beginning of the reaction a
stream of organisms is seen passing toward the centre of diffusion,
as Garrey has described (See the analogous phenomena in the
case of Paramecium in thermotaxis.[4])

[1] Italics mine.
[2] *Loc. cit.*, p. 310.
[3] Studies, etc., V. (*loc. cit.*), p. 232
[4] Studies, etc., II. (*loc. cit.*) p. 335.

It seems to me, therefore, that there is no disagreement between Garrey's observations and my own, in this matter. Chilomonas collects in regions of weak acetic or butyric acid through the agency of the motor reflex described in the fifth of my Studies, in a manner exactly analogous to the collections of Paramecia in drops of acid, described in the second of my Studies.[1]

Whether these phenomena should be called chemotropism, or chemotaxis, or chemokinesis (the term proposed by Garrey), of course depends on the definition to be given to these words. I have hitherto used the name (chemotaxis) which was employed before the exact character of the phenomenon was known, preferring to let nature supply the definition. I find it difficult to determine whether the method of reaction described above falls within the definition of chemokinesis given by Garrey or not.

[1] *Loc. cit.*, p. 315.