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LIGHT REACTIONS IN LOWER ORGANISMS.

II. VOLVOX GLOBATOR.

BY

S. O. MAST.

Professor of Biological Science at Hope College, Holland, Michigan.

WITH FIFTEEN FIGURES.

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I. INTRODUCTION.

Since the discovery of Volvox by LEEUWENHOEK, over two hundred years ago, it has been studied in detail by many investigators. Nearly all noted the effect of light on the direction of

motion, but only two of them, OLTMANNs and HOLMES, made a special study of the reactions of the organism.

OLTMANNs ('92, p. 195) concluded that the difference in light intensity was the chief factor in determining the direction of motion, thus opposing the "ray-direction" theory of SACHS, and originating the contention concerning the efficacy of ray-direction and that of difference in light intensity in producing orientation. HOLMES ('03, p. 324) after a detailed discussion leaves the question in a very unsettled condition, as is indicated by the following statement: "The direction of the rays may be the important factor in orientation irrespective of difference in intensity of light upon different parts of the organism as has been maintained by SACHS for the phototropic movements of plants."

The ray-direction theory of SACHS is still supported by LOEB and the majority of botanists, but is opposed, on the one hand by HOLT and LEE and others who support the views of OLTMANNs, and on the other hand by the "motor reaction" theory of JENNINGS and his followers, who agree with OLTMANNs in holding that difference in light intensity is the cause of the reaction, but give a different account of how the reaction occurs. They hold, however, that difference in light intensity is functional in causing reaction only in so far as such difference makes changes in intensity on the organisms possible. These theories are so well known that it will be sufficient to merely mention them here.

It has been proved by Miss TOWLE, STRASBURGER, and others that the apparatus used by OLTMANNs in studying the light reaction of *Volvox* was defective, and therefore the accuracy of his results and conclusions has been justly questioned. Moreover, the motor reaction theory was formulated to explain the behavior of unicellular infusorians, and it has not been applied to colonial forms. It therefore seemed desirable to take up the study of the light reactions of *Volvox* again, first with the view of throwing more light on the natural history of this extremely interesting organism, and secondly with the view of obtaining experimental results which might possibly give a clearer insight into the secrets of light reactions of organisms in general, and colonial forms in particular.

The experiments discussed in the following pages were carried on in the Zoölogical Laboratory of Harvard University during the summer of 1904, and in the Biological Laboratory of Hope College during the summer of 1905.

It gives me pleasure to express my appreciation to Professor E. L. MARK for encouragement in the work, both in the way of personal interest, and in very generously granting me the privileges of his laboratory and equipment during the summer vacation. I wish also to heartily thank Professor G. H. PARKER for helpful suggestions, and Professor H. S. JENNINGS for critically reading this article in manuscript.

2. NATURAL HISTORY.

Volvox is widely distributed in temperate climates. It is usually found from early May till late in November in quiet pools or lakes, containing aquatic plants, such as *Elodea*, *Myriophyllum*, *Ceratophyllum*, *Cladophora*, and the like. On cloudy days, and during the night, it collects near the surface in small open spaces between the aquatic plants where it frequently occurs in such abundance that the water appears distinctly green. But when the sun comes out it migrates from the surface and takes shelter in the shade, where it clings to small leaves and stems. It is, however, rarely found deeper than 25 to 30 cm. *Volvox* is not found in places which are densely shaded during the greater part of the day, nor is it found where it cannot get out of direct sunlight. As a matter of fact, a few hours' exposure to direct sunlight proves fatal to it. This is shown by the following experiments:

Some *Volvox* colonies were exposed to strong, direct sunlight for an hour, on July 28, 1904; at the end of this time practically all of the colonies were dead. The temperature of the water was 31° C. at the close of the experiment. It was found later that a temperature of 45° C. does not kill all colonies, so it could not have been the temperature which caused death in this experiment. Moreover, the experiment was repeated several times with a layer of water 7 cm. deep between the sun and the jar containing the colonies. The heat rays were thus largely absorbed; but the colonies were nevertheless killed. It may be of interest to cite one of these experiments in detail.

On August 18, 1904, a number of colonies were exposed to strong direct sunlight, under the conditions mentioned, from 11 a. m. until 3 p. m., when it was found that nearly all were dead. The temperature of the water containing the *Volvox* colonies was but a little higher at the close of the experiment than at the beginning. The large colonies were almost perfectly bleached, but the

smaller ones, especially such as were still within the mother colonies, appeared quite normal in color. Intense light evidently causes some change in the chlorophyll.

The specimens used in the experiments performed at Harvard University were collected in various small ponds located some little distance west of Cambridge. Some of these ponds are artificial, having formed in clay pits; others are apparently natural, being located in low, swampy land. All of the ponds contained numerous aquatic plants, and the water in them was stagnant but clear and not foul. The material used in the work done at Hope College was collected in ponds connected with a very sluggish river which runs through a marsh directly north of the city of Holland. Colonies of *Volvox* were found sparsely scattered here and there along almost the entire shore line of nearly all the ponds. In a few spots, however, they were so numerous that the water appeared green, and in these places they could readily be collected in great numbers.

There are two well defined species of *Volvox*, *globator* and *aureus* (EHRENBERG = *minor* STEIN). In the ponds near Cambridge practically all the colonies belonged to the species *globator*; but in the ponds north of Holland the two species were found about equal in number. They were usually found intermingled, but in a few places I found only *globator* and in one place nothing but *minor*.

After colonies of *Volvox* have been in the laboratory from 12 to 24 hours they become inactive, and no longer respond readily to stimuli, and are therefore not satisfactory for experimental work. This makes it necessary to collect frequently. An abundance of material close at hand is consequently almost a requisite for experimental work on this form. In the following experiments, the specimens usually were collected early in the morning and used the same day.

3. STRUCTURE.

Since the discovery of *Volvox* by LEEUWENHOEK nearly every naturalist has had something to do with the study of this exceedingly interesting organism. Most of these investigators laid greatest stress on the structure, but in spite of all this work there are still two questions with regard to structure, concerning which there is some doubt. One is the location of the eye-spot with reference

to the colony as a whole, the other, the variation in form of the vital portion of the individuals composing the colony. Since these structures are of considerable importance in the study of light reactions, I shall take up the structure of *Volvox* rather more in detail than otherwise would be necessary. The following description is the result of a review of the literature on this subject, supplemented by my own observations.

Volvox varies in form from approximately spherical to ovoid. The smallest free swimming colonies can scarcely be seen with the naked eye, while the largest are nearly, if not quite, one millimeter in diameter; KLEIN ('89, p. 143) gives 850μ , HANSGIRG ('88, p. 101) 800μ , KIRSCHNER ('79) 700μ , and FOCKE ('47) 1100μ . Some of the investigators found *Volvox globator* to be larger than *Volvox minor*, while others found the opposite to be true. KLEIN gives 800μ as the diameter of the largest colonies of *V. globator* and 850μ as that of the largest *V. minor*. HANSGIRG gives 800μ as the diameter of the former and 460μ as that of the latter. In my own collections I found *V. globator* in general much larger than *V. minor*. I did not, however, make any accurate measurements with reference to this point.

The colonies of both species are composed of numerous individuals, each of which consists of one cell. KLEIN ('88, p. 146) found from 200 to 4400 individuals in various colonies of *V. minor* and from 1500 to 22,000 in *V. globator*. The individuals consist of a central portion, composed largely of protoplasm, and a thick hyaline layer which surrounds the central portion. The central portion will be referred to as the zooid in the future description. The hyaline layers of contiguous cells usually appear continuous, one with the other, but WILLIAMS ('53) demonstrated that they are limited by cell walls. I was not able to see these in living colonies of *V. minor*, but could see them very distinctly in a few spore-bearing colonies of *V. globator*, especially at the anterior end. The hyaline layer is much thicker in *V. minor* than in *V. globator* and the zooids are much more nearly spherical in the former than in the latter, in which they are in general quite angular. The difference in the shape of the zooids forms the chief distinguishing characteristic of the two species. The cells in the colonies are arranged side by side so as to form a wall enclosing a cavity. In *V. minor* the hyaline layer is figured by MEYER ('95, p. 227) as extending nearly to the middle of the colony, thus leaving only a very small central

cavity, but in *V. globator* it is represented as being only about twice the thickness of the zooids, thus forming a comparatively large central cavity. The hyaline layer is much thinner on the outer surface of the zooids than on the inner, in both species. The cavity is said to be filled with a slimy fluid.

Each zooid contains, among other structures, an eye-spot, two

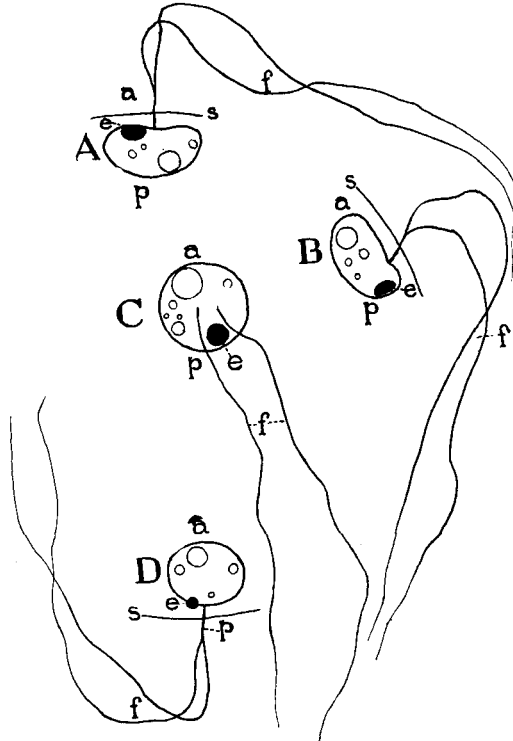


FIG. 1. Zooids as they appear in *Volvox minor*. *A*, Side view of a zooid situated in the middle of the anterior end of a colony; *B*, side view of a zooid halfway between the anterior end and the equator; *C*, surface view of the same; *D*, side view of a zooid located in the middle of the posterior end; *a*, surface of zooid facing the anterior end of the colony; *p*, surface facing the posterior end; *e*, eye-spot; *s*, surface of the colony; *f*, flagella.

flagella, a nucleus, a chloroplast and several vacuoles. In *V. minor* the zooids vary but little in form in different parts of the colony. At the posterior end they are nearly spherical, but as one proceeds toward the anterior end they become more and more flattened. At the anterior end the maximum diameter is about twice as great

as the minimum (see Fig. 1). As seen from the surface they are all nearly circular in outline.

The zooids of *V. globator* vary much more in form in different parts of the colony than do those of *V. minor*. At the anterior end they are nearly spherical, but at a short distance from this end they begin to become somewhat angular and from this point

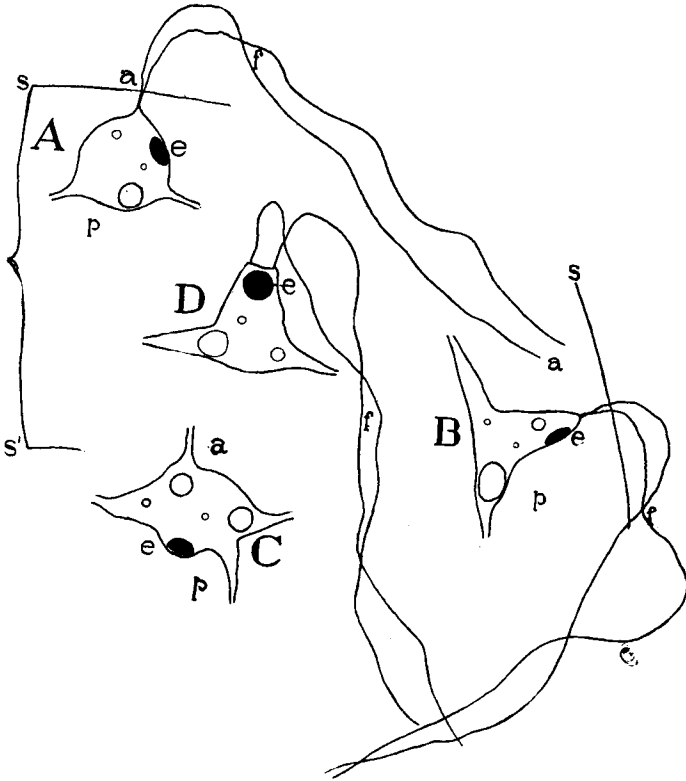


FIG. 2. Zooids as seen in a colony of *Volvox globator*. *A*, Side view of a zooid located in the middle of the anterior end of the colony; *B*, side view of a zooid situated halfway between the anterior end and the equator; *C*, surface view of same zooid; *D*, same zooid as it appeared when viewed from the posterior end of the colony; *a*, surface of zooid facing the anterior end of the colony; *p*, surface facing the posterior end; *e*, eye-spot; *s*—*s'*, exterior and interior surface of colony, showing thickness of hyaline layer; *f*, flagella. The projection extending toward the surface of the colony is longer and thinner, and the eye-spot is much smaller in the zooids at the posterior end of the colony than elsewhere.

toward the posterior end they become gradually more irregular in form. One of the angular zooids may be described as consisting of two portions: a flattened portion which is nearly parallel with

the surface, and a conical portion which projects from near the middle of the flattened portion into the hyaline layer almost to the surface of the colony. At a point about halfway between the anterior pole and the equator of the colony, the altitude of the projection is about twice as great as the diameter of its base. The ratio between these dimensions becomes gradually greater as one proceeds farther from the anterior end, until at the posterior end the altitude is four to five times as great as the diameter. It will thus be seen that the distal end of the conical projections gradually extends farther out as one proceeds from the anterior end to the posterior (see Fig. 2). The only reference to the variation in

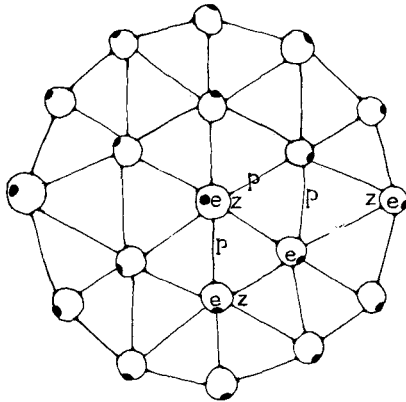


FIG. 3. View of the anterior end of a colony of *Volvox minor*, showing the location of the eye-spots. Z, zooids; e, eye-spots; p, protoplasmic fibers connecting the zooids.

form of zooids in the same colony is found in OVERTON'S article ('89, p. 70), and he states only that the projection (Schnabel) is longer in the neighborhood of injured places ("In der Nähe von verletzten Stellen verlangt sich der Schnabel").

The projection is nearly circular in outline at the base, but it becomes considerably flattened toward the distal end, so that a cross section near this end is elliptical in outline. The zooids are so arranged in the colony that one of the flattened surfaces of the projections faces the anterior end and the other the posterior. Viewed from either of the flattened surfaces, the outline of the distal end forms nearly a straight line, at either end of which is found the attachment of a flagellum. The flagella are five or six times as long as the diameter of the zooids. OVERTON ('89, p. 72)

says they are about $\frac{1}{2}\mu$ apart and 25μ long. The eye-spot is situated on the surface of the projection which faces the posterior end of the colony. It is found but a short distance from the free end, between the points of attachment of the flagella.

If the projections are short or absent and the zooids nearly spherical the eye-spots are still located in the same relative position as they are in zooids containing long projections, *i. e.*, they face the posterior end of the colony. This becomes very evident in viewing a colony from the anterior end. Under such conditions it is clearly seen that the eye-spots are situated on the surface of the zooids farthest from the middle of the anterior end, as represented in Fig. 3.

Nearly all the investigators, who have worked on the structure of *Volvox*, figure the eye-spot as situated on one side of the zooids near the outer surface, but only one, OVERTON, describes and figures it in such a way that its position with reference to the colony as a whole is made clear. OVERTON ('89) in Taf. 4, Fig. 26, and Taf. 1, Fig. 3, clearly represents the eye-spot as being located near the outer anterior surface of the zooids and says, p. 114: "Sehr bemerkenswerth erscheint, dass, wie bei einstellung auf einen Meridiankreis des *Volvox* Stockes sich ergibt, die Augen flecke (wenigstens bei *V. minor*) bei allen Zellen derjenigen Seite anliegen, die dem vorderen Pole am nahsten liegt."

During the first few days in August, 1905, I examined 30 specimens of *V. globator* and 50 of *V. minor*, with special reference to the location of the eye-spots and found that in all but one of these, they were unquestionably located on the outer posterior surface of the zooids. Furthermore, I gave the problem of locating this structure to three of my students in October, 1905. These students had never seen *Volvox* before and knew nothing about any work done on it. All of them concluded that the eye-spots face the posterior end of the colony. When they took up the problem they knew that these organisms are usually positive in their light reactions. I had given them the term, eye-spot, and it was clearly evident that they assumed that this structure functioned in directing the organisms toward the light, and consequently expected to find it on the anterior surface of the zooids, for they were all much surprised to find it on the opposite surface. It is, therefore, safe to conclude that OVERTON's observation was wrong.

The eye-spots in *Volvox* are brownish in color and lenticular in

form, but the outer surface is much less curved than the inner. They are much more distinctly seen in large than in small colonies, for the latter contain more coloring matter. In *V. minor* they appear relatively much larger and more definite than in *V. globator*. They are more nearly opaque than the rest of the zooid, and when strongly illuminated by light passing through the colony they stand out clearly and can be distinctly seen under a magnification of 65 diameters. RYDER ('89, p. 219) maintains that the eye-spots at the anterior end of the colony are eight or ten times as large as those at the posterior end. I also found this to be true. They are so small and so nearly colorless at the posterior end that they can be seen only under the most favorable conditions. This difference in size and color seems to be due to difference in illumination at the two ends, for if the colonies are kept in low light intensity for some time all the eye-spots become much lighter in color and appear to become smaller.

The zooids of contiguous cells are interconnected by fine protoplasmic strands, each cell being connected to every adjoining cell by a single strand, with an occasional exception in the posterior end where the strands are sometimes double. The earlier investigators considered these strands to be structures through which substance is transmitted from cell to cell, while later investigators claim that they function only in the transmission of impulses. It is altogether likely that they function in both ways. Impulses are unquestionably transmitted from cell to cell, otherwise the individuals composing a colony could not perform coördinated acts. It is almost certain that such impulses pass through the strands rather than through the hyaline layers separating the zooids. On the other hand it is evident that the transmission of impulses is not the only function of these strands, since there is never more than one found connecting adjacent individuals in the anterior end of the colony, while those in the posterior end are sometimes connected by double strands. The colonies, however, are stimulated at the anterior end much more frequently than at the posterior, so that if the strands function merely as structures for the transmission of impulses, we should expect to find them double at the anterior end rather than at the posterior.

4. FUNCTIONS OF EYE-SPOT.

EHRENBERG ('38, p. 70) discovered the eye-spots in *Volvox* and considered them to be light recipient organs, as is clearly shown

by the following interesting quotation: "Nie darf man auch vergessen, dass alle Einzelthierchen Empfindungsorgane besitzen, die den Augen vergleichbar sind und dass sie mithin nicht blind sich im Wasser drehen, sondern als Bürger einer unserm Urtheile fern liegenden grossen Welt den Genuss einer empfindungsreichen, so stolz wir uns auch geberden mögen, mit uns selber theilen."

The only experimental evidence we have in reference to the function of the eye-spot is presented by ENGELMANN ('82, p. 396). He says, in substance, referring to this structure in *Euglena viridis*: 'If a sharp shadow is gradually brought from the posterior end of a swimming *Euglena* toward the anterior, there is no reaction until the shadow reaches the colorless anterior portion of the organism which contains the eye-spot. In the case of large individuals moving into a shadow, the reaction could be seen to be given before the eye-spot was in darkness. The colorless anterior end is, therefore, the primary light recipient region, but the eye-spot may still function secondarily as do the pigment cells in the retina of higher animals.' It is thus clear that the idea of EHRENBERG has no experimental support. There are, however, the following reasons for believing it to be correct.

1. EHRENBERG based his conclusion, with reference to the function of the eye-spots upon their structural similarity to the eyes of rotifers and Cyclops. This is shown not only by external appearance but also by the fact that both are affected alike by certain chemical reagents.

