AN INVESTIGATION INTO THE CAUSE OF THE SPONTANEOUS AGGREGATION OF FLAGELLATES AND INTO THE REACTIONS OF FLAGELLATES TO DISSOLVED OXYGEN.

PART II.

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Previous Work.

As far as I am aware, the only workers who have attempted to find the causes of the spontaneous aggregation of flagellates are Jennings and Moore. They used a flagellate called *Chilomonas paramecium* and came to the conclusion that the organisms are attracted by the carbonic acid produced by themselves. Where by chance the flagellates are more crowded together in the liquid on a slide, more carbonic acid is produced than elsewhere and in consequence yet more flagellates are attracted into these regions. The experimental evidence on which they based their conclusion was as follows. They found: (1) that when a dilute mineral acid was let in under the cover-slip by means of a capillary pipette into a suspension of the flagellates the latter were attracted by the drop; and (2) when a bubble of carbon dioxide was introduced in the same manner the organisms crowded around it, whereas they did not collect around an air bubble. Therefore, Jennings and Moore concluded, in the formation of spontaneous collections the flagellates are attracted to regions which become more acid than the remainder of the liquid, through the accumulation of the carbonic acid produced by the individuals already there.

I have found that both the experimental data of these workers are correct, but that nevertheless they do not lead to their conclusion. I tried the effect of mineral acids on Bodo by letting the acid in under one end of a long, supported cover-slip, under which some Bodo suspension had just previously been introduced. In a zone parallel to the junction of the two liquids a band of flagellates rapidly accumulated. A series of such tests was made with acids of decreasing strengths and it was found that the reaction became feebler each time until a point was reached (at 0.0005 N HCl) when the Bodo no longer reacted to the acid at all. Nevertheless 0.0005 N HCl, tested with Congo red as indicator, is a considerably stronger acid than a saturated solution of CO₂ in water. Congo red is turned violet by 0.0005 N HCl whereas the scarlet color of this indicator in distilled water takes on a very slight bluish tinge only when the solution is saturated with CO₂.

Further it has already been shown (page 489) that the flagellates do not collect in the region next a drop of water saturated with CO₂ let in under the cover-slip. Jennings and Moore made no attempt to determine quantitatively the exact strength of acid solution to which these organisms react. In fact, we are dealing here with a phenomenon quite unconnected with spontaneous aggregation, a reaction of the flagellates to acids and the weakest effective acid is stronger than carbonic acid.

It is worthy of remark that the accumulation of a crowded zone of Bodo in the region in front of an acid introduced beneath the cover-slip is much more marked in aerated than in non-aerated suspensions, which is the reverse of the reaction of the flagellates to a region of reduced oxygen concentration. Indeed an acid as weak as 0.001 N HCl produces a slight collection in an aerated preparation only and none in one made by taking liquid straight from the Bodo culture jar. The crowded zone next the acid always dissipates before the subsequent commencement of aggregation and band formation of the flagellates in the region of low oxygen concentration.

With regard to the gas bubbles, it is true that flagellates collect around a carbon dioxide bubble and not around an air bubble, but it is equally true that flagellates collect around a hydrogen bubble. Jennings and Moore did not try the hydrogen bubble. It is evidently
to reduced oxygen concentration that the organisms are attracted both in the case of the carbon dioxide bubble and of the hydrogen bubble. Part of the oxygen in solution in the water diffuses into the gas bubble, where the partial pressure of oxygen is nil, and so a region of reduced oxygen concentration is established in the water around the bubble. The flagellates are attracted into this region just as they are attracted into a central region under the cover-glass where the oxygen concentration is lowered by their own respiration. Just as in the latter case the aggregation occurs sooner in a non-aerated than in an aerated preparation, so the response to a hydrogen bubble is found to be greater in a non-aerated suspension.

