STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS.— VII. THE MANNER IN WHICH BACTERIA REACT TO STIMULI, ESPECIALLY TO CHEMICAL STIMULI.

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IN earlier numbers of this series of studies the manner in which ciliate and flagellate infusoria react to various stimuli has been described. It has been shown, especially in the second,[1] fourth,[2] and fifth[3] of these papers, that the so-called tactic phenomena of these organisms are not as a rule due to an orientation, or direct turning of the organism to or from the source of stimulus, as has often been assumed to be the case. On the contrary, the phenomena are due to a definite movement or reflex action, produced by the stimulating agent, and always taking place in essentially the same manner. The organisms when stimulated by a chemical, by heat, by cold, by mechanical shock or other similar agent, swim backward and turn toward a structurally defined side. To this simple reaction is due the collecting of the organisms in certain regions and their apparent avoidance of other regions, — the so-called positive and negative chemotaxis, thermotaxis, etc. (The reaction to the electric current is complicated by other factors).

Early in the progress of the work, it was incidentally observed in a number of cases that the bacteria have an analogous method of reaction. At that time opposition had developed in certain quarters to the account given in these studies of the method by which the so-called tactic phenomena take place in the infusoria. In view of this fact, and the further fact that Pfeffer in his classical studies on the reactions of unicellular organisms had distinctly asserted[4] that the

[1] This journal, 1899, ii, pp. 311–341.

[2] *Ibid.*, pp. 355–379.

[3] *Ibid.*, 1900, iii, pp. 229–260.

[4] "Dass die Ansammlungen nicht etwa nur zu Stande kommen, weil die zufällig einschwärmenden Organismen beim Versuch des Entfliehens zurückschrecken, lehrt die direkte Beobachtung." —Pfeffer, Untersuchungen aus dem botanischen Institut, Tübingen, 1888, ii, 648, note. See also ibid., 1884, i, 464.

reactions of the bacteria were not of the nature which our observations showed them to be, it was not deemed worth while to publish an account of these reactions of the Bacteria until the description given of the reactions of the infusoria had been confirmed. This description has now been confirmed and extended to other organisms by various observers. A careful study of the movements and reactions of certain bacteria was therefore undertaken during the past winter, with the intention of publishing the results of the investigation.

This work was entirely finished when the valuable paper of Rothert,[1] dealing partly with the same subject, appeared. This paper, so far as it covers the same ground, agrees throughout with our own observations and clearly establishes the fact that the reaction method of the bacteria to chemicals is not by an orientation, but is analogous to that of the infusoria, as described in these studies.

As the subject is of much interest, and as our work was done from a different standpoint from that of Rothert, with different methods and, to a certain extent, different organisms, a brief account of our own observations will not be superfluous even after the publication of Rothert's paper. In a field where so much uncertainty and disagreement exists, the mutual confirmation of two investigators working independently is of importance. For a discussion of the literature and of the general bearing of the results, reference should be made to the paper of Rothert.

The bacteria studied by us were those occurring in cultures of hay and of aquatic plants decaying in water. Two species of Spirillum, apparently Spirillum volutans and S. undula, were selected for special investigation.

The gross features of the reactions of bacteria to chemicals, as usually shown, are well known through the work of Engelmann,[2] Massart,[3] Verworn,[4] and others. Particularly striking is the reaction to oxygen. The bacteria, mounted on a slide and covered with a cover glass, collect (1) about bubbles of air; (2) about the edge of the cover glass, next to the air; (3) about green plant cells, diatoms, desmids, etc., which are giving off oxygen through the action of

[1] ROTHERT: Flora, 1901, lxxxviii, pp. 371–421.

[2] ENGELMANN: Archiv für die gesammte Physiologie, 1881, xxv, pp. 285–292.

[3] MASSART, J.: Bulletin de l'Académie royale de médecine de Belgique, 1891 (3), xxii, pp. 148–167.

[4] VERWORN: Psychophysiologische Protistenstudien, 1889, pp. 103–106.

the chlorophyll. (See in Verworn's General Physiology, Figs. 211 and 214, and Davenport's Experimental Morphology, Part 1, Figs. 3–5*a*.) Such collections may be observed in any preparation containing the ordinary bacteria of decay.

How are these collections formed? Do the bacteria turn and direct their course toward the centre of diffusion of the oxygen,— proceeding directly toward the region of greatest oxygen density? Or are the collections brought about more indirectly, in a manner similar to that by which Paramecium collects in regions containing an acid?

