Resumido por el autor, S. O. Mast.

Reversión en la orientación hacia la luz en las formas coloniales Volvox globator y Pandorina morum.

1. Volvox y Pandorina son generalmente positivos en la luz débil y negativos en la luz intensa, si están adaptados a la oscuridad; pero si están adaptados a la luz, lo contrario sucede a veces. 2. Si las colonias adaptadas a la oscuridad se exponen a una iluminación constante, al principio se comportan como neutrales, después se transforman en positivas, más tarde en negativas y finalmente en positivas otra vez. Cuánto más intensa es la iluminación más corto es el tiempo necesario para pasar por todos estos estados, pero en tales intensidades es necesaria mucha más energía para producir los cambios en la orientación que en una iluminación menos intensa. 3. La reversión depende en cierto grado de la cantidad de energía recibida, pero bajo ciertas condiciones parece depender principalmente de la duración del cambio de iluminación. 4. La reversión no está regida por la fotosintesis. La luz roja y la amarilla, en las cuales la fotosintesis se verifica con relativa intensidad, producen pocos efectos sobre la reversión, mientras que en la luz verde y en la azul, en las cuales la fotosintesis es relativamente débil, son casi tan efectivas como la luz blanca. 5. Los rayos que poseen la mayor eficiencia estimulante (azul y verde) son los más potentes en la producción de la reversión. 6. El sentido de la orientación depende del estado fisiológico de las colonias, asi como de la constitución del medio de cultivo. Depende también de la edad de las colonias. Las colonias jóvenes son más aptas para ser negativas que las de más edad. 7. La reversión está probablemente asociada con cambios en la permeabilidad.

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REVERSION IN THE SENSE OF ORIENTATION TO LIGHT IN THE COLONIAL FORMS, VOLVOX GLOBATOR AND PANDORINA MORUM

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TWO FIGURES

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INTRODUCTION

The literature on reversion in the sense of orientation was briefly reviewed in a preceding paper which dealt primarily with the effect of chemicals on the sense of orientation to light in Spondylomorum (Mast, '18). The results presented in that paper indicate that reduction in alkalinity, increase in some anesthetics, increase in temperature and decrease in illumination all have the same effect on the sense of orientation, causing photonegative specimens to become photopositive. They also indicate that the same change in the sense of orientation may occur without any appreciable change in the environment. On the basis of these results it was concluded that reversion in the sense

of orientation is probably due to some specific change or state in the physiological processes of the organisms which can be induced by any one of the factors mentioned, i.e., alkalis, anesthetics, temperature, or light.

In this paper we shall deal primarily with the effect of illumination on the sense of orientation, but we shall also briefly consider some other factors.

MATERIALS AND METHODS

The specimens used in this investigation were all collected at Woods Hole in a fresh-water pond. Volvox appeared early in July and continued for nearly a month. It was found only at the edge of the pond in a few small puddles, but in these it was very abundant most of the time. Pandorina appeared early in August, shortly after Volvox had disappeared, and it continued nearly to the end of the month. It was not found in all parts of the pond, but was much more widely distributed than Volvox and equally abundant.

Pandorina thrived much better in the laboratory than Volvox, but neither lived more than a week or two. Most of the observations were consequently made on specimens within a few days after they had been collected.

The observations were all made in a large basement dark-room in which there was remarkably little variation in temperature throughout the season and practically none during the time occupied by any given experiment. The dark-room was so fitted up that either natural or artificial light could be used. Aside from ordinary electric lamps, there were two gas-filled stereopticon lamps, one 250 and the other 1,000 watt. These two lamps were mounted in an adjoining room from which light was admitted to the dark-room through a hole in the wall, which could be varied in size (fig. 1). Thus a horizontal beam of light of the desired size was produced. This extended through the dark-room parallel with the surface of a series of black tables 7 m. long The two lamps could readily be interchanged in position. By this means and by varying the distance between the organisms

and the lamps it was possible to obtain quickly a wide range in illuminations. The beam of light, before it entered the dark-room, passed through a heat-screen consisting of a Pfeifer warming-stage containing distilled water. This screen was so arranged that a stream of water continuously flowed through it so as to prevent excessive heating. The observations were practically all made in six rectangular glass aquaria which were designated observation aquaria. These aquaria were 2.7 cm. wide, 2.7 cm. long and about 1 cm. deep. They were constructed from pieces of the best-quality microscope slides and Khotinsky sealing-wax.

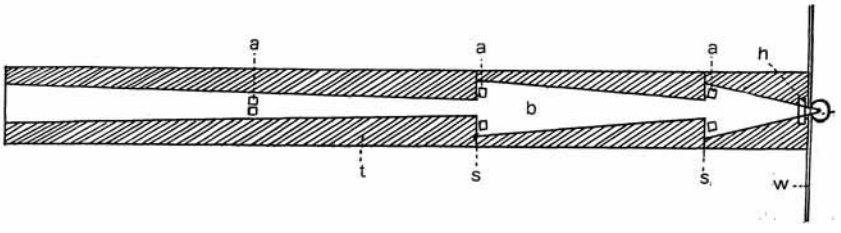


Fig. 1 Diagram representing arrangement of apparatus. *a*, rectangular observation aquaria; *l*, gas-filled steriopticon lamp; *b*, beam of light; *w*, wall between dark-room and room containing lamp; *h*, heat-screen containing running distilled water or saturated solution of chlorophyl in 96 per cent alcohol; *t*, dead black table, 7 m. long; *s*, light-screens.