The flagellates collect immediately around a hydrogen bubble but the intensity of the aggregation diminishes with time, until after a certain interval there are no more *Bodo* around the bubble than in the general suspension. This is because the bubble has now absorbed from the water enough oxygen to be in equilibrium with the dissolved oxygen and there is no longer a region of low oxygen concentration in the water around the bubble. After this the continually decreasing oxygen tension in the water, due to the respiration of the flagellates, falls below that in the bubble and the flagellates move away from the latter which is now giving back oxygen to the water. The center of the preparation is then cleared of *Bodo*, the peripheral band formed in the usual way, and in this process the bubble acts as an air bubble, becoming surrounded by an inner ring of flagellates which gradually approaches it.

Thus the experiments with acids and with gas bubbles not only dispose of the view that spontaneous aggregations are due to positive chemotropism to acid produced by the respiration of the flagellates but they also strengthen the explanation of the phenomenon given above.

*The Effects of Excess and of Absence of Oxygen.*

There is a relation between the oxygen content of the *Bodo* culture media and the preference shown by the flagellates for an optimum oxygen concentration lower than the saturation concentration of the gas in water in contact with air. For a series of determinations showed that, in the grass infusions contained in upright unstoppered
glass jars in which the *Bodo* swarmed, the oxygen content was considerably lower than that in a similar vessel containing an equal volume of tap water.

A number of experiments was made to test the effects, if any, of excess and of absence of dissolved oxygen on *Bodo*. An ordinary cover-slip preparation was made and at the same time an equal amount of *Bodo* suspension was placed on a hollow-ground slide and exposed to the air in a moist chamber. 24 hours afterwards the usual square-shaped band of flagellates was present under the cover-glass, in the zone of optimum oxygen concentration, while on the uncovered slide the flagellates were evenly distributed throughout the drop. In spite of the oxygen concentration being above the optimum in the second preparation, the swimming activity of the individuals was alike in both. The fact that a large excess of oxygen has no effect either was demonstrated at the conclusion of a gas chamber experiment such as that described on page 492. A preparation with an established *Bodo* band was placed in the chamber and oxygen passed through the latter. The square band retired towards the center becoming a ring and then a single mass in the middle after which the flagellates dissipated to become evenly distributed through the slide. This dissipation always occurs when the oxygen concentration is more than a certain amount above the optimum; it was seen also in preparations left standing for some time (page 487). In the present instance the oxygen concentration must have been very greatly above the optimum since the gas chamber contained pure oxygen. The chamber was now closed and the preparation left over night in the oxygen atmosphere. 17 hours after the commencement of the experiment the flagellates were swimming in full activity. The preparation was then removed from the gas chamber and exposed to the air. In 3 hours and 10 minutes two aggregations had formed towards the center and in 10 hours and 10 minutes the square band was reestablished. Thus 17 hours in the presence of an oxygen concentration higher than they could ever encounter in nature had no inhibitory effect on the activity of the flagellates.

To test the effect of the complete absence of oxygen an experiment was devised as follows. An ordinary preparation was made of *Bodo* suspension beneath a supported cover-slip. This was then closed
along the four sides with vaseline so that the suspension was every-
where shut off from the air (Fig. 14). For comparison a second
preparation was made which was vaselined along two of its four
open edges. This was done to eliminate any possible injurious effect
of the vaseline on the \emph{Bodo}, which would now be the same on both
slides. 24 hours afterwards almost all the flagellates in the sealed
preparation were motionless, just a few swimming feebly or vibrating.
In the open preparation there were normal bands of flagellates at a
certain distance inside the free edges (Fig. 15). 48 hours from the
start of the experiment the \emph{Bodo} in the sealed slide were in the same
motionless condition as on the previous day, while those in the open
preparation were in full activity, the bands having retracted in the
usual manner to form a ring near the center. A small aperture was
now made in the vaseline wall surrounding the sealed preparation
so that the air came into contact with the water of the suspension
at this point. Instantaneously all the \emph{Bodo} in the neighborhood of
the aperture commenced to swim actively and arranged themselves in a band at a certain distance inside the aperture (Fig. 16). This semicircular band gradually retreated inwards as the atmospheric oxygen diffused in. The effect of the absence of oxygen is thus not immediately injurious; it stills the motion of the flagellates but after 48 hours in the absence of oxygen they recover their full activity as soon as they have access to oxygen again.