2. WAGER ('00) maintains that the color of the eye-spot indicates that it absorbs blue. ENGELMANN demonstrated that *Euglena viridis* is more sensitive to light composed of short waves (the light absorbed by these structures) than to that composed of long waves, and STRASBURGER ('78) demonstrated the same for swarm spores.

3. STRASBURGER ('78, p. 18) states that the eye-spots are found only in swarm spores which are colored, and that light reactions are strong only in such as contain these structures. Two colorless *Chytridium* swarm spores which are phototactic were, however, known to STRASBURGER ('78, p. 568). ROTHERT ('01) also claims to have found a colorless phototactic flagellate among *Chlamydomonas multifilis*. I found the small, colorless flagellate *Chilomonas paramecium*, in which no eye-spot has been found, strongly negative to light under some conditions but not at

all phototactic under others. DAVENPORT ('97, p. 188) proved certain species of *Amœba* to be negative to light, and it is well known that *Stentor cœruleus* responds very definitely to stimulation by light. It is said that the *Chytridium* swarm spores have an orange colored oil globule at the base of the flagellum which may function as an eye-spot, but in the four organisms mentioned last there are no structures which appear as though they could take the place of these organs. It is, therefore, evident that we have organisms without eye-spots which are sensitive to light but as far as I know there are none with these structures that are not sensitive.

4. WAGER ('00, Pl. 32, Fig. 2) represents the flagellum in *Euglena viridis* as indirectly connected with the eye-spot, in that it has an enlargement which lies immediately over the concave surface of this structure as represented in Fig. 4. The eye-spot is supposed to absorb the blue of the spectrum and in some way to stimulate the enlargement on the flagellum.

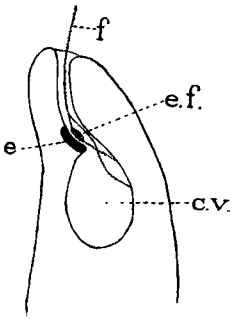


FIG. 4. Side view of anterior end of *Euglena viridis*, after WAGER; e, eye-spot; f, flagellum; e.f., enlargement in flagellum; c.v., contractile vacuole.

5. The fact that the eye-spots are larger and more highly colored at the anterior end of *Volvox* than at the posterior, that they lose their color and become smaller in the absence of light, and that they are situated near the distal end of projections which become longer as one proceeds from the anterior end toward the posterior, and thus expose the eye-spots to more light, indicates that these structures function in light reactions.

In view of the evidences presented above in favor of considering the eye-spot as a light recipient organ, and in view of the fact that there is nothing in the structure or location which indicates that it could not function in light reactions or that it has any other function, it appears safe to conclude that EHRENBERG's idea with reference to the function of the eye-spot is correct.

WAGER ('00) suggests three ways in which the eye-spot in *Euglena viridis* may function in light reactions: (1) It may absorb light and thus produce a change in the movement of the flagellum; (2) it may merely prevent the light rays from reaching one side of

the enlargement in the flagellum, while the other side is exposed, and thus produce a difference in light intensity on opposite sides of the enlargement; or (3) it may cut off the light from one side of the sensitive portion of the anterior end of the organism when it is not oriented, and thus produce unequal illumination on opposite sides of this end.

It seems impossible to test the suggestions of WAGER experimentally, but it may be possible to arrive at a tentative conclusion concerning the matter, from what we know about the structures and reactions of these organisms. JENNINGS ('04, p. 54) shows that *Euglena* swims in a spiral course with the larger lip constantly farthest from the center of the spiral. In thus swimming the longitudinal axis never points toward the source of greatest illumination, so that when the organism is oriented the side of the anterior end containing the larger lip is always more shaded by the eye-spot than that containing the smaller lip (see Fig. 4). From this it seems evident that the eye-spot in *Euglena* does not function in accordance with WAGER's third suggestion. That it does not function in accordance with this suggestion in *Volvox* is still more clearly evident, for here the eye-spots are located near the posterior surface in the projection of the zooid, so that if this projection is sensitive to light there certainly is no possibility of opposite sides being equally stimulated when the organism is oriented, for under such conditions the shadow of the pigment granule falls on the posterior surfaces while the anterior surface is fully exposed to the light. With reference to the second suggestion, it is probably true that the eye-spot does prevent the light from reaching one side of the enlargement in the flagellum in *Euglena*, but by referring to Fig. 4 it will be seen that the difference in light intensity thus produced on opposite sides of the enlargement must be practically the same when the light strikes the organism nearly parallel with the longitudinal axis, as it is when it strikes it at an angle from the side containing the smaller lip. If this be true, there is no change in stimulation when the organism is slightly thrown out of orientation. It therefore does not seem probable that the eye-spot in *Euglena* functions in accordance with the second suggestion of WAGER. In *Volvox* we know of no enlargement in the flagellum such as that found in *Euglena*, and if there were one, or some other similar structure, the criticism offered above with reference to *Euglena* would hold here also.

The first suggestion of WAGER, namely, that it is transformation of energy in the absorption of light by the pigment in the eye-spot which produces the stimulus, either directly in the eye-spot or indirectly by transferring the transformed energy to sensitive structures in the immediate neighborhood (*e. g.*, the enlargement in the flagellum of *Euglena*) seems to me to fit the structure and reactions of both *Euglena* and *Volvox*. The eye-spot in *Euglena* and in *Volvox*, especially in the zooids some distance from the anterior end is a flat structure. It is so located that when these organisms are oriented, one of its flat surfaces is fully exposed to the light and is directed toward the source of strongest illumination. In this position it absorbs a given amount of light, perhaps a maximum amount. If now the organism changes its direction of motion, the flat surface of the eye-spot is no longer directed toward the source of strongest illumination and consequently there is a reduction in the amount of light absorbed and this forms the basis for a stimulation which may result in reactions that will orient the organism again. In general, then, it may be said that any change in the relation between the source of strongest illumination and the direction of motion will, if the eye-spot functions as described above, produce a change in the amount of light absorbed, and every such change produces a stimulation.

It is quite possible, since the surfaces of all these structures are curved, that the protoplasm in the distal end of the projection of the zooids in *Volvox* and that which forms the smaller lip in *Euglena*, and even that in the enlargement of the flagellum, may act as a condensing lens, and thus increase the amount of light which reaches the eye-spot. If this be true, we can at once see some significance in the location of this structure on the posterior surface of the projection in *Volvox*, instead of on the anterior, for if the distal end of the projection acts as a lens the light on the surface directed from the source of illumination would probably be more intense than that on the surface directed toward it, and if the light comes to a focus on the pigment granule, the direction of reaction might even be regulated by the localization of difference in light intensity thus produced on the surface of this granule.

5. LOCOMOTION.

Volvox, although ovoid to spherical in form, always moves with a given fixed portion of the body ahead, that is, it has definite

anterior and posterior ends, and in locomotion it rotates on an axis passing through these ends. The direction of rotation is in general counter-clockwise as seen from the posterior end, but, as KLEIN ('89, p. 168) pointed out, it is under ordinary conditions frequently reversed. As the colonies swim through the water they may be seen to make several counter-clockwise rotations, then suddenly reverse and rotate clockwise for a time, then again reverse, and so on. The number of rotations between reversals varies greatly. This fact led me to believe that the reversal is largely due to external stimuli, and I soon noticed that it depends somewhat, at least, upon the condition of the water.

On August 29, 1904, I put a number of *Volvox globator* colonies into some thoroughly filtered water and found that they moved toward the source of light, rotating counter-clockwise almost continuously. Several were carefully watched under a hand lens and it was found that they moved from 5 to 6 cm. without changing the direction of rotation. Colonies taken from the same jar were then put into water which had not been filtered and therefore contained small particles of substance, mostly in suspension. In these colonies, reversal in the direction of rotation was frequent. This seemed to indicate that the contact stimulus produced by the small particles in the water caused the reversal. To test this matter further, clean sand was scattered on the bottom of the aquarium containing filtered water. As the colonies moved toward the source of light along the bottom of the aquarium, frequently coming in contact with grains of sand, they were seen to reverse the direction of rotation at short intervals. They appeared to rotate in one direction about as much as in the other. In thus traveling among the sand grains a colony occasionally comes squarely up against the flat surface of a grain and remains with the anterior end in contact with the surface, sometimes as long as fifteen seconds. While thus in contact with the surface its forward progress is of course stopped, but it continues to rotate on its axis, and while in this position it almost always rotates counter-clockwise without reversal. Sooner or later, apparently accidentally, it turns slightly and then it takes a course parallel with the surface of the sand grain and thus gets by the obstruction.

From these observations it is clear that contact stimulation of the anterior end, even to such a degree that progress is prevented, does not cause reversal in the direction of rotation. If a change

in the direction of rotation is caused by contact stimuli at all it must be by contact stimuli along the sides of the colonies.

Volvox colonies were subjected to such stimuli by laying a glass slide into an aquarium containing filtered water about 3 mm. deep, so that the edge of the slide made an angle of about 45 degrees with the rays of light. When the colonies moved toward the source of light and came in contact with the slide, the point of contact was not at the anterior end but some little distance from it. After being thus stimulated they immediately turned from the slide making an angle of about 95 degrees with their previous course. Then they gradually turned toward the source of light again and thus continued along the edge of the slide making a zig-zag path. In following along the edge in this way they frequently came in contact with the slide before they were perfectly oriented and were consequently stimulated at a point further from the anterior end than usual, sometimes about midway between the two ends. In all these reactions the direction of rotation was seldom changed. It is therefore clear that a single contact stimulus on the side of a colony, which does not obstruct forward progress, does not cause reversal in the direction of rotation. In the experiment just referred to a small portion of one of the upper corners of the slide was slivered off, making an incline on which the water became gradually more shallow until, at the upper end, it was not deep enough for the larger colonies to swim without difficulty. As the colonies worked up this incline, they came in close contact with the glass and the direction of rotation was frequently changed.

It may then be concluded that continuous contact stimulation on the sides causes reversal in the direction of rotation, providing the contact is such that considerable resistance is offered to forward motion.

But why should contact stimuli on the anterior end, which prevents forward motion, not cause reversal as well as similar stimuli along the sides? Considering the structure of the organism in question, it seems probable that rotation is brought about largely by an oblique stroke of the cilia along the side and that those at the ends have little if anything to do with it. Now it seems reasonable to assume that when a certain proportion of these cilia on the sides meet considerable resistance they all strike in the opposite direction and thus produce reversal of rotation. When the anterior end is in contact with an object the cilia along the sides are of

course free, and if it is the action of these cilia which causes rotation we should not expect a change in the direction of rotation when the anterior end is stimulated.

As stated above, we find reversal in the direction of rotation frequent in water containing numerous small particles. What is the cause of this? This is probably due to particles becoming entangled in the cilia and obstructing their free movement, thus causing a change in the direction of rotation.

While we have thus found that reversal of rotation is largely caused by external agents, it is unquestionably true that it depends to some extent upon the condition of the organism itself, for under similar external conditions difference in the frequency of reversal was repeatedly noted.

6. ORIENTATION—GENERAL DISCUSSION.

It is well known that if *Volvox* is subjected to light of moderate intensity, it swims toward the source of light; but if the light intensity is high, as *e. g.*, direct sunlight, it travels in the opposite direction. In casually studying such movements it appears as if the course of the colonies in either direction were nearly parallel with the light rays, and investigators have, in general, assumed this to be true. HOLMES ('03, p. 320), writes: "It is easy to determine that *Volvox* orients itself, and that very accurately, to the direction of the rays of light. If specimens of *Volvox* are taken into a dark room and exposed to the light from an arc lamp they travel towards the light in almost a straight course, swerving remarkably little to the one side or the other. They will often travel a foot without deviating as much as a quarter of an inch from a perfectly straight course."

In studying the effect on the direction of movement, of difference in light intensity on opposite sides of a *Volvox* colony, I accidentally discovered that, contrary to HOLMES' conclusion, *Volvox* very seldom orients "accurately to the direction of the rays." The colonies do, of course, swim toward or from the source of light in a general way; but movement parallel with the rays is quite the exception. In swimming toward a source of light the colonies may deflect not only to the right or left but also up or down. Deflection up or down will be discussed under the effect of gravitation on orientation (p. 122); deflection to either side will be taken up in connection with the description of the following experiments.

Most of these experiments were performed in an apparatus which I have called a "light grader." I have given a detailed description of this apparatus in another paper (MAST '06, p. 364). The

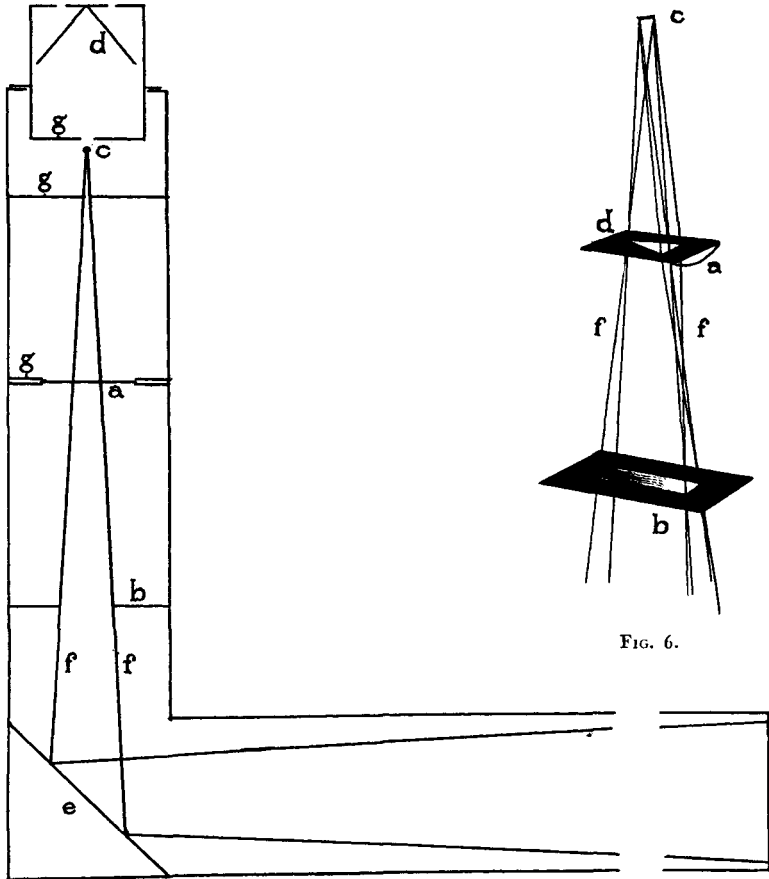


FIG. 5.

FIG. 5. A vertical section of the light grader. The lens (*a*) which is a segment of a cylinder has its longitudinal axis lying in the plane of the section; *b*, stage; *c*, Nernst glower; *d*, non-reflecting background; *e*, mirror; *f*, light rays; *g*, opaque screens. Distance from glower of lamp to stage, one meter.

FIG. 6. Stereographic view of light, lens, and image; *a*, lens; *b*, field of light produced by the image of the glower (*c*); *d*, opaque screen which lies flat on lens and contains a triangular opening which causes a gradation in the light intensity of the field (*b*).

important features of the apparatus will be readily understood, however, by referring to the accompanying figure.

An aquarium¹ 15 cm. long, 8 cm. wide and 8 cm. deep inside was placed at one of the principal foci in the light grader, which was in a horizontal position and so arranged that the Nernst glower at the other principal focal point was vertical. The light rays passed through the aquarium practically parallel with each other and the bottom of the aquarium and perpendicular to the sides. An opaque light screen, containing a rectangular slit 10 mm. wide and 13 cm. long, was fastened to the side of the aquarium nearest the light, so that the lower edge of the slit was on a level with the bottom and the ends of the slit were 1 cm. from either end of the aquarium. Filtered water was poured into the aquarium to such a depth that its surface was above the upper edge of the slit and was consequently in darkness. Since the rays were practically parallel with the bottom and perpendicular to the sides of the aquarium, it is evident that reflection from the water surfaces was practically eliminated. The light which passed through the aquarium was largely absorbed by the wall of the dark room which was over seven meters from the light grader, and since this was the only light which entered the room it is safe to conclude that the direction of movement of *Volvox* in the aquarium was influenced only by rays direct from the Nernst glower.

By placing an opaque screen containing a triangular opening over the cylindrical lens in the light grader, a field of light is produced which becomes gradually less intense from one end to the other (MAST '06, p. 364). If *Volvox* is allowed to swim toward the source of light in such a field it is evident that one side of the colonies will be more strongly illuminated than the opposite, and if difference in light intensity on the two sides, regardless of ray-direction, determines the direction of movement we should expect the organisms to move at an angle with the direction of the rays of light. This was found to be true, as will be shown later (pp. 136-141).

The first series of experiments made to ascertain the effect of difference in intensity on orientation was performed in the light grader, arranged as described above, by carefully introducing about one hundred colonies into the aquarium at a fixed point

¹ The aquarium was made of the best plate glass obtainable, accurately cut and ground, and cemented with Canada balsam boiled in sufficient linseed oil to give it the desired consistency. This cement proved very satisfactory, much more so than any other of several tried. Balsam in xylol is good but it becomes so brittle on drying that it breaks readily. Linseed oil prevents this.

near the side farthest from the light. In some conditions, the colonies thus introduced, proceed across the aquarium nearly parallel with each other, spreading but little, frequently not more than five or six millimeters. In other conditions, however, they spread out as much as three or four centimeters. By laying a straight wire on the aquarium and constantly keeping it over the middle of the group of colonies as they proceeded on their way, the average course was quite accurately ascertained. The course under most conditions, although at a decided angle with the rays, was remarkably straight; but under some conditions it curved considerably as the organisms approached the side of the aquarium nearest the light. The paths produced and the direction of the rays were transferred to paper by means of a miter square; and thus the angle of deflection was recorded for future reference.

Between August 20 and 30, 1904, seventy-three paths observed under the conditions described above were recorded, and many more were observed. In nearly all of these cases the colonies deflected strongly to the left, frequently making an angle of 45 degrees with the light rays, and rarely less than 5 degrees. This deflection to the left was brought out in a striking way, by putting the *Volvox* colonies into the aquarium a few centimeters to the right of the left edge of the light area. When introduced at this point, they soon reached the plane between light and shadow and passed into the dark area without any apparent change in their course. After they had traveled in the dark region some little distance they rose, deflected more sharply to the left, and frequently made a small circle or spiral and entered the light region again.

This deflection to the left in the light area was, of course, thought to be due to the fact that the light was graded in intensity, the more intense end of the field being to the right. It was accidentally discovered, however, that similar deflections were produced when no lens was used, and later it was found that when the screen over the lens was inverted so as to make the left end of the field the more intense instead of the right as in the previous experiment, the *Volvox* colonies still deflected to the left. It was therefore clear that this deflection was not due to difference in light intensity on opposite sides of the organisms.

One hundred and one additional paths were observed and recorded between July 18 and August 3, 1905. Some of these observations were made in the light grader; others outside. In

many cases a single colony was selected and its course studied, in place of that of a number of colonies in a group. To my surprise, I found that whereas during the preceding season, 1904, *Volvox* colonies deflected, with scarcely an exception, to the left, they now deflected to the right more often than to the left. We shall consider the cause of this somewhat in detail later. In all I have records of 174 paths, only a few of which were observed in light of uniform intensity. Seventy-eight of them deflect to the left from 2 to 45 degrees; seventy-five deflect to the right from 2 to 45 degrees; and only twenty-one are found in the area between 2 degrees to the right and 2 degrees to the left, and very few of these are parallel with the rays.

In these experiments, however, only deflections to the sides were recorded; it is important to note that marked deflection up or down was also to be observed. It becomes clear then, that the colonies which appeared to be moving nearly parallel with the rays when seen from above, were in all probability slowly ascending or descending as they proceeded toward the source of light.

The cause of deflection—the inability to orient accurately—is complicated. The direction of movement in *Volvox* is affected by internal as well as by external factors. The effect of some of these factors on orientation or deflection will be discussed under the following headings: (a) Effect of internal factors on orientation; (b) Effect of light intensity on orientation; (c) Effect of gravitation on orientation; (d) Effect of contact stimulation and rotation on orientation.

a. Effect of Internal Factors on Orientation.