RELATION BETWEEN ILLUMINATION AND REVERSION IN ORIENTATION

Both Volvox and Pandorina are very sensitive to light and they orient fairly precisely. Like a considerable number of other similar organisms, they have ordinarily been found to be positive in weak and negative in strong light. This has often been observed in previous work (Mast, '07, '11, '18) and it was repeatedly observed in the experiments performed in connection with this work. The following detailed description of a typical series of observations clearly illustrates the relation between this change in the sense of orientation and the intensity of illumination.

On August 8, 4.40 P.M., colonies of Pandorina which had been in darkness for nearly four days were exposed in strong direct sunlight. At first they were very inactive and there was no indi-

cation whatever of orientation. But after approximately one minute it could be seen clearly that they were swimming slowly from the light. Gradually they became more and more active, and as they became more active they became more strongly negative, until at 4.50 P.M. they were swimming rapidly and fairly directly from the source of light. At 4.55 P.M., they were moved 4 m. from the window and placed in diffuse light. Here they promptly became strongly positive. They were then returned to direct sunlight near the window, when they again promptly became strongly negative. These changes in illumination were repeated several times and the same results were obtained each time.

It is thus evident that strong illumination tends to make these organisms negative and that weak illumination tends to make them positive. Under certain conditions, however, just the opposite holds. In previous work this was observed only once (Mast, '07, p. 165). In the observations now under consideration it was, however, repeatedly observed both in Volvox and in Pandorina, and the conditions under which it occurs have been discovered. This is demonstrated by the results obtained in the observations described below:

On July 13, Volvox colonies were collected at 7.30 A.M., in a pool fully exposed to direct sunlight. They were taken to the laboratory, and put into two finger-bowls. One bowl was put into total darkness, the other was exposed in strong diffuse daylight. Thus one group of colonies remained light-adapted, while the other group became dark-adapted. At 3 P.M., light-adapted colonies and dark-adapted colonies were put, respectively, into each of six rectangular observation aquaria. These were then exposed in the dark-room in light from the 250-watt lamp, one aquarium containing dark- and one containing light-adapted colonies side by side in each of the following illuminations, 62.5 m.c., 250 m.c., and 4,000 m.c. (fig. 1). The dark-adapted colonies were strongly positive in all the illuminations and the light-adapted were negative in all, but they were clearly more strongly negative in the lowest intensity than they were in the highest. In 4,000 m.c. they were only slightly negative; in 250 m.c.

they were clearly more strongly negative. One of the aquaria was then moved nearer to the lamp into an illumination of 16,000 m.c. The colonies immediately became positive, although not strongly. The dark-adapted colonies were not tested in this intensity, but judging from results obtained in numerous other experiments, one of which is described in detail in the preceding pages, they probably would have been negative.

These results seem to indicate that long exposure in strong light produces changes in Volvox which make it negative in weak and positive in strong light. The following results obtained in observations on Pandorina support this conclusion.

August 4 was a bright clear day. At 11.30 A.M., Pandorina was taken in abundance from a small puddle at the edge of the collecting pond. This puddle had been fully exposed to direct sunlight all forenoon. The specimens collected were taken to the laboratory and some of them were immediately exposed to direct sunlight in an observation aquarium, surrounded by water which was changed from time to time so as to prevent excessive rise in temperature. They were strongly positive and remained so. At 3 P.M. the aquarium was placed on the stage of a microscope in direct sunlight, and a beam of direct sunlight, concentrated by means of the concave mirror, was thrown up through the aquarium. The colonies promptly aggregated in this extremely intense illumination forming a dense mass. At 5 P.M. many colonies were lying on the bottom inactive, but all the rest were still fairly strongly positive. These results indicate clearly that under certain conditions Pandorina cannot be made negative by exposure to intense light. The specimens used in this experiment were unfortunately not tested in weaker light, but judging from the results of other observations they probably would have been negative. For example, at 3 P.M., August 11, colonies adapted to strong diffuse light were exposed in an illumination of 16,000 m.c. They were inactive for a few moments and then became definitely positive. The illumination was then considerably reduced. The colonies continued to proceed toward the light, but only for a few moments, when practically all of them turned through 180 degrees and proceeded from the light.

This was repeated many times and the same results were persistently obtained. Thus it is clear that, under certain conditions, Pandorina is definitely positive in strong and definitely negative in weak light. What are the conditions under which this occurs?