**Minor Phenomena of Aggregation and Band Formation.**

There are several minor phenomena in the process of aggregation and band formation which have not been mentioned up to the present but which must now be described.

1. It should be added to the general description of aggregation and band formation that when very few flagellates are present in the liquid no central aggregation is formed, but after a certain time the flagellates simply leave the central region of the preparation and arrange themselves directly in the final stationary position of the band.

2. A vertical section of the Bodo band is not an upright wall but has the shape shown in Fig. 17. The upper part of the band is nearer to the center of the preparation than the lower part. Further, there are more flagellates present in the upper and lower regions, that is next the cover-glass and next the slide, than in the intermediate region. The latter is curved in section, the concavity being towards the edge of the preparation. The band assumes this shape as soon as it forms and keeps the same shape when in its final station-
The relative density of flagellates in the upper and lower parts of the band varies in different preparations, being sometimes more and sometimes less marked. The degree of slope of the section of the band also varies, being sometimes more and sometimes less steep. The consequence of the greater concentration of *Bodo* at top and bottom of the band and of the slope of the latter is that when the preparation is viewed from above the band appears to be double (Fig. 18). The outer and inner lines of this double band naturally appear the further separated from one another the higher the cover-glass is placed above the slide.

**Fig. 17.** Vertical section of a flagellate band.  
*a*, cover-glass;  
*b*, slide;  
*c*, water-air surface.

**Fig. 18.** Enlarged view of one corner of a square flagellate band.  
*a*, edge of cover-glass.

**Fig. 19.** Vertical section through an air bubble and a ring of flagellates surrounding it.  
*a*, air bubble.

The section of the band has a similar shape to that of the water-air surface at the edge of the cover-slip. This is shown in Fig. 17. It was thought at first that the form of the water-air surface might determine the form of the band, but this is not the case for a flagellate ring surrounding an air-bubble has the same sectional shape as the main band whereas the section of the water-air surface bordering the bubble is a symmetrical curve as shown in Fig. 19. The reason for the peculiar shape of the *Bodo* band remains undetermined. It is unknown why those individual flagellates which are negatively geotropic seem to have a lower optimum oxygen concentration than those which are positively geotropic. The possibility naturally arose that there were two species of *Bodo* present or two different stages in
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the life history of *Bodo sulcatus*. To settle this, the flagellates were fixed after they had formed a band by introducing the fixative beneath the cover-slip through a capillary tube previously placed in position. Fixed in this way many individuals remained in position attached to the glass so that the cover-slip could be lifted off the slide and then the upper and lower flagellates stained and mounted separately. The flagellates attached to the cover-glass were found to be identical with those on the slide. We are thus dealing with a single species but the possibility remains that the positively and negatively geotrophic individuals are in different stages of their life cycle.

3. A further point that has not yet been mentioned is the following. When an oxygen absorber such as reduced indigocarmine is introduced beneath a cover-slip next to a suspension of *Bodo*, a band of flagellates collects in a zone bordering the oxygen absorber in the manner already described. After this principal band has formed a subsidiary band containing relatively very few individuals makes its appearance nearer in to the oxygen absorber than the principal band. The flagellates in this inner band are close up beneath the cover-glass and move very sluggishly in striking contrast to the intense activity of those in the principal band. When the latter moves away to take up its definitive position just inside the edges of the cover-slip, the inner band remains for some time in place and then gradually the individuals composing it dissipate to join the principal band.

The reason for this inner band may be that a few individuals have a lower oxygen optimum than the rest. This explanation seems unlikely, however, because the inner band is not a permanent one; after a certain time it dissipates. More probably some individuals chance to swim into the region of lowest oxygen concentration and are trapped there because, as was shown on page 504, the effect of absence of oxygen is to inhibit swimming activity. This suggestion is supported by the fact that in ordinary spontaneous band formation an inner band is best developed in those preparations in which the central region is cleared most rapidly. Here more individuals would be left behind, trapped in the region of least oxygen.