If a number of *Volvox* colonies, varying in size, are put into the aquarium at the same time and allowed to swim horizontally toward any concentrated source of light, it will be seen that the larger colonies, especially such as contain numerous daughter-colonies, soon collect along the right side of the group, and the smaller ones, and such as contain only very small daughter-colonies along the left side. In some experiments there was such striking difference between the deflection of different colonies in a group that two distinct columns were formed, which moved across the aquarium at quite a definite angle with each other. The right column in such cases invariably contained most of the larger colonies, and the left most of the smaller ones.

On July 26, 1905, the paths of two such diverging columns, observed in light of uniform intensity, were recorded. The one containing the larger colonies deflected to the right, making an angle of nine degrees with the light rays, while the one containing the smaller colonies deflected to the left, making an angle of fifteen degrees. Both columns, however, sometimes deflect to the right or to the left of the light rays.

Deflection then varies with different periods in the life of the colonies; but it also depends upon the physiological state of the organisms, as is shown by the following observations.

In the morning, after being in the aquarium all night, *Volvox* colonies were repeatedly found lying on the bottom, apparently perfectly quiet. They were in a state which may be termed dark rigor. When light is thrown on them while they are in this condition, they do not respond at once. After a time, however, they begin to swim about, slowly at first, without orienting; but soon, more rapidly, until they become normally active, and move toward the light. Apparently there is a certain chemical change necessary to bring the organisms out of the state of dark rigor into such a condition that they can respond readily to light; and this change appears to be induced by light. The production of carbon dioxide in darkness suggests itself as the probable cause of dark rigor.

When a colony, after having been in darkness all night, first begins to respond to light, it moves toward the surface of the water and deflects strongly either to the right or left as it proceeds toward the source of light. But if it be made to cross the aquarium several times in succession, it is found that the deflection gradually decreases until it has traveled 25 to 30 cm. Then it reaches an apparently stable condition; and on the following trips it takes a fixed course which may be at almost any angle with the light rays, but is usually at an angle of from 5 to 10 degrees. Such reactions were observed many times, mostly in experiments performed for other purposes. The following detailed experiment is typical.

On August 7, 1905, *Volvox* was collected at 6.15 a. m. and left in total darkness until 8.30 a. m., at which time the colonies were still moving about, but very slowly. One of them was put into the aquarium in the light grader in a light intensity of nearly 400 candle meters. This colony moved about irregularly at first and deflected strongly to the right, but it soon became more active and moved quite rapidly toward the light. On its first trip across

the aquarium it deflected to the right 17 degrees, on its second trip 15 degrees, and on its third trip 11.5 degrees. The following thirty trips were made with so little deviation from 11 degrees that it could not be measured. The experiment was closed at 10.45 a. m., two hours and fifteen minutes after it was begun.

It appears, then, that when the internal factors have become stable, and the external factors are not changed, the angle of deflection remains constant.

b. Effect of Change in Light Intensity on Orientation.

In general a decided increase or decrease in light intensity causes an increase in deflection. This seems to be connected with the fact, pointed out by HOLMES ('03, p. 321), that in low or high light intensity the colonies are not strongly positive.

On August 3, 1905, the relation between the course of a given colony and the ray direction was obtained in a light intensity of 400 candle meters and also in an intensity of 20 candle meters. In the higher intensity, the deflection to the left was found to be 1 degree; in the lower intensity 11 degrees. The course was ascertained by letting the colony across the aquarium three times in succession in the lower intensity, then three times in the higher, then twice in the lower, and finally twice in the higher. The light intensity was reduced by cutting off part of the light with a screen, which contained a narrow slit, placed close to the Nernst glower, and so arranged that the slit was perpendicular to the glower. Neither the light nor the aquarium had to be touched in decreasing or increasing the light intensity, so the ray direction was unquestionably the same under both conditions. There was remarkably little variation in the angle of deflection in all the trips made across the aquarium in either light intensity. There can thus be no question about the accuracy of these observations. This experiment was repeated a few days later with similar results. The colony selected, however, deflected to the right instead of to the left, as the one in the first experiment had done. The deflection in the second experiment was studied in three different light intensities: 20 candle meters, 400 candle meters and nearly 2000 candle meters. The highest intensity was produced by a carbon arc. The angle between the light rays and the course taken by the colony was found to be 12 degrees in 20 candle meters intensity; 2 degrees in 400, and 40 degrees in 2000.

Moderate increase or decrease in light intensity does not appear to affect the degree of deflection, *e. g.*, the path of a given colony in a light intensity of 400 candle meters was found to be so nearly the same as that of the same colony in an intensity of 100 candle meters that the difference could not be measured. From numerous experiments, it appears that in order to influence deflection, the increase or decrease in intensity must be great enough to affect the positiveness of the organism; that is, the intensity must be decreased to somewhere near the threshold or increased to near the optimum. Now the threshold and optimum in different colonies, and in the same colony under different conditions, vary extremely. It is therefore to be expected that the effect of variation in intensity on deflection varies much. This was found to be true experimentally.

The above discussion on the effect of change in light intensity on deflection might lead one to assume that all *Volvox* colonies could be made to move parallel with the rays, if the proper light intensity were used. This, however, was not found to be true. To bring about such a reaction, not only the proper light intensity is necessary, but the organisms must also be in a certain physiological state. Immediately after taking colonies from darkness or very intense light in which they have been for some time, they are in such a condition that no light intensity was found in which they travel parallel with the rays. And many colonies under various other conditions could not be made to swim parallel with the rays. In the above discussion deflection up or down is not considered; by parallel we mean merely without lateral deflection.

c. Effect of Gravitation on Orientation.

If *Volvox* is killed in formol and then transferred to water, it gradually sinks to the bottom, showing that its specific gravity is greater than one. When first dropped into the water there is, of course, no indication of orientation; the longitudinal axis of the different colonies point in all directions, but as they sink, it is soon found that their axis becomes approximately perpendicular, *i. e.*, the colonies become oriented with the anterior end up. Such orientation is especially marked in organisms which contain numerous daughter-colonies, but it is apparently accidental or absent in those without. Since the colonies are dead this orientation can be brought about only by a difference in the specific gravity of the

anterior and the posterior half of the body, and since this orientation to gravity is definite only in specimens containing daughter-colonies it is evident that the daughter-colonies, located as they are mostly in the posterior half of the body, render it heavier than the anterior half.

The specific gravity of living *Volvox* is also greater than one. If the colonies become inactive they sink to the bottom, and it is undoubtedly due to this that they are frequently found lying quietly on the bottom of the aquarium after being in darkness all night. The fact that the specific gravity of living colonies is greater than one and that the posterior end of those which contain daughter-colonies or spores is heavier than the anterior end, has an important bearing on orientation to light and the direction of motion.

It is owing to the difference in weight of the two ends, that the anterior end turns up, if for any reason the forward motion of a colony ceases. In this position the colonies are frequently found in very dim light, apparently hanging in the water motionless. If they become active while in this position, they swim upward. Such activity may be induced by light so dim that the organisms do not orient. The degree of activity in light of low intensity, without doubt, depends upon the physiological state of the organism, for it was frequently noticed that many colonies did not become quiet in darkness, and several times after exposure to darkness for as long as four or five hours, a large majority was found at the surface of the water, apparently clinging to the surface film.

If horizontal movement of *Volvox* colonies toward a given source of light is observed from the side instead of from above, as was customary in the experiments described in the preceding pages, it can be clearly seen that the longitudinal axis of most of the specimens forms a decided angle with the bottom of the aquarium, that is, the posterior end is lower than the anterior. This angle varies from zero to 90 degrees. Contrary to the observations of KLEIN ('89, p. 169), it was found to be larger in organisms which contain numerous daughter-colonies and spores than in those which do not contain these structures. It is therefore in all probability caused by the difference in weight of the two ends.

The angle which the axis makes with the bottom of the aquarium varies also with the light intensity. The more strongly positive a given colony is, the smaller the angle; but the positiveness of

Volvox depends upon the light intensity, as was shown above (p. 121). Light, therefore, under the second conditions, tends to keep the axis horizontal, while gravitation tends to keep it vertical.

In traveling horizontally toward a source of light, then, the axis of Volvox is not parallel with its course, but if the light is suddenly decreased in intensity, as was repeatedly done, the colonies change their course and start in the direction in which the axis points. This seems to indicate clearly that they *tend* to travel in a direction parallel with the longitudinal axis. Now when they are strongly positive the axis becomes nearly horizontal and they consequently tend to move horizontally toward the source of light, but the force of gravity keeps pulling them down so that when the colonies are strongly positive they move toward the light very near the bottom of the aquarium. This was observed many times. If they are oriented in a beam of light thrown through the aquarium at some distance from the bottom, they soon sink out of the region of light into the darkness, but as soon as they get into the dark region gravity causes their longitudinal axis to take a vertical position and they swim upward again, unless darkness produces inactivity and thus causes them to sink slowly to the bottom. Thus they were frequently seen, while swimming across the aquarium, to pass from light down into darkness and back into the light again several times. If the specimens are not strongly positive the inclination of the axis toward the horizontal is not great, and they therefore tend to swim toward the surface. This upward tendency may be just sufficient to compensate the effect of gravity, and if so, the colonies appear to be moving parallel with the rays when viewed from the side. Under these conditions specimens were frequently seen to swim across the aquarium with very little deflection upward or downward.

In summing up, we find that when the colonies are strongly positive to light, the deflection to the side is reduced to a minimum, but owing to the effect of gravitation the downward deflection is marked; and when they are not strongly positive the deflection to the side is marked, while the vertical deflection may be practically zero. Thus it becomes evident that accurate orientation in horizontal movement is indeed exceptional.

If gravitation tends to keep the longitudinal axis of Volvox vertical with the anterior end directed upward, and light tends to keep it parallel with the rays with the anterior end directed toward

the source of light, and if the colonies tend to travel parallel with the axis, we should expect them to move parallel with the rays, when the rays are vertical and the source of light is above. This was found to be approximately true, as is shown by the following experiment.

On August 8, 1905, the plate glass aquarium was nearly filled with filtered water and put upon the stage of the light grader which was so arranged that the rays were vertical (see Fig. 5). A number of colonies were then put into the aquarium with a pipette and set free near the bottom in a beam of light, which was uniform in intensity and two and one-half centimeters square in cross section. After swimming upward to the surface of the water, some of the colonies wandered out into the shaded region. These could readily be forced to swim down again by reflecting the beam of light upward through the aquarium slightly to one side of the illuminated area produced by the light direct from the glower. The reflected beam could be made vertical by tipping the light grader so that the direct beam of the light made an angle of about 10 degrees with the vertical. In this way movements both upward and downward were studied.

In swimming up *Volvox* was found to travel very nearly parallel with the light rays, taking a spiral course, which was in some instances at least 2 mm. wide. In thus traveling upward, it could be clearly seen that the anterior end described a larger circle than the posterior, which in many colonies appeared to go almost in a straight line. The anterior end appeared to swing about the posterior as a pivot. While a large majority of the colonies traveled nearly parallel with the rays, there were a few which deflected considerably, some to such an extent that they passed out of the beam of light before reaching the surface of the water. That the movement parallel with the rays was due to the harmonious interaction of gravitation and light, and not to especially favorable conditions of light intensity, was demonstrated by the course of a certain colony in traveling upward toward the source of light parallel with the direction of the force of gravity, and then again in movement perpendicular to this force. When moving parallel with the direction of the force of gravity, the colony observed did not deflect more than one degree in making several trips up through the water in the aquarium, but in moving perpendicular to this force in the same aquarium and in the same light intensity, this same colony deflected 30 degrees to the right.

In swimming downward there is no evidence of a spiral course, the path, however, is much more irregular than in swimming upward; colonies on their way down were frequently seen to swerve to one side as if about to turn and go in the opposite direction. Gravitation, as has been stated, tends to keep the longitudinal axis vertical with the anterior end up, but the light from below, under the conditions of the experiment, tends to orient the organisms with the anterior end down. It is the interaction of these two opposing directive forces which brings about the swerving reaction and the irregularity in the downward course. If the light is weak its directive influence is not as strong as that of gravitation, and many colonies may be seen oriented with the anterior end up. The downward movement of specimens in this position is very slow compared with that of those with the anterior end directed down. This is evidently the result of the effect of gravity and a tendency to swim upward, *i. e.*, in the direction which the anterior end faces.

The rate of movement varies greatly in different colonies under the same external conditions. It is, however, in general, much faster toward a source of light with the force of gravitation than against it. This is shown by the following results. The time required for each of three specimens to swim downward 8 cm. toward a source of light, in a given intensity, was found to be 40 seconds for one, 32 seconds for another, and 30 seconds for the third. That required to swim up toward the light in the same intensity, was 100 seconds, 80 seconds, and 66 seconds, respectively, an average of 48 seconds longer to swim upward 8 cm. than to swim the same distance downward. It is very probable that the activity of *Volvox* in swimming upward is just as great as it is in swimming downward and that the difference in rate is entirely due to its specific gravity.

In summing up this whole matter we find: (1) That *Volvox* tends to move in a direction parallel with its longitudinal axis; (2) that gravity tends to keep this axis vertical, with the anterior end up, but owing to stimulation by light the organisms tend to orient with the anterior end facing in the direction of strongest illumination; (3) that *Volvox* travels very nearly parallel with the rays in moving up toward a compact source of light, but that it very rarely moves parallel with the rays in swimming downward or horizontally toward a source of light; (4) that in reacting to light it almost always deflects upward or downward, or to the right or left, and

that these deflections depend upon the light intensity and the physiological conditions of the organisms; (5) that it deflects most in moving horizontally when its axis is most nearly vertical and that the axis becomes most nearly vertical when the organism is not strongly positive.

In swimming downward toward a source of light, the deflections are clearly due to a tendency of the organism to orient in the direction of the force of gravity with the anterior end directed upward. In swimming horizontally it is clear that the downward deflection is due to the specific gravity of the organism, and the upward deflection to the tendency to swim parallel with the axis. The cause of lateral deflection in such movement is, however, not so evident.

Colonies swimming horizontally toward a single source of light, tend, as stated, to take a position such that the axis is parallel with the rays and the zooids on all sides are equally illuminated. If the organisms are strongly positive, the axis is nearly horizontal, so that if they turn to the right or left, one side immediately becomes shaded and thus causes a reaction which tends to keep the direction of movement parallel with the rays. But if the colonies are not strongly positive, the axis is more nearly vertical, and while they are in this position there is already a difference in light intensity on opposite sides, so that if the organism now turns to the right or to the left, this intensity difference is only slightly changed. There is consequently but little cause for reaction and therefore nothing to prevent movement at an angle with the rays. Since lateral deflection has been observed to be greater the more nearly vertical the axis, it seems probable that this is a valid explanation of the cause of such deflection. But how is it that a colony can repeatedly travel across an aquarium, making the same angle with the light rays each time; or that when the position of the source of light is changed, after it has started on a course at a given angle with the rays, it changes its course until the new one has the same angle? The only explanation I have to offer is the following: If colonies in water only a few millimeters deep, are simultaneously and equally illuminated from above and from below, they do not move in straight lines but in curves, frequently making continuous

the directive force of the light and the tendency to swim in curves. This would necessarily result in movement at an angle with the light rays. The size of this angle would depend upon the relative efficiency of the directive force of the light and the tendency to swim in circles. If the organisms are strongly positive, the directive force of the light is strong compared with the tendency to move in curves and the angle becomes small. But if they are not strongly positive, the directive force of the light is relatively weak and the angle becomes large. The theoretic results thus formulated are in accord with the experimental results described in the preceding pages.

d. Effect of Contact Stimulation and Rotation on Orientation.

When colonies of *Volvox* come in contact with the side of the aquarium nearest the light and the rays are perpendicular to this side, many of them soon begin to drift to the right along the glass wall, and in a short time a large majority are found in the right hand corner of the aquarium nearest the source of light. This movement to the right takes place in a field of graded light as well as in light of uniform intensity, and it is apparently as marked if the intense end of the field is to the left as it is if this end is to the right. Thus the organisms were frequently seen to move along the wall toward the right, on the one hand, into regions gradually decreasing in intensity until they passed into darkness and, on the other, into regions gradually increasing in intensity until they became negative. The movement to the right along the wall takes place, with much greater regularity, however, in specimens containing large daughter-colonies or spores than in young colonies. Indeed it is doubtful whether more of the young colonies turn to the right than to the left after they reach the wall of the aquarium. At any rate shortly after the introduction of a group containing both large and small colonies, practically all the large colonies, together with some small ones, have gathered in the right hand corner, some small ones have collected in the left hand corner, and a few of both kinds usually remain scattered along the entire side. What is the cause of this movement to the right along the wall?

After reaching the wall the colonies ordinarily remain with the anterior end in contact with it for some little time, but sooner or

later the posterior end begins to settle, the longitudinal axis becomes nearly vertical, and the organism begins to swim upward along the wall, deflecting to the right. The angle of deflection varies greatly. Some colonies travel nearly parallel with the bottom at once; others swim nearly straight upward. During the time that the anterior end is in contact with the wall, the colonies usually rotate counter-clockwise without reversal, and rotation in this direction frequently continues during the whole process of turning and moving to the right. It is, therefore, clear that the drifting to the right along the wall is not due to change in the direction of rotation.

After the axis becomes nearly vertical the colonies sometimes remain in close contact with the wall but continue to rotate counter-clockwise without moving forward, and thus roll along the wall to the left. Frequently after thus moving along the wall a short distance, the anterior end turns to the left and the organism begins to swim forward, but still continues to roll on the wall. This rolling along the wall, together with the effect of gravity, soon carries it to the bottom of the aquarium, where it apparently becomes lodged in the angle between the bottom and the side. Here it remains for a time, but sooner or later works its way out, usually by swimming back from the wall a short distance, after which it turns and soon comes in contact with the wall again. A colony may, as is clear from what has just been said, turn either to the left or to the right after reaching the wall, but many of those which turn to the left are prevented from continuing on their course by the effects of rotation and gravitation, as explained above; and since those which turn toward the right are not thus prevented from continuing, the result is, of course, a general drifting of the colonies in this direction. But as a matter of observation a much larger proportion of colonies turn to the right than to the left shortly after they reach the wall, so that general movement to the right cannot be primarily brought about by the prevention of continuous movement to the left. Neither can it be due primarily to the direction of rotation, for many colonies were repeatedly seen to deflect to the left in swimming across the aquarium toward the source of light, and then to the right, after coming in contact with the wall, without changing the direction of rotation. It seems then that the tendency to turn to the right after reaching the wall must be due primarily to contact stimuli. As evidence in support of this view I present the following experiments:

On August 10, 1905, between 20 and 30 colonies were put into the aquarium into an intensity of 21 candle meters. When the rays were parallel with the bottom the group spread very little and swam across the aquarium nearly parallel with the rays. But when the glower was lowered so that the rays passed up through the glass bottom of the aquarium, making an angle of 25 degrees with it, the group spread out considerably and the majority deflected quite sharply to the right. The largest colonies were found along the right side of the column and the smallest along the left, under both conditions. It is doubtful whether the smaller colonies changed their course after the position of the glower was changed, but the larger ones certainly did. Later more definite results were obtained by experimenting with a single colony. The specimen selected was of medium size and contained quite a number of rather small daughter-colonies. When the rays were parallel with the bottom this colony deflected three degrees to the right, but when the light was below the level of the bottom and came up through it so that the rays made an angle of 25 degrees with it, the organism deflected 19 degrees in the same direction.

In ascertaining these deflections the colony was allowed to cross the aquarium a few times first with the rays parallel with the bottom, then with the rays at an angle of 25 degrees with it, then again with the rays parallel with it, and finally, with the rays at an angle of 25 degrees. The deflection during the various trips under each condition, was nearly constant. It is therefore certain that the increase in deflection was not due to a possible change in the physiological condition of the organism. Neither was it due to difference in light intensity, for the strength of illumination was nearly the same under the two conditions of the experiment, and deflection is not much affected unless there is very marked change in the intensity of the light (see p. 122).