It was repeatedly observed that colonies exposed in strong diffuse light, in which they were strongly positive, usually become negative in the evening at the approach of twilight. This suggests that the phenomena may be associated with the night and day variations in illumination and that it may be analogous to the so-called sleep movements in higher plants. The fact, however, that it does not occur in dark-adapted colonies and in colonies which have been exposed to relatively weak light does not support this contention.

The physiological condition in which the colonies are positive in strong and negative in weak light is, in all probability, dependent upon the amount of light energy received in the immediate past as the following results indicate.

At 8 A.M., August 23, dark-adapted specimens in observation aquaria were exposed in illuminations of 16,000 m.c., of 4,000 m.c. and in lower intensities. Twenty minutes later, 8.20 A.M., a few of the small colonies in 16,000 m.c. were negative, the rest were all strongly positive. The aquarium was left in this illumination in the dark-room and observations were made every hour. The temperature remained practically constant throughout the day. At 9 and 10 A.M., the reactions were practically the same as they had been at 8.20 A.M. At 11 A.M. practically all of the small colonies and a few of the large ones were negative. More and more continued to become negative, until at 3 P.M., practically all were negative. At 4 P.M. a few of the colonies were scattered, all the rest were negative. From this time on gradually more and more became scattered. At 7 P.M. many of the colonies were scattered, some were negative and some were distinctly positive. At 8 P.M. there were more positive colonies, and at 9 P.M., when the experiment was closed, there was a large positive collection.

In the aquarium in 4,000 m.c. the colonies were at first all positive. An hour later, 9 A.M., a few were negative. From

this time on gradually more became negative, until at 6 P.M. nearly all were negative. At 9 P.M., when the experiment was closed, more of the colonies were scattered than earlier, but there were no positive colonies. In 62.5 and 250 m.c. many of the colonies became negative, but some of them remained positive throughout the experiment. In all intensities lower than 62.5 m.c., none of the colonies became negative at all.

This experiment demonstrates that a certain intensity of light is necessary to induce reversion in the sense of orientation from positive to negative, that the time required depends upon the intensity, and that in strong illumination the colonies, after having become negative, become positive again if they are exposed long enough. Whether or not they continue positive indefinitely after the last reversion was not ascertained, nor was it ascertained in this experiment whether or not they would have been negative in low illumination after they had become positive in high. But in all probability these colonies would have remained positive in strong and would have been negative in weak illumination indefinitely, for negative orientation in weak light was never observed in dark-adapted colonies; it was observed only after long exposure to intense light, and continued exposure to such light failed to make the colonies negative after they had once become positive in this light.

The changes that occur in the reactions of Pandorina as indicated by the results described above may be visualized by means of a graph presented in figure 2. This graph indicates that dark-adapted colonies of Pandorina when first exposed to light are neutral for a short time, i.e., they do not orient (fig. 2, *a–b*), that they then become positive, increasing rapidly to a maximum (*b–c*) then decreasing slowly to a minimum (*c–d*), after which they become negative, passing through a maximum at *e*, and that they finally become positive again (*f–g*). It is probably during this last period that the colonies are positive in strong and negative in weak light.

As previously stated, this reaction is found only in light-adapted colonies, never in dark-adapted ones. What, now, is the difference in different illuminations in the reaction of colonies in these two states?

Numerous observations were made to ascertain this. In all of these observations dark-adapted colonies were put into one rectangular aquarium and light-adapted ones into another. These two aquaria were then placed side by side at the desired distance from the source of light and the reactions of the colonies compared. Frequently three sets of such aquaria were exposed in different illuminations at the same time.

The results obtained appear to be extremely contradictory. Usually the light-adapted colonies were found to be more strongly

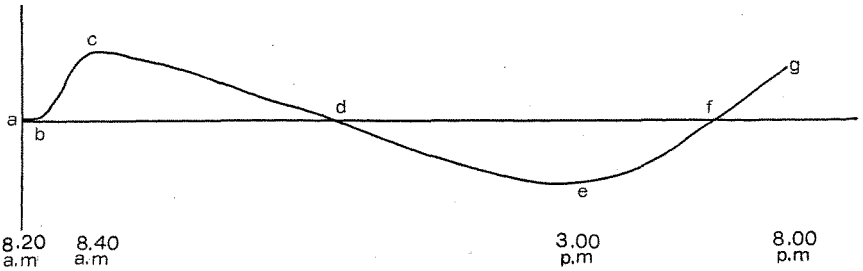


Fig. 2 Graph representing changes in the sense of orientation in dark-adapted Pandorina exposed to constant illumination of high intensity. The horizontal axis represents time, the vertical axis degree of orientation, above the base line positive, below the base line negative. The graph indicates that Pandorina was first neutral (*a–b*) then became positive, increasing to a maximum (*b–c*) then decreasing to a minimum (*c–d*), after which it became negative, increasing to a maximum (*d–e*), then decreasing to a minimum (*e–f*), after which it again became positive (*f–g*). After the colonies reach the last stage they are probably in a state such that they are positive in strong and negative in weak illumination.

positive in strong and less strongly positive in weak light than the dark-adapted ones, but in many instances precisely the opposite was found to be true and in some there was no appreciable difference in the reaction. Moreover, in a given intensity, e.g., 4,000 m.c., the light-adapted were in some instances more strongly positive and in others less strongly positive than the dark-adapted colonies. How can these puzzling results be explained?