Before describing the observations by which this question is answered, it will be necessary to give a brief account of the form and usual movements of the organism Spirillum.

Spirillum volutans forms an elongated rod, very slender, with a length of from 15 to 50 μ. It is curved into the shape of a spiral of from two to six turns, so that it resembles a corkscrew in form. At each end of the spiral are found one or two flagella. There is no observable difference between the two ends of the organism, nor is there any marked differentiation of two sides, such as distinguishes oral and aboral sides in Paramecium, for example.

Movements.— Spirillum swims in the direction of the long axis of the spiral by means of its flagella. At the same time it revolves, the revolution following the direction of the spiral, and being therefore (usually at least) from left over to right, if one faces in the direction in which the organism is swimming. At intervals the movement is reversed, the organism swimming then with the opposite end in advance, and revolving in the opposite direction. As a rule, neither end seems to be preferred as the anterior one, Spirillum swimming indifferently in either direction. Given individuals are observed, however, at times to swim for long periods with a certain end in advance, the reversals lasting but a moment.

Occasionally an individual may be seen revolving on its long axis without progressing in either direction, while in other cases there is a rapid whirling on a tranverse axis. But these methods of movement are rare.

"**Chemotaxis.**"—If the Spirilla are mounted on a slide beneath a cover-glass, in company with some desmids or other green algal cells, after a time they will be observed to have formed collections about the algæ, as illustrated in the figures referred to above. How does this occur?

Careful observation shows the course of events to be as follows: —

At the beginning the bacteria are scattered uniformly throughout the preparation. They are swimming rapidly in all directions. At first they pass close to the green plant cells without any reaction whatever. The algæ begin, in the light, to give off oxygen, so that after some time each desmid or other alga must be conceived as surrounded by a zone of water impregnated with oxygen.

Now begins the collection of the Spirilla about the algæ. The bacteria surrounding the algæ do not change their direction of motion and swim toward the centre of diffusion of the oxygen. On the contrary, all continue to swim in the same direction as before. A Spirillum passing close to the alga into the oxygenated zone does not at first change its movement in the least. It swims across the zone till it reaches the other side. It is here that the reaction occurs; the organism reverses its movement and swims in the opposite direction till it reaches the opposite boundary of the oxygenated area. It then reverses again, and this is continued, — the direction of movement being reversed as often as the organism comes to the boundary of the zone of oxygen. The Spirillum therefore remains within the area, which thus acts like a trap. Other Spirilla, swimming at random, enter the area in the same way, react at the outer edges in the same manner, and remain. In the course of time therefore the zone of oxygen swarms with Spirilla.

There is thus no orientation shown either by the organisms within the area or by those outside. *Within*, the Spirilla are swimming in all directions, crossing each other's paths at every angle, and agreeing only in the fact that the movement is reversed on coming to the boundary of the zone of oxygen. A single individual may be seen to oscillate back and forth from one side of the area to the other an indefinite number of times. *Without*, movements are occurring absolutely without relation to the position of the alga and its oxygen zone. Many Spirilla pass close to the edge of the zone, but do not enter unless their original course carries them directly into it. Many of the bacteria therefore remain scattered throughout the preparation, not gathering about the algæ, no matter how long the slide is allowed to stand. But through their continued movement in all directions, dense groups are soon formed about the algal cells.

It is evident therefore that the collections of bacteria arise through the agency of a "motor reflex" essentially similar in character to that of the infusoria, described in previous numbers of these studies.

This motor reflex consists in the bacteria in a reversal of the direction of movement, upon stimulation. The direct cause of the reaction is a change in the nature of the surrounding medium. In the cases already described, it is the change from water containing *much* oxygen to water containing *little* oxygen. The "boundary" of the oxygen zone, above spoken of, is of course merely the region where the change in oxygen density is sufficiently great to cause the reaction.

The bacteria collect in exactly the same manner about air bubbles, and about the edge of the cover-glass, next to the air. In these cases the bacteria usually collect in a narrow zone a short distance from the air surface. If their movements be observed here, it will be found that the reversal of motion is brought about in two different regions. (1) The passage from the optimum zone of oxygen to a region having *less* oxygen pressure causes the reaction. (2) Passage from the optimum into a region having *greater* oxygen pressure, — next to the air surface, — causes the reaction with even greater precision than the opposite change. The Spirilla therefore remain in the narrow optimum zone a short distance from the bubble or the edge of the cover-glass.