In moving toward the light in rays parallel with the bottom, the axis of this colony was at an angle of about 12 degrees with the bottom. The organism moved near the bottom of the aquarium so that the posterior end appeared to be slightly in contact with it. But when the light came from beneath at an angle of 25 degrees the axis of the colony was nearly horizontal and the organism moved so near the bottom that the cilia must have come in close contact with it. As the specimen thus swam across the aquarium the axis could be clearly seen to swing at short intervals, from a posi-

tion nearly parallel with the general direction of motion to a position nearly perpendicular to it. This swinging of the axis, it is thought, was due to contact with the bottom and counter-clockwise rotation, owing to which the posterior end seemed to roll to the left more rapidly than the anterior. This appeared to turn the anterior end of the axis sharply to the right, and since the colonies tend to move parallel with their axis, it would cause deflection to the right. Some such reaction must be at the basis of the deflection to the right when the organism is in contact with the vertical wall nearest the light. It may also explain why the larger colonies are found to deflect more to the right than the smaller, since the specific gravity of the two is different.

I have discussed the cause of the movement of *Volvox* to the right along a vertical wall at some length because of its importance in the study of the reactions of the colonies in aggregating in regions of optimum intensity in graded light, which will be taken up later.

7. ORIENTATION TO LIGHT FROM TWO SOURCES.

In the preceding pages we have conclusively demonstrated that while *Volvox* moves in general toward a given source of light, it seldom travels parallel with the rays, excepting when they are vertical, and it swims upward. But while the colonies do not usually swim parallel with the rays they still orient in a definite way. That is, if a colony is swimming at a given angle with the rays and the source of light is moved, it so changes the direction of motion that its course again makes the same angle with the rays that it did before the position of the source of light was changed. What is the cause of orientation?

OLTMANN'S, as has been stated (p. 100), came to the conclusion that difference in light intensity is the principal cause of orientation of *Volvox*, but he presented no direct evidence in favor of this view, and his indirect evidence is based upon experiments which have since been proved to be defective. HOLMES was not able to explain orientation by assuming difference in light intensity on opposite sides of the organism to be the cause, and he is inclined to believe that it is due to the direction of the rays. He writes ('03, p. 324): "It seems not altogether improbable that light in passing through a nearly transparent organism like *Volvox* exercises a directive effect upon its movements, in a similar way, whatever it may be, to that produced by the current of electricity.

The direction of the ray may be the important factor in orientation irrespective of difference of intensity of light upon different parts of the organism, as has been maintained by SACHS for the phototropic movements of plants. I am not ready to adopt the theory of SACHS, but I feel that it is a view that is not entirely out of court."

The following experiments on the movement of *Volvox* when exposed to light from two different sources, and on the orientation of *Volvox* in light graded in intensity seem to me to settle this question conclusively.

On August 18, 1904, a single 222 volt Nernst glower was fixed in a vertical position 70 cm. from the middle of the plate glass aquarium, so that the lower end of the glower was level with the bottom of the aquarium and the rays perpendicular to the side at a point 4 cm. from one end. A single 110 volt glower was arranged like the 222 volt glower, but in such a position that the light rays were perpendicular to the end of the aquarium at the middle and, therefore, perpendicular to the rays from the 222 volt glower at a point 4 cm. from the end, and half way between the two sides, as represented in Fig. 7. The 222 volt glower was stationary, but the 110 volt glower could be moved to any desired distance from the aquarium. These glowers were both carefully screened so that the only light which escaped passed through a rectangular slit a trifle larger than the glower. The side and end of the aquarium facing the glowers was also screened, with the exception of an opening one centimeter wide and six centimeters long, at the bottom of the aquarium, as indicated in Fig. 7. The aquarium contained thoroughly filtered water 1.5 cm. deep. Thus, practically all reflection from the sides of the aquarium and the surface of the water was eliminated.

The direction of movement of *Volvox* was ascertained, first with the 222 volt glower exposed alone, then with both glowers exposed, the 222 volt glower 66 cm. from the side, and the 110 volt glower 24, 49, 99, and 199 cm. from the end of the aquarium. In order to ascertain the direction of motion under the various light conditions, a considerable number of colonies were carefully dropped into the corner of the light area farthest from the glowers. Among the specimens used in this experiment there were about as many that deflected to the right as to the left, so that when one glower only was exposed the center of the group of colonies moved across the aquarium practically parallel with the light rays. Sev-

eral trials were made under each light condition and each path, as recorded in the table below and in Fig. 7, is the average of several such trials. There was, however, surprisingly little variation in the direction of motion of different groups when subjected to the same light condition. The light intensity was measured with care. Both glowers were on the same circuit so that variation in voltage could not have affected markedly the relative intensity of the light from the two sources. There can thus be no question about the approximate accuracy of the experiments, the results of which will be readily understood by referring to Table I, in connection with Fig. 7.

| TABLE I. | | |
|---------------------|----------------------|-------------|
| I. | II. | III. |
| 82.4 candle meters. | .0 candle meters. | 0 degrees. |
| 82.4 candle meters. | 6.0 candle meters. | 9 degrees. |
| 82.4 candle meters. | 23.5 candle meters. | 25 degrees. |
| 82.4 candle meters. | 89.0 candle meters. | 47 degrees. |
| 82.4 candle meters. | 318.8 candle meters. | 59 degrees. |

Table I represents the effect of light from two sources on the direction of movement of *Volvox*. Column I gives the light intensities at the middle of the light area in the aquarium, which were produced by the 222 volt glower under the five different conditions. Column II gives light intensities produced by the 110 volt glower, and column III the angles between the rays produced by the 222 volt glower and the course taken by the organisms under the different light conditions.

In these five experiments the direction of the rays from the two sources of light was practically constant, but the direction of movement of the *Volvox* colonies varied 50 degrees. This variation was certainly not primarily due to any influence of the ray direction; for when the relative intensity of light affecting different sides of the organism was changed the orientation changed, though the direction of the rays remained the same. It can, therefore, be considered fully demonstrated that *difference in light intensity on different sides of the colonies may determine orientation independently of the direction of the rays*. Additional proof of this conclusion will be given later, in experiments of a different character.

This conclusion is not in harmony with the dictum of LOEB, repeatedly expressed in a recent work (1905), in which he writes, "It is explicitly stated in this and the following papers that if there are several sources of light of unequal intensity, the light with the strongest intensity determines the orientation and direction of

motion of the animal. Other possible complications are covered by the unequivocal statement, made and emphasized in this and the following papers on the same subject, that the main feature in all phenomena of heliotropism is the fact that symmetrical points of the photosensitive surface of the animal must be struck by the rays of light at the same angle. It is in full harmony with this fact that if two sources of light of equal intensity and

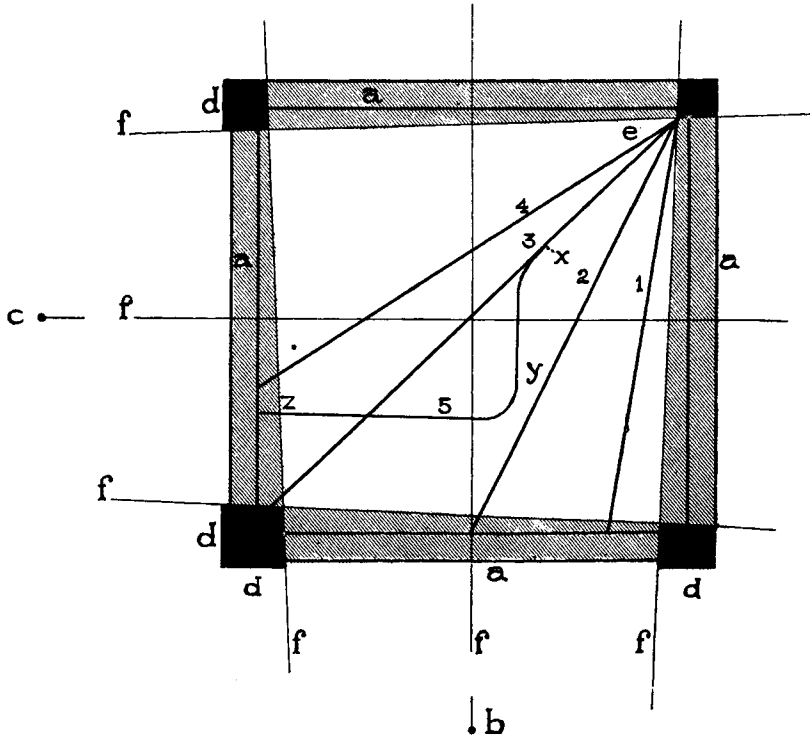


FIG. 7. Representation of the direction of movement of *Volvox* when subjected to light from two sources. *a*, plate glass aquarium 8 cm. long and 8 cm. wide; *b*, 222 volt Nernst glower, 66 cm. from aquarium (distance from aquarium constant); *c*, 110 volt glower, (distance from aquarium variable); *d*, screen; *e*, point of introduction of *Volvox*; *f*, direction of light rays; 1, 2, 3, and 4, courses of *Volvox* exposed to light from both glowers: 1, with 110 volt glower 199 cm. from aquarium; 2, with 110 volt glower 99 cm. from aquarium; 3, with 110 volt glower 49 cm. from aquarium; 4, with 110 volt glower 24 cm. from aquarium; *xy*, course of *Volvox* when exposed to light from glower *b* only; *yz*, course when exposed to light from glower *c* only.

distance act simultaneously upon a heliotropic animal, the animal puts its median plane at right angles to the line connecting the two sources of light. This fact was not only known to me but

had been demonstrated by me on the larvæ of flies as early as 1887, in Würzburg, and often enough since. These facts seem to have escaped several of my critics" [p. 2]. "When the diffuse daylight which struck the larvæ [*Musca larvæ*] came from two windows, the planes of which were at an angle of 90° with each other, the paths taken by the larvæ lay diagonally between the two planes. This experiment was recently published by an American physiologist as a new discovery to prove that I had overlooked the importance of the intensity of light!" (p. 61-62). "The direction of the median plane or the direction of the progressive movements of an animal coincides with the direction of the rays of light, if there is only a single source of light. If there are two sources of light of different intensities, the animal is oriented by the stronger of the two lights. If their intensities be equal, the animal is oriented in such a way as to have symmetrical points of its body struck by the rays at the same angle" (p. 82). "Attention need scarcely be called to the fact that if rays of light strike the animal [*larvæ of Limulus polyphemus*] simultaneously from various directions, and the animal is able to move freely in all directions, the more intense rays will determine the direction of the progressive movements" (p. 268).

It is evident without further discussion that the reactions of *Volvox* do not fit the statements by LOEB, given in the above quotations. Upon what experimental evidence does he base these statements? Those with reference to orientation when the animals are subjected to light from two or more sources are based largely, if not entirely, upon the following observations: (1) "When the diffuse daylight which struck the larvæ (*Musca larvæ*) came from two windows, the planes of which were at an angle of 90° with each other, the path taken by the larvæ lay diagonally between the two planes." (2) "Hawk moths were brought into a room with the single window at one end, and a petroleum lamp at the opposite end. It was found that, as twilight came on, the moth flew to the window, or to the light, according to the relative intensity of the one or the other at the point where the moth was liberated."

In the first place I am unable to understand how the direction of rays can be ascertained in diffuse daylight coming through a window; and in the second place, it is certainly not difficult to see that an object placed between two windows, or between a

window and a petroleum lamp, in an ordinary room, is illuminated by light rays striking it from every conceivable direction, for light under such conditions is reflected from practically all surfaces in the room as well as from those outside. Under the conditions of the experiments cited above, then, the larvæ and moths were not exposed to light from two sources but to light from an infinite number of sources, and the direction of the rays was not known. How then, can it be concluded from the results of these and similar experiments (1) "That if there are several sources of light of unequal intensity, the light with the strongest intensity determines the orientation and direction of movement of the animals;" (2) "that symmetrical points of the photosensitive surface of the animal must be struck by the rays of light at the same angle;" and (3) "that if two sources of light at the same intensity and distance act simultaneously upon a heliotropic animal, the animal puts its median plane at right angles to the line connecting the two sources of light?"

Let it be clearly understood that in the criticism of LOEB's conclusions, I do not wish to intimate, that because the reactions of *Volvox* or any other organism do not take place in accord with those conclusions, they necessarily cannot hold for the organisms LOEB worked with. I do, however, wish to state and emphasize that in my opinion his experimental results as quoted above, do not warrant his conclusions, even for the animals worked on, much less for all organisms which orient in light.

The experiments upon which LOEB bases his theory of orientation to a single source of light will be discussed later (see p. 142).

8. ORIENTATION IN LIGHT GRADED IN INTENSITY.

The reaction of *Volvox* to light from two sources varying in relative intensity seems to me to prove conclusively that orientation is determined by the relative intensity of the light on opposite sides of the organism, while there is no evidence that the direction of the rays has anything to do with orientation in this organism except in so far as it may affect the relative light intensity on opposite sides. If, however, difference in light intensity on opposite sides of a colony can be produced with the rays of light approximately parallel, and such intensity difference affects the direction of motion, the verdict must be considered final.

By means of the light grader referred to several times in the preceding pages, I was able to subject colonies to rays which were nearly parallel but decreased in intensity from one end of the field to the other, so that when the longitudinal axes of the colonies were parallel with the rays, one side was more strongly illuminated than the other; and I found that this intensity difference did affect the direction of motion, as will be shown in the following detailed account of the experiment.

The light grader was so arranged that the Nernst glower was vertical and the rays and the long axis of the lens horizontal. The plate glass aquarium was so placed that the rays were parallel with the bottom. Now by fastening over the lens a screen, which contained an opening in the form of two truncated triangles with their apices in contact, a field of light was produced which was of high intensity at either end and gradually became lower toward the middle. Two methods were used in ascertaining the direction of movement in such a field of light.

In the first method a large number of colonies were taken up in a pipette and half of them introduced into the aquarium near the side farthest from the glower at a fixed point some distance from one end of the field, and the other half in a similar place near the opposite end. Thus the organisms in one group as they swam across the field were more intensely illuminated on the right side, while those in the other group were more intensely illuminated on the left side.

In the second method a single colony was selected and allowed to cross the aquarium toward the source of light several times, first near the right end of the field so that the lower light intensity was to the left and then near the left end of the field so that the lower light intensity was to the right. This alternating process was continued until the path in the two different positions was definitely established. The angles of deflection were read and recorded as described on p. 117. Those obtained by the first method may be found in Table II and those by the second method in Table III. The negative numbers indicate deflection to the left of the ray direction and the positive to the right.

Table IV represents the effect of difference in light intensity on deflection in graded light. The course taken by the colonies was obtained by studying the reactions of single colonies, just as in the experiments of Table III. This table shows that an increase

TABLE II:
Light Intensity 333± candle meters.

| Angle of deflection with strongest illumination to the left. | Angle of deflection with strongest illumination to the right. | Difference in angle of deflection |
|--|---|-----------------------------------|
| 6° | 11° | 5° |
| 7 | 11 | 4 |
| 8 | 13 | 5 |
| 8.5 | 15 | 6.5 |
| 11 | 13 | 2 |
| 14 | 16.5 | 2.5 |
| 15 | 15 | 0 |
| 19 | 25 | 6 |
| 19 | 23 | 4 |
| 21 | 27 | 6 |
| 7.5 | 10 | 2.5 |
| 7.5 | 10 | 2.5 |
| 9 | 9 | 0 |
| 9 | 10 | 1 |
| -17.5 | -15 | 2.5 |
| -13 | -12 | 1 |
| 10 | 12 | 2 |
| 10 | 15.5 | 5.5 |
| 11 | 15.5 | 4.5 |
| 13.5 | 18.5 | 5 |
| 13.5 | 20 | 6.5 |
| 16.5 | 20 | 3.5 |
| Average difference | | 3.6 degrees. |

TABLE III.
Light Intensity 333± candle meters.

| Angle of deflection with strongest illumination to the left. | Angle of deflection with strongest illumination to the right. | Difference in angle of deflection |
|--|---|-----------------------------------|
| 3° | 5° | 2° |
| 7 | 9.5 | 2.5 |
| 0 | 2 | 2 |
| -1 | 4 | 5 |
| -5.5 | -1.5 | 4 |
| 3 | 6.5 | 3.5 |
| 13.5 | 16.5 | 3 |
| Average difference | | 3.1 degrees. |

| Angle of deflection in 142 candle meters of light. Strongest illumination to the left. | Angle of deflection in 380 candle meters of light. Strongest illumination to the left. | Difference in angle of deflection |
|--|--|-----------------------------------|
| 6.5° | 4° | 2.5° |
| 8 | 9 | -1 |
| 10 | 10 | 0 |
| 10 | 6 | 4 |
| 14.5 | 11.5 | 3 |
| -20.5 | -18 | 2.5 |
| Average difference | | 1 $\frac{5}{8}$ degrees. |

in light intensity from 142 candle meters to 380 candle meters causes an average decrease in deflection of $1\frac{5}{8}$ degrees.

By referring to the above tables and text figures it will be noted: (1) that *Volvox*, in swimming horizontally toward a source of light, seldom moves parallel with the rays. There is striking individual variation in the angle of deflection, the variation in these experiments being from 16 degrees to the left to 24 degrees to the right; (2) that in a field of light graded in intensity there is a tendency to deflect toward the brighter end of the field, an

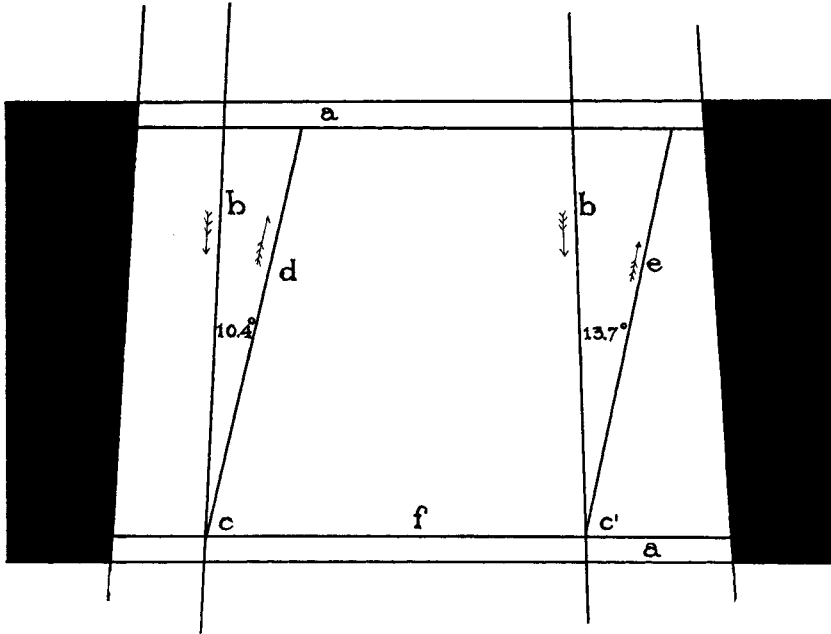


FIG. 8. Graphic representation of the total average difference in deflection due to difference in light intensity on opposite sides of the colonies, as indicated in Tables II and III. *a*, plate glass aquarium 8 cm. wide and 15 cm. long; *b*, light rays; *c*, *c'* points where the colonies were introduced; *d*, average course with the region of highest light intensity to left; *e*, average course with strongest illumination to the right. Light intensity at (*f*) the middle of field 57.12 candle meters. From the middle the intensity gradually increased toward either end where it was 442.68 candle meters. Intensity at *c*, 327 candle meters, at *c'*, 263 candle meters.

average of over $1\frac{1}{2}$ degrees under the conditions of these experiments; (3) that the degree of deflection in a field of light graded in intensity depends upon the strength of illumination, it being greater in a low light intensity than in a high one. A decrease in intensity from 380 candle meters to 142 candle meters without

change in the grade of intensity caused an average increase in deflection of $1\frac{1}{8}$ degrees.

Cause of Deflection Toward the More Strongly Illuminated Side in Graded Light.—If a colony of *Volvox* deflects to the right in light of uniform intensity it will deflect more in a field of light graded in intensity, provided the more highly illuminated end of the field is to the right, but not as much if this end is to the left. This fact is clearly expressed in Fig. 8. Under the conditions of the experiments described above, this difference in deflection must have been primarily due to one of three factors: (1) difference in total light intensity under the two conditions; namely, with the more highly illuminated end of the field to the right and with this end to the left; (2) refraction or reflection as the light passes through the aquarium; (3) difference in light intensity on opposite sides of the colony. A discussion of these three factors follows.