Dark-adapted specimens, as we have seen, are frequently neutral when first exposed to light, then they become strongly positive and later negative. If they are in the strongly positive stage when their reactions are compared with those of light-

adapted specimens, they are likely to be more strongly positive than the light-adapted specimens; whereas if they are in the negative stage they will be found to be less strongly positive. Other contradictory features in these results can be similarly explained.

The cause of the passage through the various stages mentioned above, especially the return to positive orientation after having been negative, is not known. It may be associated with what is ordinarily called acclimatization or adjustment or with some sort of a periodicity. However this may be, reversion is undoubtedly associated with physiological processes or states and these processes are dependent both upon time and intensity of illumination. This the evidence presented clearly indicates. How are these processes related to the amount of light energy received?

RELATION BETWEEN REVERSION IN ORIENTATION AND THE QUANTITY OF LIGHT ENERGY

The relation between reversion and the amount of light energy received was ascertained as follows: Colonies of Pandorina adapted to darkness or to weak light were put into each of seven observation aquaria. These were then exposed in various intensities of light from the 1,000-watt lamp as indicated in table 1. Observations were then made, first at intervals of thirty minutes and later at intervals of one hour. After each observation the position of the colonies in each aquarium was recorded. From these records the approximate time at which reversion occurred in each intensity was ascertained. The results thus obtained appear in table 1.

When first exposed the colonies in all of the aquaria were neutral and relatively inactive. Gradually they became more and more active, and as they became more active they began to swim toward the side of the aquarium nearest the source of light, where in the course of several minutes practically all had collected in a dense mass. This occurred first in the aquarium in the highest and last in that in the lowest illumination. Later

the colonies began to scatter, evidently becoming neutral again. Thus they remained for some time, then they became negative, and finally collected along the side of the aquarium farthest from the source of light as definitely as they had formerly collected on the opposite side. This again occurred first in the highest and last in the lowest illumination.

In all of the aquaria the smaller colonies invariably became negative before the larger ones. Thus there was often found in

TABLE 1

Relation between time of exposure, intensity of illumination and reversion in orientation

	INTENSITY OF LIGHT IN METER-CANDLES	TIME OF DAY REVERSION OCCURRED	TIME IN HOURS REQUIRED TO INDUCE REVERSION	ENERGY IN METER-CANDLES HOURS REQUIRED TO INDUCE REVERSION
August 22. Specimens which had been in weak light several days	16,000.0	3.45 P.M.	5	80 000
	4,000.0	4.45	6	24,000
	1,000.0	5.45	7	7,000
	250.0	6.45	8	2,000
	111.0	6.45	8	888
	27.7	6.45	8	444
	20.4	(?)	?	160(?)
August 23 Same specimens after having been in darkness overnight	16,000.0	3	7	112,000
	4,000.0	4	8	32,000
	1,000.0	7	10	10,000
	250.0	8	11	2,750
	111.0	9(?)	13(?)	1,443(?)
	27.7	9	No reversion	
	20.0	9	No reversion	

aquaria a very definite positive collection and at the same time an equally definite negative collection. Later, however, practically all of the colonies became negative, and the record in the table indicates the time when this occurred in each aquarium.

The table mentioned contains the results obtained in two experiments made on two successive days with the same organisms. By referring to this table it will be seen at once that reversion from positive to negative orientation depends upon the intensity of illumination and upon the time of exposure, but that the energy

required to induce reversion varies with the intensity of the illumination and with the condition of the colonies. In one experiment it required in all intensities much more energy than it did in the same intensities in the other and in both experiments it required much more energy in the higher than in the lower intensities. In one experiment no reversion was observed in illuminations below 250 m.c., and longer exposure probably would not have induced it, for at the close of the experiment, 9 P.M., the colonies were inactive. In the other experiment reversion was observed, to some extent, in the lowest illumination tested, 20.4 m.c. The question as to whether or not it occurs in all intensities that induce positive orientation is consequently not definitely settled.

Why it requires more energy to induce reversion from positive to negative orientation in strong than it does in weak light is not clear. It is, however, well known that in Euglena and Volvox heat and light energy have opposite effects on reversion (Mast, '11, p. 300). The same is true for Pandorina as we shall demonstrate later. Now, a certain amount of light which is absorbed is doubtless transformed into heat. This probably occurs in all illuminations in the same proportion, but in the lower illuminations it occurs so slowly that radiation may have relatively a much greater effect than in the higher. Consequently, the heat produced would be more effective in the higher illuminations than in the lower, and since heat tends to make the colonies positive, it would require more light energy to overcome its effect in the higher than in the lower illuminations. The value of this suggestion could doubtless be ascertained by studying the effect of different regions in the spectrum on reversion.