The above phenomena are cases of what has been spoken of as "positive chemotaxis." "Negative chemotaxis," or the avoidance of regions containing certain chemicals, takes place in the same manner, save that the reaction occurs when the organism comes, from the outside, against the outer boundary of the area in question. Thus, if a drop of a $\frac{1}{5}$ per cent solution of sodium chloride be introduced beneath the cover-glass by means of a capillary pipette, the following phenomena will be observed. The bacteria do not orient themselves and move in radial lines away from the centre of diffusion of the salt solution. On the contrary, all move in random directions, as before. But on coming against the outer boundary of the salt solution, the organism reacts by reversing the direction of its movement. Hence it does not enter the drop. As every Spirillum that comes in contact with the drop reacts in the same way, the drop remains empty.

Solutions of most acids, alkalies, and salts act in the same manner, so that a drop of any of them (of sufficient concentration) remains empty when introduced beneath the cover-glass.

In addition to the observations on Spirilla, the reactions of a number of the other bacteria found in decaying vegetable matter were studied. In every case the reactions took place in the manner above described for Spirillum, so that there can be no doubt that this method is of general occurrence among the bacteria. In this respect our results agree throughout with those of Rothert.[1]

The same method of reaction often occurs when the bacteria strike against a solid obstacle. The movement is reversed, the organism swimming in the opposite direction.

This manner of reaction was first observed by Engelmann in the reaction of Bacterium (Chromatium) photometricum to light.[2] If a small circumscribed area on the slide is lighted from beneath, the bacteria, swimming at random, pass into this area in the same manner as described above for an area of oxygen. On attempting to pass from this light area into the dark, this organism, according to Engelmann, suddenly reverses its movement and swims backward, — thus remaining in the lighted area. This reversal lasts in the case of Bacterium photometricum but a short time, the organism beginning soon to swim forward again. This is due to the fact that this bacterium has flagella only at one end and normally swims with that end in advance. The reversal of the movement is therefore soon followed by a return to the original direction. This reaction was called by Engelmann a "Schreckbewegung;" it is clearly identical with the "motor reflex" described in these studies.

Bacteria thus react to chemicals, to mechanical obstacles, and to light (or darkness) in the same way, — by a "motor reflex," comparable to that of the ciliate infusoria.

This method of reaction is denominated by Rothert *apobatic* taxis, in contradistinction to *strophic* taxis, which consists in a turning of the organism toward or from the source of stimulus, so as to bring the axis of the body into a definite orientation with respect to the stimulus. The bacteria would thus show apobatic chemotaxis and apobatic phototaxis, using this method of denomination. The really fundamental phenomenon in these cases is the definite reflex action produced by the stimulus; whether aggregation or scattering of the organisms occurs and where it occurs, depend merely on what agencies produce this reflex.

The "motor reflex" of the bacteria differs from that of the infusoria in the same way that the form and structure of the body differ in the two cases. In such bacteria as Spirillum there is no differentiation as between the two ends, or between the two sides of the organism. In correlation therewith, movement takes place indifferently in the

[1] *Loc. cit.*

[2] ENGELMANN: Archiv für die gesammte Physiologie, 1883, xxx, 95–124.

direction of either end, and the motor reflex consists merely of a reversal of the direction of the movement, — without subsequent return to the original direction except as a response to a new stimulus. In the infusoria there is a differentiation both between the ends and between the sides of the animal. The movements reflect these differentiations. The organism swims normally with a certain end in advance, and usually swerves toward a certain side. The motor reflex consists in a reversal of the direction of movement, so as to swim toward the opposite end, together with a turning toward a definite side, and this is always followed soon by a return to the original motion with the anterior end in front. In the case of Engelmann's Bacterium photometricum we have an interesting intermediate condition. Here there is a differentiation between the two ends of the organism, only one bearing flagella, while apparently all sides are alike. The reaction to a stimulus consists in a reversal of the direction of movement, as in the other bacteria, but without any turning toward a certain structurally defined side, such as occurs in the infusoria. But the reaction is followed, as it is in the infusoria, by a return to the original direction of movement. The reactions thus give throughout, in their simplicity or complexity, a faithful reflection of the structure.