1. We have demonstrated (see Table IV) that an increase in light intensity, without change of grade, causes a decrease in deflection. Now, as represented in Fig. 8, the colonies, as they deflect in crossing the aquarium with the brighter end of the field to the right, gradually pass into regions of higher light intensity, but when the brighter end of the field is to the left, they gradually pass into regions of lower intensity. This consequently tends to cause a decrease in deflection under the former conditions and an increase under the latter, but the angle of deflection is greater under the former condition than under the latter. The difference in deflection under the two conditions, therefore, cannot be due to the higher light intensity to which the organisms are exposed when the more strongly illuminated end of the field is to the right than when it is to the left.

2. As the light passes through the glass wall of the aquarium and the water in it, some is reflected and some refracted thus producing lateral rays. This reflection and refraction cannot be entirely eliminated even with the utmost precaution. May not these lateral rays have been of sufficient intensity to cause deflection toward the brighter end of the field as was found to be true in case of OLTMANN'S apparatus?²

² OLTMANN'S (92) produced a field of light graded in intensity by placing a hollow prism filled with a mixture of gelatine and India ink between the source of light and the aquarium. He assumed that the rays in the aquarium were all perpendicular to the wall facing the source of illumination. This, however, is not true, for the particles of ink in the prism disperse the light before it gets into the aquarium.

The field of light in which the colonies were exposed in the above experiments was high in intensity at either end and low in the middle. In such a field of light it is clear that an organism swimming toward the light in the middle is stimulated alike on both sides, since the lateral rays necessarily come in equal numbers from both ends of the field. Consequently the direction of motion cannot be influenced by these rays. But if the organism in traveling toward the light swims nearer one end of the field than the others, the lateral rays might influence the direction of motion. If, however, the lateral rays do affect the direction of motion under such conditions, we should certainly expect to be able to detect it when all lateral rays on one side of a colony swimming toward the source of light are eliminated by shading the entire portion of the field either to the right or to the left of the colony. I repeated the above experiments many times with a portion of the field thus shaded, but was unable to detect any effect on the angle of deflection. It must therefore be concluded that the difference in deflection, represented in columns I and II of Tables II and III, was not caused by lateral rays.

THE DIRECTION OF MOTION IN VOLVOX EXPOSED TO LIGHT IS CONSEQUENTLY REGULATED BY THE RELATIVE INTENSITY OF THE LIGHT ON OPPOSITE SIDES OF THE COLONIES REGARDLESS OF THE DIRECTION OF THE RAYS.

Cause of the Effect of Change in Intensity Upon the Degree of Deflection in Graded Light.—The difference in intensity of illumination on opposite sides of the colonies exposed in the light grader under the conditions of the experiments just discussed, can readily be calculated. The light intensity was 442.6 candle meters at either end of the field, from which it gradually decreased toward the middle, where it was 57 candle meters. The distance from the middle to either end was 60 millimeters. We have therefore a change of 385+ candle meters in 60 millimeters or 6.4 candle meters per millimeter. The largest colonies are nearly a millimeter in diameter and the average light intensity to which they were exposed was about 333 candle meters. In the largest specimens, then, one side was exposed to an intensity of about 330 and the other to an intensity of about 336 candle meters.

If WEBER'S law holds true, as we have good reason to believe, (see p. 171), we should expect this difference in intensity on opposite sides to be more effective in weak light than in strong and we should consequently expect a greater deflection in regions in the

field where the light intensity is low than in those where it is high. As is clear from Table IV this was found to be true. But since we have demonstrated (p. 121) that deflection in light of uniform intensity can be increased either by decreasing or increasing the intensity, it may be maintained that the difference in deflection recorded in Table IV is due to the difference in light intensity in the field regardless of difference in intensity on opposite sides of the organisms. It must be remembered, however, that deflection in a field uniformly illuminated, is increased only if the intensity is decreased to a point near the threshold or increased to a point near the optimum. In the experiments just referred to, the intensity,

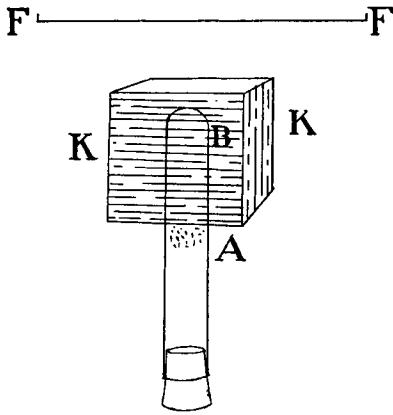


FIG. 9. After LOEB, 1905, p. 25, Fig. 1.

in all probability, was far below the optimum and above the threshold, so that it is not likely that mere reduction in illumination affected the deflection to any considerable extent. The difference between the degree of deflection in 142 candle meters and 380 candle meters of light, graded in intensity, must, therefore, have been due to the greater effect of the differences in light intensity on opposite sides of the organism when exposed to weak light than when exposed to strong. The experimental results recorded in Table IV therefore support our previous conclusion, that the direction of motion in *Volvox* is regulated by the relative intensity of light on opposite sides of the colonies.

LOEB, however, as is well known, asserts that orientation is caused by the direction of the rays regardless of the difference in light intensity. He bases his assertion largely on the results in the three following experiments on *Porthesia* larvæ (LOEB '05, p. 25-28).

Experiment 3.—The test tube is placed perpendicular to the plane *F* of the window, and at the beginning of the experiment the animals are collected at the window side *B* of the test tube. Now if the half near the window is covered, the animals soon collect at *A*. “As soon as they emerge from the box *K* into *A* they turn about,

direct their heads toward the window, move to the edge of the pasteboard and remain at the boundary between the covered and uncovered portions of the tube at *A*, and especially at the top of the test tube.

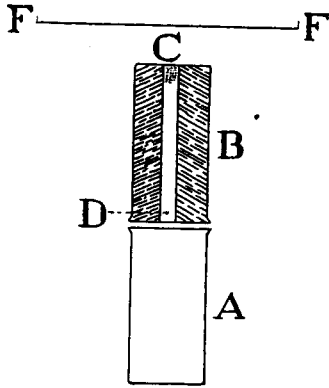


FIG. 10. After LOEB, 1905, p. 27, Fig. 2.

shaded as represented in the figure, the light intensity is lower than in the test tube *A*, not shaded, but the larvæ go to *C*.

Experiment 5.—The animals move from direct sunlight at *A* to *B* into the diffuse daylight. They pass from the direct sunlight into diffuse daylight without even attempting to return into the sunlight.

In these, as in other experiments of LOEB referred to on p. 135, the animals were exposed to light, the ray direction of which must have been exceedingly complicated, since the light was diffused before it reached the tube in which the animals were.

Moreover, the walls of the tube caused still further diffusion by refraction and reflection. How, then, could it be ascertained in any of these experiments whether the animals moved in the direction of the rays or not?

is that they are not distributed evenly over the whole brightly illuminated part of the test tube. The explanation is as follows: As soon as the animals near the window at *B* are covered by the pasteboard, the weak rays of light reflected from the walls of the room fall upon them. *The animals follow the paths of these rays and arrive at the uncovered portion of the tube*" [Italics ours].

Experiment 4.—The larvæ were found to move to *C*, toward the window *F—F*. In the test tube *B*,

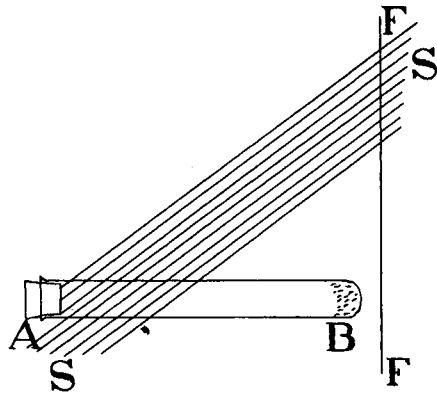


FIG. 11. After LOEB, 1905, p. 28, Fig. 3.

In Experiments 4 and 5, the animals moved from a region of higher light intensity to one of lower. Now from this the author concluded that difference in intensity does not cause orientation, for if it did, the animals, being positive, would remain in the region most highly illuminated.

In discussing the effect of difference in light intensity it is necessary to define the sense in which this is meant. There is a vast difference between the difference in light intensity in a given field and the difference in intensity on different areas of the surface of a particle in the field. For example, hold an opaque piece of paper in direct sunlight so that the rays strike it at right angles and you will find almost an infinite difference in the light intensity on the two sides, but remove the paper and you will find that the intensity difference in the field is actually infinitesimal. It is evident then that an organism can move from regions of higher to regions of lower light intensity in a field produced by apparatus arranged as represented in Figs. 10 and 11, and still have the anterior end constantly more highly illuminated than the posterior. LOEB evidently did not recognize this in the experiments cited above, for he accepts the theory of SACHS, who ('87, p. 695) defines his position very clearly, as follows: "I came to the conclusion that in heliotropic curvatures, the important point is not at all that the one side of the part of the plant is illuminated more strongly than the other, but that it is rather the direction in which the rays pass through the substance of the plant."

In moving toward the window in the test tubes arranged as represented in Figs. 10 and 11, the anterior end of the animal was very likely more highly illuminated than the posterior. On the assumption that difference in intensity on the surface of the organism causes orientation, the larvæ would consequently be expected to move toward the window. I can, therefore, see nothing in these experiments which in any way indicates that difference in light intensity on the surface of the body, regardless of the direction of the light rays, is not the cause of orientation.

9. ORIENTATION OF SEGMENTS.

In working on *Volvox* it was noticed that colonies with various portions missing still appeared to respond to stimulation by light. Such colonies were most frequently found after heavy rain storms or other rather violent disturbances. On July 28, 1905, a colony

was found, in which the anterior end and a narrow portion of the side extending nearly to the posterior end, were missing. This segment oriented quite definitely. In swimming horizontally toward a source of light it moved approximately parallel with the rays, deflecting but little. When exposed to light from two sources of equal intensity, it took a course about midway between them. If the light from one of the sources was cut off after the segment had thus oriented, it continued on its original course for a few millimeters, then changed the direction of motion until it was oriented once more. Its light reactions in general were like those of intact colonies, but the path of this segment instead of being straight as is true in case of entire colonies, was in the form of a spiral. This was evidently the result of the mechanical effect of the gap in the side and rotation on the longitudinal axis.

The reactions of many other segments of colonies were studied later. Most of these segments were made by cutting the colonies in pieces. In performing these operations a considerable number were put under a cover glass which was then carefully pressed down until the colonies split open. Under these conditions they usually split at the posterior end, but sometimes at the side. By inserting a needle ground to a knife-edge, the wall could be cut in any direction desired without much difficulty.

It was found that segments of practically all forms and sizes responded to stimulation by light, but owing to their form and the effect of gravitation, many could move only in small circles, and were unable to orient.

It can be stated definitely, however, that among segments of various forms and sizes, such as are produced by cutting the colonies in half, either parallel or perpendicular to the longitudinal axis, respond in general like whole colonies, with the exception that most of the segments take a spiral course, the width of which depends upon the form of the segment. It is thus clear that a colony of *Volvox* can orient when the anterior or the posterior end or one side is missing. A theory of orientation must be broad enough to explain not only the reactions of entire colonies but also those of any segments.

10. MECHANICS OF ORIENTATION.

JENNINGS ('04, p. 32-62) found that *Stentor cœruleus* and *Euglena viridis* orient by means of motor reactions when exposed

to light. If stimulated they turn toward a structurally defined side regardless of the direction of the rays or difference in light intensity on opposite sides of the organisms. If they fail to become oriented by a single motor reaction they repeat the reaction, turning successively in different directions, until they turn in the right direction; this direction they hold, and thus become oriented. The process of orienting in these organisms is, therefore, strictly on the trial and error basis.

In *Volvox*, taking a colony as a whole, there is no evidence of motor reactions, nor is there any hit or miss method about its orientation. It makes no mistakes in the process. If exposed to light it turns toward the source of light without error. What sort of mechanism has this organism, by means of which it can thus regulate the direction of its motion?

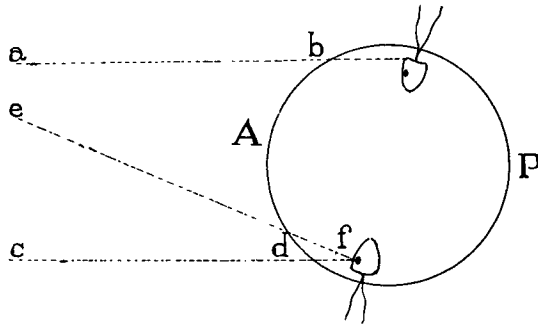


FIG. 12. After HOLMES, 1903, p. 325.

A colony of *Volvox* may be conceived to turn in its course by decreasing or increasing the backward stroke of the flagella on one side or the other, or by using the flagella on either or both ends as rudders, or even by directing the stroke of these flagella in such a way as to turn the organism. But since the organisms orient when either the posterior or the anterior end is missing, and probably also when both ends are missing, it is clear that the flagella on the ends do not function primarily in changing the direction of motion. Such changes must, therefore, be the results of inequality of the strokes of the flagella on opposite sides. What then is it that causes the strokes on opposite sides to become unequal?

HOLMES ('03, p. 325) after concluding that it cannot be caused by difference in light intensity on opposite sides, suggests the

following explanation. "The orientation of the colony may be accounted for, if we suppose that the eye-spots are most sensitive to light striking them at a certain angle such as is indicated in the diagram by the lines $a-b$ and $e-f$. If rays of light enter the colony in the direction of the lines $a-b$ and $c-d$ somewhat obliquely to the long axis, $A-P$, the flagella of the cells represented on the upper side of the diagram would beat more vigorously and accelerate the motion of that side of the organism. The opposite cell being struck by rays in the direction $c-d$ would be less stimulated, and, as the flagella would beat less strongly than those on the other side of the colony, the organism would swing about until its long axis is brought parallel with the rays when, being equally stimulated on both sides, it would move in a straight course towards the light. We do not have to suppose that each cell makes a special effort to orient itself at a particular angle to the rays, but that it is so organized that the effective beat of the flagella is most accelerated by light striking the cell at a certain angle. If the cells were most stimulated by light falling upon them at such an angle as would result if the rays diverged from a spot in front of the colony and in line with its long axis the conditions for orientation would be fulfilled. Since the eye-spots in all the cells face the anterior end of the colony this supposition appears very probable. The foregoing explanation of the orientation of *Volvox* may or may not be the true one, but it enables us to see a significance in the peculiar arrangement of the eye-spots in this form and is consistent with the results of the experiments we have described." Is it also consistent with the results of the experiments described in the preceding pages?

In the first place the eye-spots, upon the arrangement of which HOLMES places considerable importance in his theory, are not so situated that they all face the anterior end; quite the contrary, they face the posterior end of the colony, as pointed out on p. 107; and in the accompanying diagram by HOLMES they should be on the side of the zooids nearest the end P , instead of on that nearest the end A . They do, however, probably function as light recipient organs, as already stated (p. 108). Let us then assume that the zooids are influenced by the direction of the rays as HOLMES suggests, even if the eye-spots do face the posterior end of the colony, and see if the theory fits our experimental results.

1. It was clearly demonstrated (p. 139) that if specimens of

Volvox be exposed to parallel rays of light so that there is a difference in intensity on opposite sides of the organisms when the longitudinal axis is parallel with the rays, they do not move directly toward the source of light but deflect toward the side most highly illuminated. In accordance with HOLMES' theory we should expect them to move parallel with the rays under these conditions.

2. HOLMES states that the condition for orientation, according to his theory, would be fulfilled "if the rays diverge from a spot in front of the colony in line with its long axis." If this be true, we should certainly expect the conditions for orientation also to be fulfilled, if the rays converge from two luminous points in front of the organism and if "the eye-spots are most sensitive to light striking them at a certain angle" we should expect the organisms to move toward a point nearly, if not exactly, midway between the two sources of light regardless of their relative intensity. But it has been demonstrated (p.133) that if Volvox colonies be exposed to light from two sources of unequal intensity, they orient and swim toward a point nearer the more intense source. It is, therefore, evident that the explanation of orientation in Volvox, suggested by HOLMES, is not consistent with the experimental results which I have presented.

I have demonstrated beyond a reasonable doubt that the difference in intensity on opposite sides of Volvox modifies its direction of motion regardless of the direction of the light rays, and since the direction of motion is changed by difference in the effective stroke of the flagella on opposite sides, it must be difference in intensity which influences the stroke of the flagella. But HOLMES, as stated above, concluded that the reaction of Volvox cannot be explained upon the assumption that difference in intensity on opposite sides of the body causes the flagella to beat with unequal vigor. Upon what does he base this conclusion and wherein lies the fallacy of his argument?

I can present his line of thought best by quoting verbatim ('03, p. 321-322): "Let us consider a *Volvox* in a region of sub-optimal stimulation and lying obliquely to the rays of light. If it orients itself to the light the backward stroke of the flagella, *i. e.* the stroke that is effective in propelling the body forward must be more effective on the shaded side than on the brighter side. This may conceivably occur in the following ways, which, however,

amount practically to the same thing: the diminished intensity of light on the shaded side of the body may act as a stimulus to the backward phase of the stroke, or decrease the efficiency of the forward phase of the stroke of the flagella; or the light on the brighter side of the body may inhibit the backward phase or increase the forward phase of the stroke of the flagella; In any case, if the organism is passing into regions of ever-increasing intensity of light, we should expect its rate of speed would be lowered. If the orientation is affected by a shading of the side away from the light it would follow that in a region in which the shading were less the speed of the travelling body would be diminished. If the parts of the body which are most shaded are the parts where the effective beat of the flagella is the strongest, then, as the organism passes to a point where the illumination on both sides of its body is increased, its rate of transit would be diminished. If we suppose that the forward stroke is most stimulated, or the backward stroke most inhibited on the brightest side of the body we should expect that with more illumination the more inhibition there would be, or the more the backward phase of the stroke would be increased, and the rate of locomotion would likewise be reduced. If we imagine a machine in the form of a *Volvox* colony and provided on all sides with small movable paddles so adjusted that when they come into regions of diminished light as the machine rolled through the water their effective beat would be increased, it is clear that such a machine might orient itself to the direction of the rays and travel towards the source of illumination, but its rate of locomotion would be diminished the brighter the light into which it passed. We may conceive the light to increase or decrease the backward or forward stroke of the paddles in any way we please and we cannot explain how such a machine can orient itself and go towards the light and at the same time move through the water more rapidly as it comes into regions of greater illumination."

It is evident that the crux of this whole argument is the relation between rate of movement and light intensity. This relation was worked out in detail by HOLMES ('03, p. 323) with the following results: "It was found that, as the *Volvox* travelled towards the light, their movement was at first slow, their orientation not precise, and their course crooked. Gradually their path became straighter, the orientation to the light rays more exact and their

speed more rapid. After travelling over a few spaces (centimeters), however, their speed became remarkably uniform until the end of the trough was reached." Unfortunately, HOLMES does not give the length of the trough, but he says the distance over which there is a marked increase of speed is considerably less than the space over which the speed is nearly uniform.

HOLMES concludes from these results that the increase in rate of speed is due to increase in light intensity and consequently that orientation cannot be due to difference in intensity on opposite sides of the organism, because if it were, the backward stroke of the flagella would have to be more effective on the side in the higher light intensity than on the side in the lower, and this would cause the organism to turn from the source of light instead of toward it. Are these conclusions correct?

If the increase in rate of speed is due primarily to increase in light intensity, one would certainly not expect the rate to become uniform after the colonies have traveled a few centimeters in the trough, nor would one expect it to increase if the colonies are exposed to light of a given intensity for some time. But HOLMES states that the rate does become uniform, and I frequently observed that if relatively quiet colonies in an aquarium containing water a few millimeters deep, are illuminated from above, they gradually become more active. Since, under these conditions, they cannot move toward the source of light, it is evident that this increase in activity is not due to increase in light intensity. It is very probable then, that the increase in rate of movement is more dependent upon the time of exposure to light than upon the increase in intensity. Moreover, HOLMES states that orientation is more exact after the colonies have traveled some little distance, *i. e.*, after the rate has become nearly uniform. It must, therefore, be least exact when the increase in rate of speed is greatest. If this be true, it follows that the factors which regulate rate of speed are quite different from those which regulate orientation. We have demonstrated that difference in light intensity on opposite sides of the colonies modifies the direction of movement. And since the factors which regulate the direction of motion and those which regulate the activity of the colonies are different, we may conclude, from this point of view, as well as from what has gone before, that the increase in the rate of speed is not primarily due to increase in light intensity. Such being the case, the argument of HOLMES

cited above cannot be valid, for it is based upon the supposition that increase in speed in *Volvox* is due to increase in light intensity. We shall refer to this question again (p. 153).