RELATION BETWEEN REVERSION IN ORIENTATION AND THE TIME RATE OF CHANGE IN ILLUMINATION

In the experiments just discussed, it required to induce reversion, sufficient time to indicate that it is dependent upon the quantity of energy received. Under certain circumstances, reversion is, however, of such a nature that it appears to be asso-

ciated with the time-rate of change in illumination rather than with the quantity of light. For example, on August 8 colonies of Pandorina which had been in darkness four days were exposed to direct sunlight at 4.40 P.M. They were inactive for about one minute, then they began to swim, slowly at first and gradually more and more actively, and in practically every case from the light. They were definitely negative. The observation aquarium was now moved 4 m. from the window. Here the colonies were strongly positive. This change in illumination was repeated several times with the same results. The aquarium was then exposed in direct sunlight near the window a few centimeters from the diffuse light. The colonies soon began to swim rapidly from the light. The microscope was then carefully moved into the diffuse light without changing the distance from the window. The colonies immediately became strongly positive, but after they had proceeded toward the window about 1.5 c.m., they became neutral, and approximately one minute later they were strongly negative again. The aquarium was now returned to direct sunlight. The colonies remained negative. They were then again moved into diffuse light where they immediately again became strongly positive, than neutral and later negative. This change in illumination was repeated many times and the same results were always obtained.

Now, the reversion from negative to positive orientation observed in this experiment, after sudden reduction of intensity, was in all probability dependent upon the time-rate of change in illumination, for without any further change the colonies, in the course of about one minute, again became negative. This seems clearly to indicate that if the reduction had consumed sufficient time there would have been no reversion.

EFFECT OF THE AGE OF THE COLONIES ON REVERSION

In the preceding section it was pointed out that in the experiments on the relation between the intensity of the illumination the smaller colonies of Pandorina invariably became negative before the larger ones did. No observations in reference to this

were made on Volvox, but it was repeatedly observed in various cultures of Pandorina, especially in those left uncovered for some days, permitting evaporation.

Whether or not the younger colonies in dark-adapted cultures exposed to light became active and positive before the older ones was not ascertained.

EFFECT OF PHOTOSYNTHESIS ON REVERSION IN THE SENSE OF ORIENTATION

It is well known that acids added to the culture solution tend to make Volvox and similar organisms positive in their reactions to light and that under certain conditions low illuminations also tend to make them positive, while high illuminations tend to make them negative. In low illuminations there is, owing to limited photosynthesis and continued respiration, a tendency toward an accumulation of carbon dioxid, resulting in an increase in acidity, while in high illumination there is, owing to rapid photosynthesis, a tendency toward a reduction in carbon dioxid. This seems to indicate that the tendency toward positive orientation in low and negative orientation in high illumination may be dependent upon photosynthesis. If this is true, then red and yellow light in which photosynthesis is relatively strong should be more effective in producing reversion in the sense of orientation than blue and green in which photosynthesis is relatively weak. Numerous observations on both Volvox and Pandorina adapted to the colors mentioned were made as follows:

Some of the colonies to be tested were put into jars in each of four black light-tight boxes containing a large window made of blue, green, yellow, and red glass, respectively. Others were put into jars in absolute darkness and still others into jars in strong diffuse sunlight. After having been in these conditions eight hours or longer specimens were taken from each jar and put respectively into six observation aquaria. These aquaria were then placed side by side in the same illumination in the dark-room, others similarly treated were placed in other illuminations. In this way the reactions in different illuminations of the

colonies adapted to the various colors could readily and accurately be compared with each other and with those of the colonies adapted to darkness or to strong diffuse light.

Without going into details regarding the results obtained, it may be said that in practically every test the red- or yellow-adapted colonies responded essentially like dark-adapted colonies and the blue- or green-adapted ones responded essentially like light-adapted colonies. The red- and yellow-adapted colonies were usually negative in strong and positive in weak illumination, never the reverse; while the green- or blue-adapted colonies, like light-adapted ones, were frequently positive in strong and negative in weak illuminations.

The colors to which these colonies were adapted were not spectroscopically tested and the illumination was not measured. The question, consequently, arises as to whether or not, under the conditions of the experiments, photosynthesis in the red and the yellow was actually greater than in the blue and the green. This question was answered as follows:

A given amount of pond-water taken from a vessel containing colonies equally distributed was put into each of six 100-cc. wide-mouthed bottles. One of these bottles was now placed in each of the four boxes mentioned above, i.e., in the red, the yellow, the green, and the blue light used in the preceding experiments; one was put into darkness and the remaining one into strong diffuse light. After having been in these illuminations one or more days a given amount of solution was removed from each bottle and put into a test-tube. A drop of neutral red was now added to the solution in each tube. This solution was found to be distinctly akaline in every case. Hydrochloric acid was then added to each tube until all were practically neutral and the same in color. The solution in the tube which required the greatest amount of hydrochloric acid was, of course, the most strongly alkaline, and in this solution photosynthesis had been most rapid, for carbon dioxid is consumed in the process of photosynthesis and the alkalinity is dependent upon the amount of carbon dioxid present.