4. There are of course many kinds of bacteria present in the *Bodo* cultures. In the preparations on slides many of the bacteria arrange themselves in zones of optimum oxygen concentration in the manner
originally described by Beyerinck. The bacteria, however, do not commence by forming obvious central aggregations which then become converted into rings. This is, perhaps, because they are less motile than the flagellates: they collect more slowly than the latter into their zones of optimum oxygen.

Now when a bacterial optimum position coincides with that of Bodo, there is frequently an antagonism between the two organisms, the bacteria preventing the flagellates from taking up their normal position. In particular, there were frequently present certain cocci the optimum position for which was situated at the middle depth of the Bodo band and certain bacilli whose optimum coincided with the lower and outer portion of the Bodo band. When a preparation was made from a culture in which these bacteria were present in quantity the Bodo were excluded from the middle and lower parts of their zone and all forced to occupy the upper and inner part. The bacteria took up their position more slowly than the flagellates, consequently when the band first formed the flagellates occupied both upper and lower regions, as shown in Fig. 17, but when the bacteria arrived they drove the flagellates out of the lower region. When a preparation having a Bodo band with these bacteria in its lower part and a piece of moss cutting across the band as described on page 498 is removed from darkness to light, all the flagellates and bacteria move inwards to take up a new position, as shown in Figs. 11, 12, and 13 (Part I). But the Bodo move back much more rapidly than the bacteria and, arriving first at the new position of optimum oxygen, are able to place themselves in a band extending in depth from cover-glass to slide. Later on the bacteria arrive and drive out the flagellates from the lower and middle parts of their zone.

SUMMARY.

Spontaneous aggregations of flagellates are formed under the cover-glass because the organisms are attracted to and remain in regions where the concentration of dissolved oxygen is less than the saturation concentration under atmospheric partial pressure. These regions of

lessened oxygen content arise towards the center of the liquid beneath
the cover-glass, owing to the oxygen consumed by the flagellates in
respiration not being replaced here by the solution of atmospheric
oxygen, as it is along the edges of the liquid. The flagellates, however,
are insensitive to the attraction of regions of lessened oxygen con-
centration when the oxygen concentration throughout the liquid is
above a certain value. Therefore, for the aggregations to form,
either the initial concentration of dissolved oxygen must be below this
limiting value, or an interval of time must first elapse after the making
of the preparation until the respiration of the organisms has reduced
the oxygen concentration throughout the liquid down to this limiting
value. The aggregations will then form because the flagellates have
become positively chemotropic to the lower concentration of oxygen
at the center of the liquid.

Once established, such an aggregation of flagellates does not remain
long in the same form. An area free from flagellates appears at the
center of the aggregation so that the organisms lie in a circular band
surrounding the clear area. The latter increases in size and its
bordering band of flagellates in diameter, the band gradually becom-
ing less circular and more square in shape, if the cover-glass is a square
one: The clear central area is a region where the oxygen consumption
of the flagellates has reduced the oxygen content to such a low value
that the organisms are forced to leave the region. They collect in
a band where the concentration of dissolved oxygen is an optimum
for them. It is the equilibrium position between the oxygen consumed
at the center and that diffusing in from the edges of the liquid. As
the consumption at the center is more rapid than the replacement
from the edge, the flagellate band moves outwards until it becomes
stationary at a position where the rates of consumption and replace-
ment of oxygen are equal.

Although the flagellates collect in this manner in regions of optimum
oxygen concentration, yet greater concentrations of dissolved oxygen
have no injurious effect on them. Concentrations of dissolved oxygen
lower than the optimum have the effect of inhibiting the movement
of the flagellates. They recover their activity, however, immediately
they are given access to dissolved oxygen again.
Work done in the past on chemotropism of flagellates will have to be revised in the light of the above facts, since the oxygen content of solutions used has never been taken into account.

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