If a colony which is not oriented turns toward the source of light, it is clear that the stroke of the flagella on the shaded side must be more effective in driving the organism forward than that on the illuminated side. This may be conceived to be caused directly by the difference in light intensity on opposite sides, or indirectly in that a *Volvox* colony may possibly act as a lens and thus cause the light on the side opposite that most highly illuminated to become most intense; or, since the zooids are intimately connected by protoplasmic strands, it is not impossible that impulses produced by excessive photic stimulation may be transmitted to the opposite side and result in action there. At any rate, it is undoubtedly true that these strands serve to transmit impulses from zooid to zooid, and thus bring about coördinate action.

It was found, as previously stated, that segments, *e. g.*, halves produced by cutting specimens parallel to the longitudinal axis, orient essentially like normal colonies. Such segments, however, cannot act as lenses, nor can impulses originating on one side be transmitted to the opposite side. The last two of the possible explanations suggested, therefore, must be abandoned, and it must be concluded that the unequal effect of the stroke of the flagella is due directly to difference in light intensity on opposite sides of the organism. But this unequal effect of the stroke on opposite sides may be caused, as HOLMES pointed out, by an increase in the backward phase of the stroke on the shaded side, or a decrease in the same phase on the illuminated side or a decrease in the forward phase on the shaded side, or an increase in this phase on the illuminated side. Can it be ascertained which of these is the cause of the difference between the effect of the stroke of the flagella on the shaded sides and that of those on the illuminated side of the colonies?

If the light intensity of the field is suddenly decreased while colonies of *Volvox* are swimming horizontally toward it, they stop forward motion, the longitudinal axis takes a vertical position due to the effect of gravity, and then the colonies swim slowly upward. It is not at all difficult to find specimens in which this upward swimming is just sufficient to overcome the effect of gravity, and

under such conditions they appear to be hanging in the water motionless. They are, however, rotating on their longitudinal axis. If now the light intensity, to which these apparently motionless organisms are exposed, is increased they soon begin to turn toward its source; but in so doing they swim upward, as represented in the accompanying diagram.

In thus swimming upward and horizontally toward the source of light, it is clear that the effect of the backward stroke of the flagella increases both on the shaded side and on the illuminated side, for both sides move forward. But the shaded side moves farther than the illuminated side, consequently the increase in the effect of the backward stroke must be greater on the former than on the latter. The difference in the effect of the stroke of the

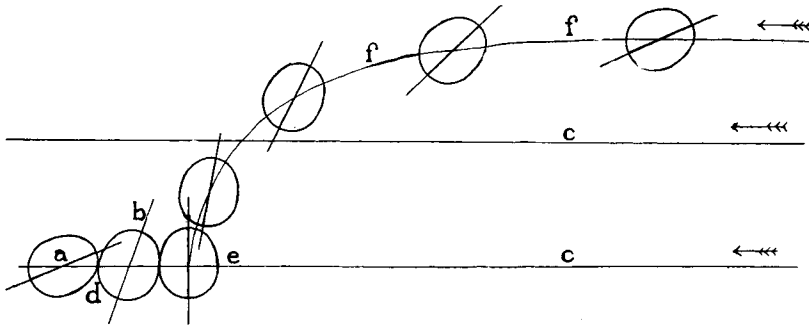


FIG. 13. Diagram representing the reaction of a *Volvox* colony when the light intensity is suddenly changed. *a*, outline of colony; *b*, longitudinal axis; *c*, light rays; *d*, point in the course where the light is suddenly decreased; *e*, point where it is suddenly increased; *f*, course taken by colony. In continuing from *e*, the side of the colony facing the source of light travels over a shorter distance than the shaded side. Consequently the backward stroke of the flagella on the latter side must be more effective than that of those on the former.

flagella on opposite sides which results in orientation of positive *Volvox* colonies is, therefore, due to a greater increase in the backward stroke of the flagella on the shaded side than of those on the illuminated side.

If the light thrown upon apparently motionless colonies is quite intense, they frequently may be seen to sink 4 or 5 mm. immediately after the light is turned on, but while they are sinking this short distance, they apparently become acclimated and soon turn toward the light, and at the same time swim upward, just as described above. During the time in which these colonies sink they continue to rotate in the same direction as before. The

sinking must then be due to a decrease in the effect of the backward stroke of the flagella on all sides, and this decrease is due to an increase in light intensity. But when the colonies turn toward the source of light, and at the same time swim upward, it is evident that the increase in light intensity must cause an increase in the backward phase of the stroke of the flagella on all sides, for if this were not true there could be no upward motion. The side nearest the source of light, however, passes over a shorter distance than the opposite side, as will readily be seen by referring to the diagram, and therefore the increase in the effect of the backward phase must be greater on the latter than on the former. But the light intensity is greater on the former than on the latter (a paradox). When the light intensity in the field is increased the effect of the backward phase of the stroke of the flagella may be increased or decreased on all sides. If it is increased the effect is most marked on the side in lowest light intensity. Furthermore, if the light is strong the colonies turn toward its source more rapidly and do not swim upward so far and thus make a sharper curve than when it is weak; but the stronger the light the greater the difference between the intensity on the shaded and that on the illuminated side. It, therefore, follows that the greater the difference in intensity on these sides, the greater the difference in effect of the backward phase of the stroke of the flagella, the effect being greatest on the side least illuminated. These considerations support the conclusion arrived at above, *i. e.*, that the factors which regulate the activity of the colonies, as a whole, are different from those which regulate the direction of motion.

We have thus demonstrated that while orientation is due to difference in light intensity on opposite sides of the colonies, it is brought about in positive specimens by the flagella striking backward with greater effect on the side in lowest light intensity than elsewhere. I suggest the following explanation of this:

First, it must be remembered that the organism constantly rotates on its longitudinal axis. If then a colony is so situated that one side is more highly illuminated than the opposite, it is clear that the zooids will constantly be carried from a region of higher to a region of lower light intensity, and vice versa. They are thus subjected to constant changes in strength of illumination. As stated above, the flagella strike backward with greater vigor on the shaded side than on the opposite one and, therefore, it is

evident that as the zooids reach the region of lower light intensity, in other words when the light intensity to which they are subjected decreases, they increase the effect of the backward stroke of the flagella, *i. e.*, they attempt to turn toward a structurally defined side (the side facing the anterior end of the colony). This is precisely what *Euglena* does when it passes from a region of higher to one of lower light intensity, *i. e.*, it turns toward a structurally defined side, the larger lip. The individuals in a colony then respond with a motor reaction induced by change in light intensity; they react on the same basis as do *Euglena*, *Paramecium*, *Stentor* and other unicellular forms, in their trial and error reactions, but owing to the way in which they are inter-related, and to the rotation of the colony on the longitudinal axis, this reaction of the zooids causes orientation in the colony as a whole, without error.

This explanation of orientation in entire colonies holds also for orientation in segments. As previously stated, only those segments orient which have such a form that they can rotate. As they rotate the cut surface constantly faces the center of the spiral, so that if the axis of the spiral is not directed toward the source of light, the outer surface where the zooids are situated is alternately turned toward the light and away from it. Thus the zooids are carried from regions of higher to regions of lower light intensity and vice versa, and the motor reaction is induced just as it is in entire colonies.

Orientation in negative colonies can be explained in precisely the same way as that in positive ones, assuming merely that in this condition the zooids respond with the motor reaction when they pass from lower to higher light intensity instead of when they pass from higher to lower (as is true when the organisms are positive). The backward stroke then becomes most effective on the side most highly illuminated.

II. REACTION OF NEGATIVE COLONIES.

Volvox becomes negative when exposed to light of a certain intensity. The intensity, however, varies greatly in different colonies and in the same colony under different conditions. RADL ('03, p. 103) concludes his discussion on the difference between positive and negative phototropism with the following paragraph: "Ich glaube nun, dass der Unterschied zwischen positivem und negativem Phototropismus ähnlich wie beim Menschen nicht ein

Unterschied in der Orientierung, sondern nur in der Lokomotion ist; dass das Tier in beiden Fällen gegen die Lichtquelle orientiert ist, jedoch nicht gleiche Muskeln spannt."

This explanation will not hold for *Volvox* or *Euglena*, for both of them turn the anterior end from the source of light when they are negative.

When *Volvox* colonies are negative they orient in all essentials as they do when positive, except that they direct the anterior end from the source of light. In swimming horizontally from a source of light they seldom move parallel with the light rays. If the position of the light is changed after they have oriented, they change the direction of motion until the course again bears the same relation to the ray-direction it did before. If exposed to light from two sources, so arranged that the rays make a definite angle with each other, they move from a point between the two. If one source is more intense than the other, the point from which they move is nearer that source.

These facts and others are established by the following experimental results, which are presented in graphic form (Fig. 14).

By referring to path *A* it will be seen that the colony introduced at *n* was positive to light from the three glowers as well as to that from the arc, but that it became negative after swimming toward the arc for a short distance from *c*, turned about and moved across the aquarium to *c'*. That is, at the end of the experiment the colony was negative to a much lower light intensity than at the beginning. The arc was approximately 250 candle power. It was 15 cm. from the point where the organism became negative. The light intensity at this point was therefore $11,111 \pm$ candle meters. But the colony was still negative after having crossed the aquarium, a distance of nearly 8 cm., or nearly 23 cm. from the arc, *i. e.*, in an intensity of $4726 \pm$ candle meters, which is $6385 \pm$ candle meters less than the intensity in which it first became negative. Similar results are represented in path *B* and the paradoxical nature of the results is even more striking than in the case of path *A*. Unfortunately, the distances between the sources of light and the aquarium, in this exposure, were not recorded.

The colony which produced path *B* was positive to the light from the arc when first put into the aquarium at *c*, but after moving toward the source of light a few centimeters, it became negative,

turned about and moved in the opposite direction. When it reached c' the glowers were exposed and the colony promptly changed its direction of motion and proceeded on a course directed from a point between the two sources of light. This point, however, was much nearer the arc than the glowers, the light from the

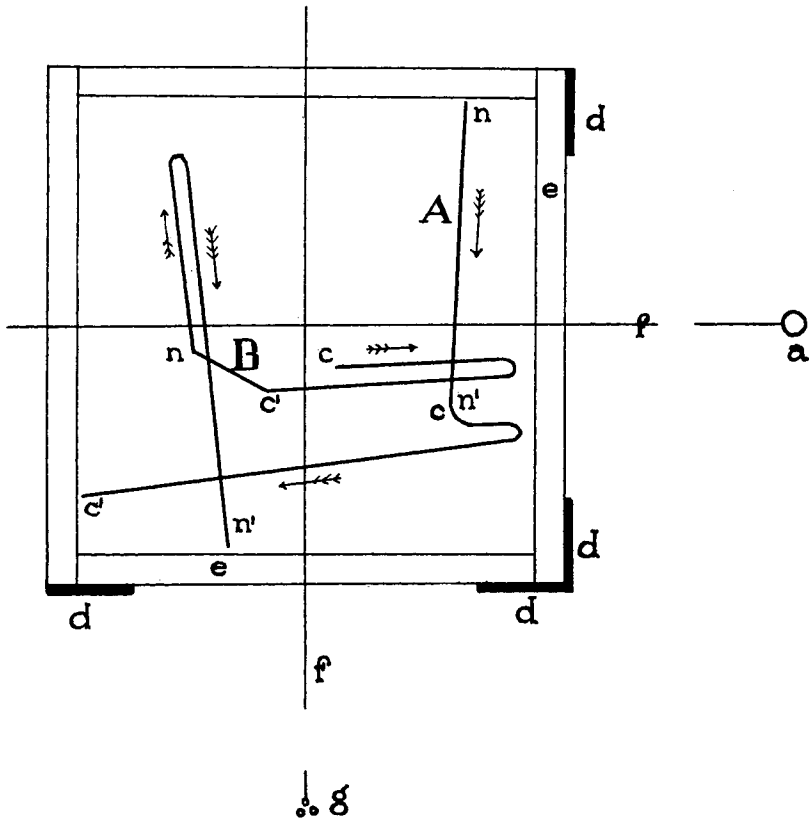


FIG. 14. The lines A and B represent the course taken by single colonies as seen in water 2 cm. deep in the plate glass aquarium, e (the paths are represented in approximately accurate proportions); g , a group of three 222 volt Nernst glowers in a vertical position; a , carbon arc; f , direction of light rays; d , opaque screens; $n n'$, path with glowers exposed and arc shaded; $c c'$, path with arc exposed and glower shaded; $c' n$, path with both glowers and arc exposed.

former being much more intense than that from the latter. When the light from the arc was cut off at n , the colony was found to be negative to the comparatively weak light from the glowers. It consequently changed its course and moved from this source; but

after continuing about 3 cm. it became positive, turned about and moved toward the glowers to n' , and probably would have continued farther had it not been prevented from doing so by the wall of the aquarium. It will be noticed that the point n' , where the colony was still positive at the end of its course, was about 3 cm. nearer the glowers than n , where it proved to be negative, and nearly 7 cm. nearer than the point where it changed its course from negative to positive. That is, the organism was positive at n' in a much higher light intensity than that in which it was negative at n and at the point where it changed from negative to positive.

12. CAUSE OF CHANGE IN SENSE OF REACTION.

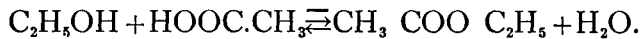
The results presented above demonstrate that *Volvox* may be either negative or positive in a given light intensity. This will be brought out more clearly later where it will be shown that *Volvox*, in certain conditions, is negative to light of all intensities to which it responds at all.

Since a *Volvox* colony may be either positive or negative in the same environment, it is clear that the transformation from positive to negative or vice versa must be due to some internal change. This change, whatever it may be, is induced by light. It is dependent upon the intensity and also upon the time of exposure, as is shown by the fact that when specimens are exposed to intense light they may be positive for a time and then negative to a much lower intensity than that in which they were positive when first exposed. Weak light tends to induce the change which causes the colonies to become positive, whereas strong light tends to induce the change which causes them to become negative.

Some photosynthetic process in chlorophyll bearing organisms, suggests itself as the probable condition upon which the sense of reaction depends. It might be assumed that the organisms are positive when a given amount of synthesized substance, such as carbohydrates, proteids, or fats, is present, and negative when this amount is decreased. This assumption fits the observed reaction in that such substances are formed in the presence of light, and in that they disappear in darkness, being either further synthesized to form protoplasm, or, perhaps, directly oxidized. But the short time and the slight change in light intensity necessary to produce a change in the sense of reaction is entirely inadequate

to cause the formation and destruction of photosynthetic substances, such as those mentioned above. The inversion of the sense of reaction, therefore, cannot be due to a photosynthetic process. May it not be due to the effect of light on the chemical equilibrium of some other substance ?

One of the more important results of recent investigation in physical chemistry is the establishment of the fact that substances in chemical equilibrium are dynamic and not static, as had formerly been supposed. If, for instance, alcohol be added to acetic acid, it is well known that water and ethyl acetate will be formed; but it is also true that if water be added to ethyl acetate, the formation of alcohol and acetic acid results, that is, the former reaction is reversed. When the reaction in both of these cases has reached a state of equilibrium, there is a certain amount of each of the following substances present: Alcohol, acetic acid, ethyl acetate, and water, and this amount remains constant; but the reaction continues; alcohol and acetic acid react to form ethyl acetate and water just as fast as ethyl acetate and water react to form alcohol and acetic acid. These reactions are expressed as follows:



This indicates that two reactions are taking place simultaneously in opposite directions. The relative amount of substance indicated in the two members of an equation representing equilibrium in chemical reaction, depends upon the nature of the substances and the environment, *i. e.*, the temperature, pressure, etc. If, for instance, the temperature of compounds in equilibrium be raised, the equilibrium will be destroyed and the reaction in one direction will take place faster than that in the other. When equilibrium is again restored the relation of the amounts of the different substances will no longer be the same as it was at the lower temperature. If the temperature is lowered, the rate of motion will increase in the opposite direction. JONES ('02, p. 514) states this as follows: "The effect of a rise in temperature is to favor the formation of that system which absorbs heat when it is formed. . . Increase in pressure diminishes the volume and, therefore, favors the formation of that system which occupies the smaller volume."

Reversible chemical reactions were formerly supposed to be quite exceptional, but it is now known that they are not. JONES ('02, p. 481) writes: "We must regard chemical reactions in general as reversible."

No work, as far as I know, has been done directly on the effect of change in light intensity on equilibrium in chemical reaction; but we know that light does affect many chemical reactions, and since we must regard chemical reaction in general as reversible, it seems reasonable to assume that the relative amount of different substances present in a mixture is dependent upon the light intensity, provided the chemical reaction between the substance is at all affected by light. This means that substances in chemical equilibrium in one light intensity will not be in equilibrium in another, so that the direction in which the reaction takes place faster depends upon the light intensity.

To explain reversal in the sense of reaction on the basis of chemical reactions induced by light let us assume: (1) That *Volvox* contains substances *X* and *Y*, the chemical reaction between which is regulated by the intensity of light; (2) that a sub-optimum intensity favors the formation of substances represented by *X* and a supra-optimum those represented by *Y*; and (3) that the colonies are neutral in reaction when there are *Y* substances in one member of the equation and *X* in the other; positive when one member contains (*X* +) substances and the other (*Y* -), and negative when one contains (*X* -) and the other (*Y* +). Can the change in sense of reaction as represented in paths *A* and *B*, Fig. 14, p. 156, be explained on the basis of these assumptions?

The colony which produced path *A* was positive when put into the aquarium at *n*. In accordance with our assumption it, therefore, contained (*X* +) and (*Y* -) substances. The intensity at *n* was relatively low so that the chemical reaction favored the formation of compounds represented by *X*. This may be expressed thus $(X+) \rightleftharpoons (Y-)$, indicating that the reaction toward *X* takes place faster than that toward *Y*. The increase in the *X* and decrease in the *Y* substances continued until a state of equilibrium was attained or the organism reached *n'* and *c*, where the light from the glower was turned off and that from the arc turned on, and the colony was thus exposed to light of supra-optimum intensity. Why did it not then turn from the source of light at once? According to our assumption, because it contained (*X* +) and (*Y* -) substances. But since the colony was in a supra-optimum intensity, the chemical reaction favored the formation of *Y* substances at the expense of *X*, represented thus $(X+) \rightleftharpoons (Y-)$. As soon as this reaction had continued far enough so that (*X* +)

was decreased to X and $(Y -)$ increased to Y , the colony became neutral. The point where this took place is represented in the path by the sharp curve. But why did the colony not remain neutral? Because it was in a supra-optimum light intensity and, therefore, in accordance with our assumption, X continued to decrease and Y to increase, $X \rightleftharpoons Y$ resulting in $(X -)$ and $(Y +)$ compounds which caused the organism to become negative and it remained so to the end of its course. Had the aquarium been wider it would have reached a point at which it would have been neutral in an optimum light intensity. If the reactions are regulated as assumed, it would have reached this point as follows: $(X -) \rightleftharpoons (Y +)$ expresses the condition of the colony as it proceeded from the source of light toward c' , but as the intensity decreases the rate of formation of X increases and that of Y decreases until the colony reaches the point of optimum intensity, when the rate in opposite directions is equal $(X -) \rightleftharpoons (Y +)$. The organism, however, is still negative at this point, since it contains $(X -)$ and $(Y +)$ substances, and it therefore proceeds into a region of sub-optimum intensity, where $(X -)$ increases and $(Y +)$ decreases $(X -) \rightleftharpoons (Y +)$. This results in X and Y substances and the colonies consequently become neutral. The chemical reaction, however, continues to favor the formation of X , since the light is sub-optimum, and this soon results in $(X +)$ and $(Y -)$ substances, which causes the organism to become positive. It therefore turns and proceeds toward the source of light again, but owing to the accumulation of $(X +)$ and $(Y -)$ substances, it passes the region of optimum intensity before it becomes neutral, and therefore becomes negative again. It may be conceived to thus pass back and forth several times, like a pendulum, before being neutral in the optimum region.