There was considerable difference in the results obtained in the different tests, but taken as a whole they show conclusively that photosynthesis was most rapid in the diffuse white light and less rapid in the other illuminations in the following order: yellow, red, blue, green, darkness.

These results, consequently, demonstrate that photosynthesis was more rapid in the red and the yellow light used in these experiments than in the blue and the green. And since the blue and the green were more effective in producing reversion in the sense of orientation than the red and the yellow, it is evident that the effect of light on reversion is not determined by photosynthesis unless the photosynthesis which occurred during the exposure to white light in making the tests in the dark-room is involved. This, however, does not seem probable since all of the aquaria were exposed to the same illuminations. Moreover, essentially the same results were obtained in observations in which the aquaria were exposed to green light produced by means of passing the beam of light in the dark-room through a saturated solution of chlorophyl in 95 per cent alcohol. Now, since photosynthesis is reduced to a minimum in green light produced in this way and since the reactions of the colonies in the different aquaria were essentially the same as in white light, it is evident that photosynthesis during exposure in the dark-room is of no practical consequence. The conclusions, therefore, that reversion in the sense of orientation is not determined by photosynthesis appears to be valid.

The region in the spectrum of maximum stimulating efficiency lies in the green near wave-length 524 $\mu\mu$ for Pandorina (Mast, '17, p. 509) and in the blue-green near wave-length 494 $\mu\mu$ for Volvox (Laurens, '18). The results of the experiments described above indicate that the regions of maximum efficiency in producing reversion in the sense of orientation in Pandorina and Volvox probably have the same location as the regions of maximum stimulating efficiency. If this is true, it is probable, although by no means certain, that the processes involved in stimulation are also involved in reversion.

EFFECT OF TEMPERATURE ON REVERSION

In nearly all of the organisms that have been tested increase in temperature tends to induce positive and decrease in temperature negative orientation to light There are only a few forms in which changes in temperature appear to have the opposite effect, but there are a considerable number in which changes in temperature have no effect on the sense of orientation (Mast, '11, pp. 272–279).

The sense of orientation is probably not specifically related to the temperature in any of the forms studied. For example, the results obtained in observations on Euglena (Mast, '11, pp. 274–277) indicate that this form may be, under certain conditions, negative or positive in practically all temperatures in which it orients at all.

The effect of changes in temperature on the sense of orientation in Volvox and Pandorina was ascertained as follows: The colonies were mounted on a Pfeifer warming-stage under a binocular. This stage was so arranged that hot or cold water could be passed through it at any rate desired. The whole apparatus was then exposed to constant illumination of the desired intensity.

The results obtained are in harmony with those obtained in the study of Euglena. The reactions of Volvox were, however, only superficially studied. Without going into details, it may be said that the results clearly show that a rise in temperature tends to produce positive, and a fall in temperature negative photic orientations, and that the sense of orientation is not directly dependent upon the temperature. For example, in one experiment it was found that Pandorina continuously exposed in a given illumination was, at 10.43 A.M., negative in 16 degrees, neutral in 17 degrees, and positive in 18 degrees; at 11.19 A.M., the same colonies were negative in 13.5 degrees, neutral in 14 degrees, and positive in higher temperature, and at 11.28 A.M. they were positive in 14 degrees. Thus the point at which they became positive changed from 18 degrees at 10.43 A.M. to 14 degrees at 11.28 A.M.

EFFECT OF CHEMICALS ON REVERSION

It is well known that acids and some narcotics tend to make many organisms that orient to light, photopositive. Salts and alkalis, on the other hand, rarely have any effect on the sense of orientation. In a recent paper on Spondylomorum ('18) it was fairly clearly demonstrated that the effect of the addition of acids is due to the reduction in the alkalinity of the culture medium and not to the acids as such. The results obtained in experiments on Volvox and Pandorina support this contention and they show that the response of the organism is not specifically dependent upon the chemical constitution of the surrounding medium.

The effect of acid on the sense of orientation in Volvox and Pandorina is in all essentials precisely the same as it is in Spondylomorum (Mast, '18). If a trace of acid is added to a solution containing negative colonies they become strongly positive, remain so a few moments and then become negative again. If more acid is now added they again become positive and later negative, just as they did after the first addition of acid. Thus they continue to become positive and negative after each addition of acid until the solution becomes fatal.

The water in the pond in which the Volvox and the Pandorina used in these experiments appeared gave, in every instance, a very definite alkaline reaction with neutral red, and when acid was added the sense of orientation was reversed long before the alkalinity was neutralized. In fact, sufficient acid to give even the slightest acid reaction invariably proved fatal. This seems to indicate that reversion in these forms, just as in spondylomorum, is due to a reduction in alkalinity, and not to the effect of acid as such. It also indicates that the sense of orientation is not directly related with the concentration of the alkalis.

The amount of reduction in alkalinity required to produce reversion varies with the concentration of the solution and the physiological state of the organisms. It is usually very small. For example, in one experiment titration against HCl showed that the water in which the colonies (Pandorina) lived was 0.0019

N alkaline. In this solution, under the conditions of the experiment, the colonies were strongly negative. After the addition of sufficient acid to make the colonies positive, the solution was 0.0015 N alkaline, and in a few other tests the reduction necessary was even less. In some it was, however, considerably more. This shows that the condition of the organism is involved in the process.

In Spondylomorum it was found that reduction in alkalinity produced by the addition of distilled water had little, if any, effect on the sense of orientation (Mast, '18, p. 512). Similar results were obtained in Volvox and Pandorina. Both of these forms live for several days in chemically pure water and respond normally, but only in relatively few tests was there any indication whatever of reversion due to the dilution of pond water with pure water, and in these tests the effect of the dilution was very slight. But that there was actually an effect was shown by the fact that in diluted pond water it required considerably less acid to induce positive reactions than it did in normal pond water. For example, in a given test in a solution consisting of one part of pond water and nine parts of pure water, it required only one-third as much acid to induce reversion as it did in the pond water. The dilution consequently seems to tend to make the colonies positive, but not in proportion to the degree of dilution.

If a reduction in alkalinity produces reversion from negative to positive orientation, it seems reasonable to expect the reverse if the alkalinity is increased by means of adding alkalis. This, however, does not appear to occur. I repeatedly added sodium hydrate to solutions containing positive colonies of Volvox or Pandorina, but never obtained any indication of reversion except in case the alkali was added immediately after the colonies had been made positive by the addition of acids, and in such cases there was always some question as to the actual effect of the alkali. However, an increase in the concentration of the solution due to slow evaporation clearly tends to make the colonies negative. These results are in full harmony with those obtained on Spondylomorum (Mast, '18, p. 512).

Chloroform has the same effect on reversion that acids have, but ether and alcohol have very little, if any effect. With ether, no clear case of reversion was obtained at all, and with alcohol reversion occurred only in colonies that were almost neutral. The concentration of alcohol necessary to kill these colonies, especially Volvox, is very surprising. In equal parts of pond water and 96 per cent alcohol they live for several hours.

The cause of the effect of anesthetics on reversion is not known, but it is certainly not dependent upon reduction in alkalinity, for the chloroform used was clearly slightly alkaline. This question will be discussed in the following section.

The evidence presented thus far indicates that the sense of orientation is dependent upon the constitution of the culture medium. This contention is further supported by the fact that ordinarily if colonies are negative in one jar and positive in another in the same illumination, as often happens, the solution in the former is more strongly alkaline than that in the latter. This is, however, by no means always true, and in some instances in which it was found to be true it was also found that the colonies retained their sense of orientation after they were interchanged. That is, the colonies which had been positive in the weaker solution were now positive in the stronger, and those which had been negative in the stronger were now negative in the weaker solution. This demonstrates conclusively that the state of the colonies may determine the sense of orientation.

DISCUSSION

It has been demonstrated in the preceding pages that decrease in illumination, decrease in alkalinity, increase in temperature, increase in anesthetics, and increase in the age of the colonies all tend to make them positive. It has also been demonstrated that light-adapted colonies are, under certain conditions, positive in strong and negative in weak light, and that reversion at times depends upon the time-rate of change in illumination. It has, moreover, been demonstrated that the region in the spectrum of maximum efficiency in producing reversion probably coincides with that for maximum stimulating efficiency. Reversion

in the sense of orientation, consequently, can be induced by a number of different factors, and this seems to indicate, as concluded in the preceding paper in this series ('18, p. 518) that it is due to some specific physiological change which can be produced by any one of the factors referred to.

The fact that the region of maximum efficiency for reversion probably coincides with that for stimulation indicates that the physiological phenomena associated with these two processes may be the same. Stimulation, however, is usually if not always accompanied with an increase in permeability and a decrease in electrical potential. Reversion, then, if our contention is correct, should also be accompanied with changes in permeability and all of the factors which produce positive orientation should induce changes in one direction while all those which produce negative orientation should induce changes in the opposite direction. As previously stated, decrease in illumination, decrease in alkalinity, increase in anesthetics, increase in temperature, and increase in age all tend to produce positive orientation. What effect have these factors on permeability?

Krabbe ('96) found in stems of Helianthus annuus that a rise in temperature greatly increases permeability, and Rysselberghe found the same in experiments on Tradescantia and Spirogyra (Tröndle, '10, p. 173). He maintains that the permeability for water, potassium nitrate, glycerine and urea increases slowly from 0 to 6 degrees, more rapidly from 6 to 20 degrees, and again more slowly from 20 to 30 degrees, and that it is eight times as rapid at 30 degrees as it is at zero. This shows clearly that increase in temperature produces increase in permeability. We have demonstrated, as stated above, that increase in temperature causes positive photic orientation. Therefore, if reversion is due to change in permeability, positive orientation must be associated with increase in permeability, and if this is true then all of the factors which produce positive orientation should cause increase in permeability.