In accordance with our assumption, the conditions of the colony in producing the path B could be represented as follows:

- $(X +) \rightleftharpoons (Y -)$ from c to the beginning of the curve;
- $(X) \rightleftharpoons (Y)$ at the point in the curve nearest the arc;
- $(X -) \rightleftharpoons (Y +)$ from this point to n ;
- $(X -) \rightleftharpoons (Y +)$ from n to the beginning of the curve beyond;
- $(X) \rightleftharpoons (Y)$ at the point in the curve farthest from the glower;
- and $(X +) \rightleftharpoons (Y -)$ from this point to n' , the end of the course.

We have thus presented a formal explanation of these paradox-

ical reactions, based upon possible chemical changes in the organism. But since the chemical changes are purely hypothetical, this explanation must be, of course, considered merely as a suggestion.

If our explanation proves to be correct, the process of acclimatization must be the process of such changes in the organism that the neutral condition will be produced when the relative amount of the substances represented by X and Y is changed.

Temperature changes, mechanical agitation, or any other agent which would in any way affect the chemical reaction between X and Y would, of course, influence the change in the sense of reaction, and thus we should have a possible explanation of the effect of such agents on the change from positive to negative reaction and vice versa, recorded in the literature on the subject.

13. EFFECT OF TEMPERATURE ON CHANGES IN SENSE OF REACTION.

On August 17, 1904, *Volvox* colonies which were strongly positive were put into a small aquarium containing water about 5 mm. deep and exposed to light from a group of three 222 volt glowers, 15 cm. from the aquarium. The light intensity in the aquarium was approximately 4000 candle meters. The colonies were therefore in an intensity which was nearly optimum. The water in the aquarium was then slowly heated to 45° C. As the temperature increased the organisms became slightly more active but showed no indication of becoming negative. When the temperature reached 45° nearly all were dead. This experiment was repeated and the temperature raised to 51°, a temperature which proved fatal to all the colonies. The results in the second experiment were similar to those in the first. It therefore seems evident that change in temperature does not induce reversal in the sense of reaction in *Volvox*. This, however, does not mean that change in temperature may not affect reaction to light; indeed, it is more than probable that it does, for at low temperature all light reactions cease.

These results agree with those obtained by PARKER on Copepods ('02, p. 117) and by YERKES ('03, p. 375) on *Daphnia pulex*, but they do not agree with those obtained by LOEB ('93, p. 91), who found that the sense of reaction in *Polygordius larvæ* was changed from positive to negative by a change in temperature from 24° C.

to 29° C.; MASSART ('91, p. 164), who found *Chromulina*, a flagellate, to be positive at 20°, and negative at 5; and STRASBURGER ('78, p. 605), who states that swarm-spores, positive to a given light intensity at 16 to 18° C. are negative to the same intensity at 40. It seems strange that organisms so nearly alike as *Chromulina*, *Volvox* and swarm-spores should be affected so differently by change in temperature.

14. EFFECT OF MECHANICAL STIMULI ON THE CHANGE IN SENSE OF REACTION TO LIGHT.

In working on the light reactions of *Temora longicornis*, a copepod, LOEB ('93, p. 96) noticed that the animals, ordinarily negative, were frequently positive immediately after being caught. This change in the sense of reaction was due probably to mechanical agitation. Miss TOWLE ('00) found that the light reaction of *Cypridopsis* could be changed from positive to negative by taking the animals up in a pipette or by making them pass through a maze constructed with needles. HOLMES ('01) thinks that the fact that *Orchestia gracilis* is positive in air and negative in water, may be due to the contact stimulus of the water. It was demonstrated by PARKER ('02, p. 117) that certain forms of tactual stimulation cause the light reactions in the copepod, *Labidocera*, to change from positive to negative.

The effect of stimulation by light on *Volvox* can readily be overcome momentarily by mechanical stimulation, but it was found impossible to change the sense of reaction by such stimuli. In attempting to do this various methods were used, as, for example, shaking the organisms violently, lifting them in a pipette and squirting them into water, and making them swim toward the source of light among numerous large sand grains with which they came in contact.

Whatever the cause of reversal in the sense of light reaction in *Volvox* may be, it is clear that such reversal is of primary importance in the life of the organism. While continuous exposure to very intense light is fatal to *Volvox* colonies, they must have a certain amount of light, since they depend upon photosynthesis in the process of feeding. It is therefore evident that it is of great advantage to them to be able to move into regions of comparatively high intensity during dark, cloudy days, early in the morning, and late in the evening, and into shaded places when the light becomes very intense.

15. THRESHOLD.

In ascertaining the threshold of photic stimulation for *Volvox*, the colonies were put into a small glass aquarium constructed so as to reduce reflection from the exposed surfaces as much as possible and thus avoid excessive variation in intensity. A description of this aquarium was published in a preceding paper (MAST '06, p. 386). The aquarium containing the colonies was then moved from the source of light, a Nernst glower, until the light intensity became so low that the organisms no longer responded to it. The point at which reaction ceased could, however, be only approximately ascertained, owing to marked individual variation in the readiness with which they became acclimatized, to unavoidable variation in the intensity of the source of light, and to the difficulty of deciding, without the use of statistical methods, just where the response to light ceased. But since the reaction of *Volvox* depends quite as much upon its physiological condition as upon the intensity of the light, it is evident that it is of no particular importance to ascertain with great accuracy, either the threshold or the optimum, unless the variations thereof can be correlated with the physiological changes which cause them. We have no methods of measuring the physiological condition of this organism with any degree of accuracy, and therefore at present can hope to do no more than study the effect of various stimuli on the threshold and optimum. The following observations were made with the view of ascertaining the general effect of exposure to light on the variation in the threshold and optimum.

On July 30, 1904, at 5 p. m., it was found that *Volvox* which had been collected at 6 a. m. and kept in the dark all day responded definitely to light of 0.16 candle meters intensity, and rather definitely to light of 0.14 candle meters. This is the lowest intensity to which any response was obtained at any time. Specimens collected shortly after 12 m., July 14 and 15, respectively, and tested as soon as brought into the laboratory responded to light of 0.50 to 0.83 candle meters. The sky was clear on both of these days, but the organisms were found among the water plants in more or less shaded places.

It was found at different times that after being exposed to direct sunlight a few moments the colonies did not respond even to an intensity as high as 500 candle meters. We have thus observed the threshold to vary from 0.14 to 500 candle meters, and this

variation seems to have been due largely to preceding exposure to light. The threshold is higher in colonies previously exposed to strong light than in those exposed to weak light.

16. OPTIMUM.

The optimum light intensity for practically all *Volvox* colonies is somewhat lower than that of direct sunlight, $5000 \pm$ candle meters, but sometimes it is very much lower; it varies greatly. This variation is clearly shown in the following observation.

After a few very cloudy days the sun came out at 11 a. m., July 24, 1904, and the sky became exceptionally clear and remained so the remainder of the day. At 2 p. m. *Volvox* colonies were found in abundance freely exposed to the sunlight. Some of the colonies were collected and taken to the laboratory where it was accidentally discovered that they were negative in light intensities in which this organism had formerly always been found to be strongly positive. I then tested the colonies for the optimum and was greatly surprised to find that they were negative to all light intensities above 0.57 candle meters. In light from 0.57 to 0.29 candle meters, the lowest intensity to which they were exposed, their reactions were indefinite. There was no indication of any positive reaction whatever.

At different times a number of colonies were taken from a given jar and half of them put into each of two similar vessels containing equal amounts of water. One of the vessels was then exposed to direct sunlight and the other covered so as to exclude all light. After having been in this condition a short time the reactions of the colonies in the two vessels were compared by exposing both to the same light intensity. In such cases it was always found that the specimens which had been in direct sunlight were negative to light of lower intensity than those which had been in darkness. These results indicate that exposure to light of high intensity causes a lowering of the optimum. OLTMANN'S did not find this to be true. He states ('92, p. 190) that he covered two lots of *Volvox* with the same kinds of prisms, July 31, in the evening. One of these lots with its prism was kept in darkness until 9 a. m., August 1, the other was exposed to light. During the following three days it was found that those which were in the darkness until 9 a. m. collected in regions of lower light intensity than the others. STRASBURGER found the same to be true with reference

to the reactions of swarm-spores. It is difficult to criticise these experiments, since the light intensity and time of exposure are not definitely stated. However, it seems utterly impossible that the effect upon the optimum in colonies exposed for so short a time could, as OLTMANN'S states, be observed after three days. For purely *a priori* reasons we should, nevertheless, expect exposure to light to cause the optimum intensity to be higher, provided it is exposed to light in which acclimatization takes place. It may be, then, that the reason why the exposure to light in my experiments caused a decrease in the intensity of the optimum, is because the organisms were exposed to very intense light for but a comparatively short time, in other words, because they did not become acclimatized. If our explanation of the cause of reversal in the sense of reaction is correct, we should expect exposure to intense light for a short time, to lower the optimum. This is expressed in Fig. 14, path *B, n n'*, which indicates that the colony was negative to a much lower light intensity immediately after it had been exposed to light of high intensity than later. In accordance with our assumption, in attempting to explain the reaction represented by this figure it would mean an accumulation of the hypothetical substances ($X -$) and ($Y +$) during the time of exposure to a supra-optimum intensity.

There are some indications that when *Volvox* is negative to light of low intensity, it becomes positive when exposed to a much higher intensity. This is shown by the following observations:

August 23, 1904, was a bright clear day. At 4 p. m. specimens were collected in a place which had been well exposed to the sun much of the afternoon. Soon after reaching the laboratory, these specimens were found to be positive in light intensities varying from 230 to 1400 candle meters. The colonies not used in these tests were put into a liter jar and placed in strong diffuse sunlight in a west window. Here many of the colonies soon aggregated on the side of the jar farthest from the source of light. At 5.45 p. m., after having been in the window about an hour, they were found to be negative to an intensity of 230 candle meters and at 6.45 p. m. to an intensity as low as 3 candle meters. They seemed to become more strongly negative the longer they were left in the window, although the light from 6.30 p. m. on was quite dim. At the close of the experiment, 7 p. m., certain colonies which had been strongly negative to an intensity of 230 candle meters were

found to be positive to an intensity of 400 candle meters. The following day these organisms were exposed again to light of 1400 candle meters and to various lower intensities, but there were no indications of negative reactions.

I have no explanation to offer with reference to these reactions. The observations were not repeated.

17. REACTIONS ON REACHING THE OPTIMUM IN A FIELD OF LIGHT GRADED IN INTENSITY.

OLTMANN'S ('92) found that *Volvox* colonies collected and remained in a given light intensity, if put into an aquarium illuminated by light which first passed through a prism such that the light became gradually more intense from one end of the aquarium to the other. If, however, clouds passed over the sun or if the aquarium was in any way shaded, they hurried (*streben*) toward the more highly illuminated end of the aquarium, but when the clouds disappeared or the shading was removed, they returned to their former positions. If the prism was put between the source of light and a vessel containing *Volvox* which had a given direction of motion, the colonies changed their direction of motion almost instantly and moved toward the region of optimum intensity. OLTMANN'S writes ('82, p. 195): "So kann man leicht constatiren, dass die einzelnen Kugeln ihre ursprüngliche Bewegungsrichtung fast momentan verlassen und dann direct auf diejenige Region im Apparat zusteuern, in welcher sie später verweilen."

Was the course taken in the apparatus used by OLTMANN'S due, as he supposed, to difference in light intensity on opposite sides of the organism, resulting from rays perpendicular to the sides of the aquarium? It is impossible to calculate the difference in intensity produced by such rays, at any given point in the apparatus but it can be estimated with a sufficient degree of accuracy for our purpose. Let x represent the intensity of the light before entering the prism. OLTMANN'S states that 80 to 90 per cent of this was absorbed at one end of the prism and 30 to 50 per cent at the other. We shall assume it to have been 90 and 40, respectively. The intensity in the aquarium then, due to rays perpendicular to the sides, was $\frac{1}{10} x$ candle meters at one end and $\frac{6}{10} x$ candle meters at the other, a difference of $\frac{1}{2} x$ candle meters. The length of the aquarium was 200 mm. The decrease in intensity was, therefore, $\frac{1}{400} x$ candle meters per millimeter. If the intensity

of the light was 5000 candle meters, the general estimate of the intensity of the strongest direct sunlight, the decrease per millimeter in the aquarium was 12.5 candle meters. The difference in light intensity on opposite sides of the largest colonies due to direct light could, therefore, not have been greater than 12.5 candle meters. It probably was much less. As previously recorded (pp. 139-141), I found that if the decrease in light intensity is 6.4 candle meters per millimeter in a field of graded light, the deflection is only 1.5 ± 0 . It is consequently evident that if the colonies in OLTMANNS' apparatus moved directly toward the region of optimum light intensity, the direction of such movement was not caused by the difference in light intensity due to rays perpendicular to the sides of the aquarium. It is clear, then, that there must have been sufficient diffusion in OLTMANNS' apparatus to affect the direction of motion of the organisms.

If diffusion is practically eliminated, will *Volvox* still be able to reach the region of optimum intensity in graded light, and if so by means of what reactions? These questions are answered in the recorded observation and results of the following experiments. These experiments were performed in the light grader so arranged that the rays of light were horizontal and nearly perpendicular to the sides of the aquarium which contained water 1.5 cm. deep. The field of light gradually decreased in intensity from $238 \pm$ candle meters at one end to total darkness at the other. It was not quite as long as the aquarium, and was a little narrower than the depth of the water, so that the surface of the water and the sides of the aquarium were not illuminated and thus reflection was prevented.

At 10 a. m., August 26, 1904, a large number of *Volvox* colonies were evenly scattered in the aquarium along the entire side farthest from the source of light. They started toward the opposite side almost as soon as they reached the water and all deflected to the left, moving across the aquarium in nearly parallel lines, reminding one of columns of soldiers. Those in the region of higher light intensity, however, swam noticeably faster than those in regions of lower. The deflection was toward the darker end of the aquarium, but it must be remembered from what has been stated in preceding pages, that this deflection was not in the main due to the difference in light intensity. It would have been in the same direction and only a little greater if the more highly illuminated end of the field had been to the left instead of to the right.

Owing to the deflection to the left there were but very few colonies within 2 cm. of the right end of the field immediately after they had crossed the aquarium; but a few minutes later it was clearly seen that a large majority were swimming toward the right along the glass wall. In this movement, some followed the wall closely but most of them made a zigzag course coming in contact with the wall at short intervals. This zigzag course seems to have been the result of the interaction between contact and light stimuli. In about 15 minutes most of the colonies collected within 5 cm. of the brightest end of the aquarium. At first they were closely packed together near the wall, but after a short time they began to spread out in the form of a right angled triangle, the perpendicular of which coincided with the end of the aquarium. Some entered the dark region near the end of the aquarium and thus no longer stimulated by light wandered back from the side of the aquarium facing the light, others left this side without entering the dark region. These evidently became acclimatized or negative after exposure for some little time. Thus they continued to move back and forth, gradually spreading out toward the darker end of the aquarium, until finally they began to become less numerous along the bright border of the field of light, the region of highest intensity. Then the whole aggregation appeared to work itself very slowly into the regions of lower light intensity, gradually spreading back from the side of the aquarium facing the source of light; thus at the close of the experiment, five hours after it was begun, most of the colonies were within 5 cm. of the darker end of the aquarium. Here they were scattered over a triangular area which extended from the side of the aquarium nearest the source of light almost to the opposite side. The light intensity within the limits of this area varied from zero at the left to $47 \pm$ candle meters at the right. The organisms were, however, most numerous in the portions most strongly illuminated. The limits of the area which contained most of the colonies were, in every instance, very indefinite. There was always quite a number scattered about in other parts of the aquarium.

This experiment was entirely, or in part, repeated seven times and the reactions and results in each repetition were in general like those described above. The optimum intensity, as was to be expected, varied greatly, as did also the time it required the colonies to reach the optimum. Thus on August 9 it required only

about three hours for the organisms to collect in the region of optimum illumination. The light intensity of this region was $16 \pm$ candle meters at the left side and $71 \pm$ at the right. In repeating this experiment, the apparatus was several times so modified that the more highly illuminated end of the aquarium was to the left. Under these conditions the colonies reacted precisely as they did when this end was to the right. All of them aggregated in the right hand corner of the aquarium, now the region of lowest light intensity, and then gradually spread out until they reached the optimum.

The reactions of *Volvox* were also studied with the light grader in such a position that the rays were perpendicular to the bottom of the aquarium in place of parallel with it as they were in the preceding experiments, and with so little water in the aquarium that the organisms were forced to swim at right angles with the rays. In some instances under these conditions, there was no evidence of any aggregation whatever, but in others the colonies collected in regions of optimum light intensity. The limits of the regions in which they collected were, however, not well defined. In a few of the exposures some specimens of *Euglena viridis* were put into the aquarium with the *Volvox* colonies. These aggregated in a very definite narrow band, the center of which was in a light intensity of approximately 35 candle meters in every exposure. The *Euglenæ* reached the region of optimum intensity in the course of a few minutes, but it required one hour for any indication of aggregation of *Volvox* in any of these experiments.

There was absolutely no evidence of orientation and direct movement toward the region of optimum intensity, neither when the light rays were parallel with the bottom of the aquarium, nor when they were perpendicular to it. If there had been, we should certainly expect the colonies to have reached the optimum in much less time than was required in any of the above experiments. The fact that the colonies reach the optimum seems to be a matter of mere chance, the result of swimming about aimlessly. They are more active in sub- and supra-optimum light intensities than in the optimum and, therefore, tend to come to rest in the latter. It is evident that this would tend to cause them to aggregate in the region of optimum intensity.

OLTMANN'S ('92, p. 186) states that he found the optimum light intensity for colonies bearing asexual cells to be higher than that

for those containing fertilized eggs. I found, as stated above, that specimens which contain large daughter-colonies or spores deflect to the right more, in moving horizontally across the aquarium, than do those containing small daughter-colonies; and also that the former move to the right along the wall of the aquarium nearest the source of light, more definitely than the latter. OLTMANN'S may have been misled in his conclusions by some such reactions. The effect of such reactions on the place of aggregation of *Volvox* colonies is strikingly brought out in the following observations:

After bringing specimens of *Volvox* to the laboratory, they were usually put into 4 liter battery jars, which were exposed to the light from a 16 candle power electric bulb placed at any desired distance from the jars. Under such conditions it was frequently noticed that the major portions of the colonies aggregated in a region some little distance to the right of the point in the jar directly opposite the bulb. At first this was thought to be due to reflection from the table or wall and other objects about, but after all such reflection was eliminated this reaction was still found to take place. It was also found that if the colonies were put into the plate glass aquarium and exposed to light from a Nernst glower situated so that the rays entered the aquarium at right angles to the side, many more collected along the side nearest the source of light, to the right of the middle than to the left, being most numerous but a short distance from the end of the aquarium.³ The specimens to the right of the middle of the aquarium, in every instance, were nearly all large and contained well developed daughter-colonies or spores, while those to the left were nearly all small. The difference in size between those to the right and those to the left could be clearly seen with the naked eye, but they also showed a marked difference in reaction. Colonies taken from the right edge of an aggregation in a battery jar, July 26, 1905, deflected on an average 8° to the right in swimming horizontally toward a source of light, while the average deflection of others taken from the same jar near the left edge of the aggregation was 15° to the left. This accounts, in part at least, for the collection of the smaller colonies to the left and the larger ones to the right, but the chief reason why the larger ones are found to aggregate to the right is because they turn to the right, after coming in con-

³ In all these experiments especial precautions were taken to eliminate reflection and refraction.

tact with the wall of the jar nearest the light, more definitely than do the smaller ones. The cause of this has been discussed elsewhere (pp. 128-131).