The work of Lillie ('09, p. 248) indicates that this is true for decrease in alkalinity; Tröndle maintains that moderate illumination, such as favors positive orientation, causes increase in

permeability, and that strong illumination, in which negative orientation is usually found, causes decrease in permeability; and according to McClendon ('17, p. 141), a considerable number of investigators hold that anesthetics in low concentration stimulate. The concentrations of anesthetics that produce reversion, therefore, probably cause increase in permeability, although Osterhout ('13) in his ingenious experiments did not discover any such effect. We thus have considerable evidence in favor of the idea that positive orientation is dependent upon increase in permeability. However, if this is true, then permeability ought, under certain conditions, depend upon the time-rate of change in illumination; it ought to be greater in old than in young colonies and it ought, under certain conditions, be greater in intense than in moderate illumination. Moreover, all conditions which cause increase in permeability, e.g., NaCl, ought to produce positive orientation, while all those which cause decrease in permeability ought to produce negative orientation. With these questions, concerning which there is at present no trustworthy evidence, I hope to deal in the following paper in this series.

Some investigators maintain that in many of the unicellular and colonial forms orientation is due to a series of shock-reactions; others maintain that there is no definite relation between shock-reactions and orientation. However this may be, it is certain that Euglena and Gonium and probably all other similar organisms usually, if not always, respond with the shock-reaction to a sudden increase in illumination if they are negative and to a sudden decrease if they are positive. That is, the same reaction may be induced either by a sudden increase or by a sudden decrease in illumination, depending upon whether the organisms are negative or positive.

If shock-reactions are due to increase in permeability then increase in permeability must be due, in negative specimens, to increase and, in positive specimens, to decrease in illumination. And if orientation is due to shock-reactions, then reversion in orientation must be due to the phenomena which produce this change in the cause of increase in permeability. In relatively high temperature or moderate illumination and in solutions rela-

tively weak in alkalinity or relatively strong in narcotics, in all of which orientation is positive and permeability relatively high, a sudden *decrease* in illumination must produce an increase in permeability, and in relatively low temperature or intense light and in solutions strong in alkalinity or anesthetics, all of which tend to produce negative orientation and low permeability, a sudden *increase* in illumination must produce increase in permeability.

There is at present no evidence which bears on these problems. I have stated them in order to emphasize the fact, that whether orientation depends upon shock-reactions or not, any theory that accounts for reversion in orientation should account for the changes in the cause of the shock-reactions which accompany it.

SUMMARY

1. The reactions to light in Volvox and Pandorina are practically the same. Both forms orient fairly precisely and both may be either negative or positive.

2. Dark-adapted colonies are usually positive in weak and negative in strong illumination, never the opposite. Light-adapted colonies are sometimes positive in strong and negative in weak illumination.

3. If dark-adapted colonies are exposed to continuous illumination they are neutral for a short time, then they become positive, passing through a maximum, after which they become neutral again, then they become negative, passing through a maximum, after which they again become neutral and finally positive again. After they have reached this final state they remain positive no matter how intense the light may be, and they probably are negative in weak light.

4. The time required to pass through these various stages depends upon the intensity of the light. The higher the illumination, the shorter the time. Reversion is, therefore, dependent upon the time of exposure as well as upon the intensity of the illumination. But it requires much more energy to induce reversion in high that it does in low illumination.

5. Under certain conditions, sudden decrease in illumination makes negative colonies momentarily positive. This change in the sense of orientation is dependent upon the time-rate of change in the intensity of the illumination.

6. Reversion is not primarily dependent upon photosynthesis. Red and yellow light in which photosynthesis is relatively strong have little effect on reversion, while green and blue, in which photosynthesis is relatively weak, are nearly as effective as white light.

7. The rays of light which have the greatest stimulating efficiency (green and blue) are the most potent in producing reversion in the sense of orientation.

8. Increase in temperature causes negative specimens to become positive and decrease causes the opposite, but neither the degree nor the extent of change in the temperature is specific in its effect. Under certain conditions, the colonies may be negative or positive in practically all temperatures in which they orient at all.

9. Alkalis have little, if any effect on reversion. Acids and some anesthetics, especially chloroform, cause negative colonies to become strongly positive, but reversion is not specifically dependent upon the concentration of the chemicals. Colonies which are positive in a solution having a given chemical concentration may be negative in the same solution or even in a weaker solution. The effect of acids is probably due to the accompanying reduction in the alkalinity of the cultural solution.

10. The sense of orientation is dependent upon the physiological state of the colonies as well as upon the constitution of the culture medium.

11. The sense of orientation is dependent upon the age of the colonies. Young colonies are more likely to be negative than old ones. In a given solution the young specimens frequently collect at the side of the dish farthest from the light while the old ones collect at the opposite side.

12. Reversion in orientation is probably associated with changes in permeability, positive orientation being associated with an increase and negative orientation with a decrease in permeability.

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