It was found by OLTMANN'S ('92, p. 191) that the optimum light intensity for *Volvox* changes during the day. He discovered on August 4, that the colonies aggregated in a darker part of the aquarium at 4.30 a. m. than at 8.30 a. m., although it was not yet daylight at 4.30. On another day, however, the aggregation was found in a still darker region between 11 a. m. and 5.30 p. m., and this day it was found in a region slightly lower in light intensity at 5.30 p. m. than at 12 m. in spite of the fact that the sunlight was unquestionably stronger at 12 than at 5. OLTMANN'S thought this variation in optimum intensity to be due to a periodicity analogous to that found in higher plants. I found no evidence of such periodicity. The change in position during the day noted by OLTMANN'S corresponds to change in the sense of reaction, which can be induced at any time of the day by exposure to light of proper intensity. I did not, however, go into detail with reference to this point; it is therefore desirable to have more experimental results along this line before coming to definite conclusions.

18. WEBER'S LAW.

"On comparing objects and observing the distinction between them, we perceive, not the difference between the objects, but the ratio of the difference to the magnitude of the objects compared" (TITCHENER, '05, p. xvi).

This law was formulated by WEBER in 1834 with especial reference to the senses of touch and sight. DAVENPORT ('97, p. 43) has worded it as follows: "The smallest change in the magnitude of a stimulus which will call forth a response always bears the same proportion to the whole stimulus."

By means of his well known capillary tube method PFEFFER ('84) proved the law to hold approximately for the reactions of fern spermatozoids to malic acid, and later ('88, p. 634) also the reaction of *Bacterium termo* to meat extract. MASSART ('88) proved it to hold for the light reactions of *Phycomyces*, by placing the plants between two flames and thus obtaining the minimum difference in light intensity on opposite sides which induced a response. He found the minimum intensity difference to be 18 per cent of the total light intensity, and this held true for all degrees

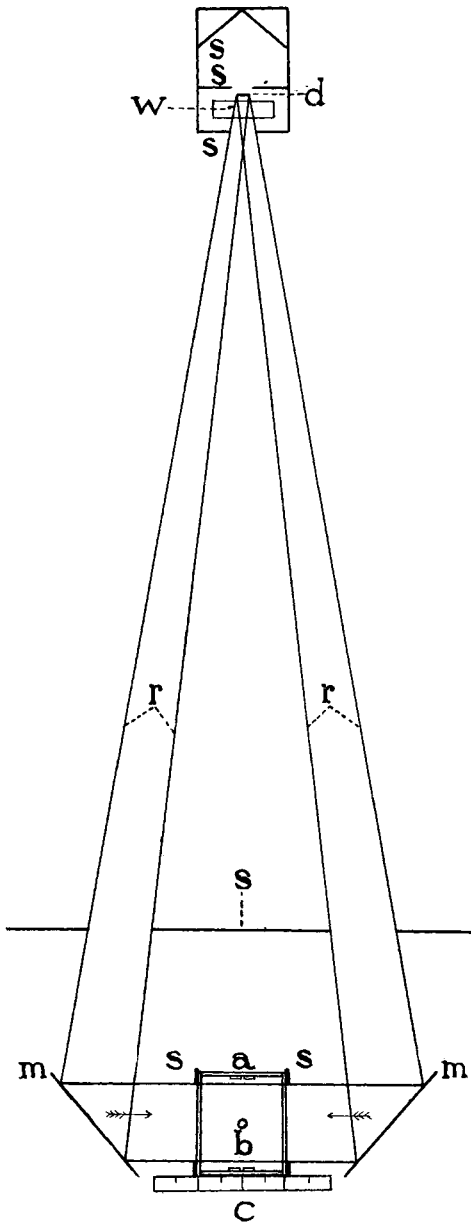


FIG. 15. Representation of apparatus and arrangement as used in ascertaining the minimum difference in light intensity on opposite sides which induces reaction in *Volvox*, in various intensities of illumination. *a*, glass aquarium 4 cm. long and 3 cm. wide; *b*, glass tube through which the colonies were introduced; *c*, metric gauge; *d*, Nernst glower, horizontal; *m m*, mirrors; *r*, light rays; *s*, dead black opaque screens; *w*, water screen.

of illumination which he used. SHIBATA ('05, p. 573) repeated PFEFFER'S experiments on the reactions of fern spermatozoids to malic acid, using the capillary tube method. He also ascertained the threshold for this organism when stimulated by potassium fumarate, succinate, or tartarate, in various degrees of concentration. He found the reactions to all of these chemical compounds to take place in accordance with the law of WEBER.

A number of other investigators have worked on this subject, but thus far no one has tested the validity of the law for the light reactions in motile organisms. In other words, no one has ascertained the minimum difference in light intensity on opposite sides which will cause a response of motile organisms in different degrees of total illumination. The following experiments were undertaken for the purpose of getting evidence concerning this matter.

The use and arrangement of apparatus used in these experiments will readily be understood by referring to the accompanying diagram.

The box, containing a small opening in front of which the Nernst glower was mounted, served as a non-reflecting background. The screens surrounding the glower were so constructed and arranged that no light escaped excepting that which passed through the opening represented in Fig. 15. This light was absorbed after being used to illuminate the aquarium, and since no other light entered the room in which the experiments were performed, it is clear that the reactions observed, and recorded in the following tables, were induced only by light directly from the glower.

The glass tube, represented in the center of the aquarium, Fig. 15, by a ring, extended about 2 cm. above the upper edges of the glass walls and was so fastened that it could be easily raised vertically. In each exposure enough filtered water was put into the aquarium to fill it to a point a few millimeters above the upper edge of the opening in the screen *s*, on either side of the aquarium. The glass tube was then put in place and a number of colonies introduced. The tube formed such close connections with the bottom of the aquarium that the colonies could not get out, but the water introduced with them could. As soon as a state of equilibrium was established, the colonies were set free by carefully raising the tube. After they had been exposed to the light from opposite directions for a few moments, the contents of the aquarium was divided into two equal parts by means of a piece of tin

made to fit the groove represented at the middle of the ends of the aquarium, Fig. 15, *a*. The colonies in each half were then counted and the numbers recorded. The aquarium could be moved to the right or left along the metric gauge and in this way the intensity of the light entering opposite sides of the aquarium could be regulated. The candle power of the glower and the distances between it and each side of the aquarium being known, the difference in light intensity on opposite sides of any object in the middle of the aquarium could easily be calculated.

TABLE V.
Distance from glower to center of gauge 100 cm. Light intensity 27 candle meters.

| Position of aquarium. | Number of colonies in left half of aquarium. | | | | Number of colonies in right half of aquarium. | | | | Ratio between totals. | Differential threshold. | | |
|-----------------------|--|----------------|----|--|---|----------------|----|----|-----------------------|-------------------------|--------|---|
| | cm. | In each trial. | | | Total. | In each trial. | | | | | Total. | |
| To the right. | 1 | 18 | 11 | | | 29 | 30 | 26 | | 56 | 1.931 | 1.080 candle meters = 4 per cent of light intensity at center of gauge. |
| | 0.5 | 29 | 19 | | | 48 | 31 | 22 | | 53 | 1.104 | |
| At center of gauge. | | 27 | 18 | | | 45 | 29 | 21 | | 50 | 1.111 | |
| To the left. | 0.5 | 37 | 25 | | | 62 | 34 | 22 | | 56 | 1.107 | |
| | 1 | 32 | 24 | | | 56 | 21 | 12 | | 33 | 1.696 | |

Distance from glower to center of gauge 200 cm. Light intensity 6.75 candle meters.

| | | | | | | | | | | | | | |
|---------------------|-----|----|----|----|----|----|----|----|----|----|----|-------|---|
| To the right. | 2 | 12 | 11 | 17 | | 40 | 20 | 20 | 17 | | 57 | 1.425 | 0.337 candle meters = 4.9 per cent of light intensity at center of gauge. |
| | 1.5 | 14 | 18 | 18 | | 50 | 20 | 23 | 22 | | 65 | 1.300 | |
| At center of gauge. | 1 | 23 | 19 | 15 | | 57 | 21 | 37 | 20 | | 78 | 1.364 | |
| | 0.5 | 27 | 30 | 16 | | 73 | 25 | 38 | 13 | | 76 | 1.041 | |
| To the left. | 0.5 | 18 | 9 | 14 | 13 | 54 | 18 | 14 | 18 | 16 | 66 | 1.222 | |
| | 1 | 23 | 17 | 26 | | 66 | 24 | 13 | 21 | | 58 | 1.138 | |
| To the left. | 1 | 36 | 22 | 17 | | 75 | 30 | 14 | 21 | | 65 | 1.153 | |
| | 1.5 | 14 | 18 | 37 | | 69 | 8 | 16 | 24 | | 48 | 1.437 | |
| | 2 | 29 | 34 | 16 | | 79 | 17 | 21 | 11 | | 49 | 1.612 | |

Distance from glower to center of gauge 400 cm. Light intensity 1.6875 candle meters.

| | | | | | | | | | | | | | |
|---------------------|---|----|----|----|--|----|----|----|----|--|----|-------|--|
| To the right. | 6 | 19 | 37 | | | 56 | 34 | 47 | | | 81 | 1.446 | 0.0842 candle meters-- 4.9 per cent of light intensity at center of gauge. |
| | 5 | 19 | 25 | 26 | | 70 | 27 | 29 | 38 | | 94 | 1.342 | |
| At center of gauge. | 4 | 21 | 11 | 41 | | 73 | 22 | 14 | 42 | | 78 | 1.068 | |
| | 3 | 34 | | | | 34 | 34 | | | | 34 | 1.000 | |
| To the left. | 2 | 15 | | | | 15 | 16 | | | | 16 | 1.066 | |
| | 3 | 30 | | | | 30 | 31 | | | | 31 | 1.033 | |
| To the left. | 4 | 38 | | | | 38 | 34 | | | | 34 | 1.117 | |
| | 5 | 26 | | | | 26 | 20 | | | | 20 | 1.300 | |

TABLE VI.

Distance from glower to center of gauge 100 cm. Light intensity 27 candle meters.

| Position of aquarium. | Number of colonies in left half of aquarium. | | | | | | | Number of colonies in right half of aquarium. | | | | | | | Ratio between totals. | Differential threshold. | |
|------------------------------|--|----------------|----|----|----|----|-----|---|----------------|----|----|----|----|-----|-----------------------|-------------------------|--|
| | cm. | In each trial. | | | | | | Total | In each trial. | | | | | | | | Total. |
| To right of center of gauge. | | a | b | c | d | e | f | | a | b | c | d | e | f | | | |
| 4.5 | 11 | | | | | | | 11 | 26 | | | | | | 26 | 2.354 | 4.104 candle meters = 15.2 per cent of light intensity at center of gauge. |
| 3.5 | 14 | 8 | 18 | 24 | 15 | 11 | 90 | 24 | 16 | 19 | 35 | 19 | 23 | 136 | 1.511 | | |
| 3 | 21 | 24 | 26 | 17 | 13 | 13 | 114 | 20 | 32 | 24 | 15 | 17 | 16 | 124 | 1.087 | | |
| 2.5 | 16 | 22 | 18 | 15 | 23 | 16 | 110 | 16 | 19 | 20 | 17 | 25 | 12 | 109 | 1.009 | | |
| 1.5 | 19 | 16 | | | | | 35 | 16 | 16 | | | | | 32 | 1.093 | | |

Distance from glower to center of gauge 200 cm. Light intensity 6.75 candle meters.

| To right of center of gauge. | cm. | In each trial. | | | | | | Total | In each trial. | | | | | | Total. | Ratio between totals. | Differential threshold. |
|------------------------------|-----|----------------|----|----|----|---|----|-------|----------------|----|----|----|---|-----|--------|--|-------------------------|
| | | a | b | c | d | e | f | | a | b | c | d | e | f | | | |
| 6.5 | 8 | 11 | | | | | 19 | 31 | 28 | | | | | 59 | 3.105 | 0.743 candle meters = 11 per cent of light intensity at center of gauge. | |
| 5.5 | 12 | 21 | 18 | 11 | | | 62 | 19 | 19 | 34 | 21 | | | 93 | 1.500 | | |
| 4.5 | 13 | 12 | 29 | 24 | | | 78 | 15 | 14 | 25 | 32 | | | 86 | 1.102 | | |
| 3.5 | 21 | 14 | 17 | 12 | | | 64 | 20 | 18 | 22 | 9 | | | 69 | 1.078 | | |
| 2.5 | 12 | 34 | 19 | 24 | 10 | | 99 | 22 | 37 | 15 | 19 | 10 | | 103 | 1.040 | | |

Distance from glower to center of gauge 400 cm. Light intensity 1.6875 candle meters.

| To right of center of gauge. | cm. | In each trial. | | | | | | Total | In each trial. | | | | | | Total. | Ratio between totals. | Differential threshold. |
|------------------------------|-----|----------------|----|----|---|---|-----|-------|----------------|----|----|---|---|-----|--------|---|-------------------------|
| | | a | b | c | d | e | f | | a | b | c | d | e | f | | | |
| 11 | 13 | 29 | 18 | 26 | | | 86 | 26 | 39 | 23 | 28 | | | 116 | 1.348 | 0.1689 candle meters = 10 per cent of light intensity at center of gauge. | |
| 10 | 18 | 12 | 22 | 18 | | | 70 | 20 | 28 | 26 | 22 | | | 96 | 1.371 | | |
| 9 | 25 | 20 | 14 | 21 | | | 80 | 20 | 16 | 22 | 25 | | | 83 | 1.037 | | |
| 8 | 26 | 19 | 22 | 33 | | | 100 | 23 | 19 | 24 | 27 | | | 93 | 1.075 | | |
| 7 | 28 | 48 | | | | | 76 | 24 | 47 | | | | | 71 | 1.070 | | |

The results recorded in Table V were obtained in experiments performed on August 22, 1904, and those recorded in Table VI in experiments performed on August 25. The experiments in both cases extended over a period of several hours. The specimens were collected at 6 a. m. on the day during which they were exposed. After being brought to the laboratory, they were kept in darkness or very dim light until used.

By referring to Table V, it will be seen that the minimum difference in light intensity on opposite sides of Volvox colonies which induced a reaction is approximately 4 per cent of the illumination on either side when the aquarium is 100 cm. from the glower, but 4.9 per cent when it is either two or four times as far away. Table VI shows that reaction is induced by a difference of 15.2 per cent when the distance between the aquarium and glower is 100 cm., 11 per cent when this distance is 200 cm., and 10 per cent when it

is 400 cm. In accordance with WEBER'S law, the proportion between the difference and intensity on opposite sides and the intensity on either side should be the same in all degrees of illumination. The reactions of *Volvox*, as recorded in these tables, therefore, are not in perfect accord with this law. But in Table V the threshold is smallest in the highest light intensity, while in Table VI it is smallest in the lowest intensity. There was also a surprising difference in the threshold of the organisms used on the two different days, confirming the statement made elsewhere, that the reactions of these organisms at any given time depend largely upon previous environmental conditions. Considering these facts, it seems almost certain that the difference between the results recorded in the tables and those demanded by WEBER'S law are within the limits of error. If this be true the light reactions of *Volvox* may be considered to be in accord with this law.

19. SUMMARY.

1. The eye-spots in *Volvox* are located on the outer posterior surface of the individuals of which the colonies are composed, not on the outer anterior surface as represented by OVERTON.
2. They are much larger in the individuals at the anterior end than in those at the posterior end, and they probably function as light recipient organs.
3. In moving forward *Volvox* usually rotates on its longitudinal axis counter-clockwise, as seen from the posterior end. But under certain conditions the direction of rotation is frequently reversed. This is caused by continuous contact stimulation of the individuals located along the sides of the colonies.
4. In swimming horizontally *Volvox* colonies seldom move parallel with the light rays when exposed to light from a single source. They deflect upward or downward as well as to the right or left.
5. Specimens containing large daughter-colonies or spores deflect more strongly to the right than others. The degree of deflection depends upon the light intensity and the physiological condition of the organism as well as upon its contents. The more strongly positive they are, the more nearly parallel with the rays they appear to move as seen from above. When exposed to light of very low or very high intensity they deflect more than when exposed to light of moderate intensity.

6. The specific gravity of *Volvox* is greater than one. When not active or when dead the colonies slowly sink with the longitudinal axis vertical and the posterior end down. The vertical orientation under such conditions is much more precise in specimens containing large daughter-colonies than in others.

7. *Volvox* tends to swim in the direction of its longitudinal axis. Gravitation tends to cause this axis to take a vertical position. If the colonies are not strongly positive the anterior end is directed nearly straight up. If such colonies swim toward a source of light, the rays of which are horizontal, they deflect upward. But if the colonies are strongly positive the axis becomes nearly horizontal, and they tend to swim parallel with the rays. Under these conditions gravity causes them to sink gradually, so they deflect downward.

8. If the rays of light are parallel with the direction of gravitation, *i. e.*, vertical, and the source of light is above, the colonies swim upward in a narrow spiral course nearly parallel with the rays, but if the source of light is below and they swim downward, there is a tendency to turn over, owing to the difference in weight of the two ends, and this causes them to swerve to the side frequently.

9. Deflection to the right or left as well as deflection upward or downward, is caused by the effect of gravitation on the direction of the longitudinal axis in connection with rotation on this axis.

10. Deflection in negative colonies is in all essentials like that in positive.

11. If a colony is swimming at a given angle to the light rays and the direction of the rays is changed, the organism changes its direction of motion until it again takes a course which makes an angle with the rays equal to that it had before the ray direction was changed, *i. e.*, *Volvox* orients, but not necessarily so as to swim parallel with the rays.

12. If exposed to light of equal intensity from two sources, *Volvox* swims toward a point nearly half way between the two sources, provided it is strongly positive, but if the lights are unequal in intensity the colonies direct their course toward a point nearer the more intense light than the other.

13. If exposed to parallel rays such that one side of a colony, swimming toward the source of light, is more strongly illuminated than the other, it deflects toward the more strongly illuminated side.

14. Segments of a colony orient, in general, like normal colonies, but they usually take a spiral course, the width of which depends upon the form and size of the segment and the part of the colony from which it was taken.

15. The direction of motion in *Volvox* is regulated by the relative light intensity on opposite sides of the colony, regardless of the ray direction.

16. Orientation is not the result of "trial and error" reactions as in *Stentor*, *Euglena* and other forms. *Volvox* colonies make no errors in this process.

17. There is no evidence of motor reaction in a *Volvox* colony, taken as a whole. Orientation is, however, brought about by motor reactions in the individuals which constitute the colony. If opposite sides of a colony are unequally illuminated, the individuals in the colony continually pass from regions of higher to regions of lower light intensity and vice versa, as the organism rotates. This change in light intensity induces motor reactions in the individuals, which result in orientation of the colony. The motor reaction in positive specimens is induced only when the intensity to which the zooids are exposed is decreased, and in negative colonies only when it is increased.

18. In general, *Volvox* is positive in comparatively low and negative in comparatively high light intensities; that is, it has an optimum, but the optimum varies in the extreme. Colonies were found to be negative in intensities ranging from 57 to 5000 candle meters. The threshold also varies greatly, the lowest found being 0.14 candle meters.

19. Change in the sense of reaction can be induced by change in light intensity. It depends upon the physiological condition of the organism and the time of exposure as well as upon the intensity of the light. It cannot be induced by mechanical stimulation or change in temperature.

20. When compelled to move practically perpendicular to the rays, *Volvox* can still find its optimum in a field of light graded in intensity. Under such conditions it collects in the optimum intensity by merely wandering movements. There is no evidence of orientation or "trial and error" reactions of the kind that were found in *Stentor* under similar conditions (MAST '06, pp. 366-377).

21. If jars containing *Volvox* colonies are exposed to light from a single source, those specimens which contain large daughter-

colonies or spores, collect to the right of the region in the jars nearest the source of light; those without daughter-colonies or spores or with small ones collect nearest the source of light, but they spread out considerably both to the right and left. A majority of all the colonies are, therefore, usually found in that part of the jar to the right of the region of strongest illumination.

22. The cause of this collection to the right is found in the fact that when the specimens containing large daughter-colonies strike the wall of the jar, in swimming toward the source of light, they usually turn to the right. This is caused by the effect of gravitation, rotation, and contact stimulation.

23. Since the ratio between the difference in light intensity on opposite sides, which is sufficient to induce a reaction, and the intensity on either side is nearly the same for different degrees of illumination, WEBER'S law holds approximately for the light reactions of *Volvox*.

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