VII. *On the Effect of Gravity upon the Movements and Aggregation of* Euglena viridis, *Ehrb., and other Micro-organisms.**

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[PLATES 32—36.]

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INTRODUCTION.

IN the course of an investigation upon the effect of various physical forces upon the movements of *Euglena viridis*, I found that under certain conditions a peculiar aggregation into networks or separate groups occurs in which the Euglenæ remain in an actively motile condition. NÄGELI in 1848 had observed groupings of a similar kind in other organisms, and SACHS in 1876 found that certain green swarm cells exhibited a peculiar net-like pattern, which he ascribed to currents set up in the water by the unequal distribution of heat. The aggregations described in the present paper are, however, as I shall endeavour to show, not due to heat, but are in large measure the mechanical result of the action of gravity combined with cohesive forces acting upon organisms heavier than water.

In the first part of the paper a description is given of the movements of Euglena and the various experiments made to determine the conditions under which the aggregations of this and similar organisms occur. The second part contains a general discussion of the facts observed and the conclusions drawn from them.

EUGLENA VIRIDIS.

1. *Seasonal Distribution and Movements.*

Euglena viridis is a very common and widely distributed organism which may be found at any season of the year in water which contains much organic matter in solution, but is usually more abundant during spring and autumn than at any other time, and may entirely disappear during the summer months. Its normal cycle of seasonal distribution is as follows:—In February, or even earlier if the season is a mild one, the surface of the pools where Euglena is found becomes covered, on fine days, with a green scum composed of motile cells which have been liberated from cysts contained in the mud at the bottom of the pool. If the weather becomes dull or cold they may sink again, and form a green layer of motile cells on the surface of the mud. But their distribution in the liquid varies continually; sometimes they may be seen forming cloud-like aggregations below the surface; at other times they form a coarse mottled network-like aggregation, or a more or less homogeneous layer at the surface. During a wind they may remain at the bottom of the pool, even if the weather is fine, owing to the movements and ripples preventing their accumulating on the surface. In cold weather they also remain below, and in prolonged spells of cold may become almost lost to view through sinking in the mud, but warm mild weather tends to bring them to the surface again. If fine weather continues they round themselves off, become covered with a thick cell wall, and thus form a more or less dense scum of encysted cells in which vegetative reproduction goes on rapidly. In rough, windy and rainy weather this scum becomes broken up and sinks in the liquid, but a fresh crop of motile cells, produced by the bursting of these cysts, appears as soon as the weather again becomes favourable.

In the summer months the liquid containing the Euglenæ gradually evaporates, and the encysted cells become incorporated with the dried-up mud. If small particles of this are examined under the microscope, the encysted Euglenæ can still be seen, slightly contracted and irregular in shape, with very thick, yellowish-brown cysts, but with green chlorophyll still visible inside. During a wet summer fresh crops of Euglenæ are commonly found, but are not so abundant as in the spring, and in a very dry summer are very rare.

During the rains of autumn the Euglenæ once more re-appear in abundance, and may be found until November or December, and in a mild season all the winter, but they disappear as a rule during the winter, probably owing to the cold. They become encysted and sink to the bottom of the liquid ready to re-appear during the first mild weather in the spring.

During the motile stage the Euglenæ are subject to very keen competition, and may at any time entirely disappear from view to give place to a crop of Spirilla or other organisms. This is commonly observed under ordinary conditions in nature, and was very noticeable in a tank in my garden, in which, during the early spring, a quantity of manure and refuse of various kinds, together with mud containing Euglenæ, had been placed. The Euglenæ at first came to the surface in large numbers, but the conditions were apparently not so favourable for them as for other organisms, for in the course of a week or ten days, they had almost entirely disappeared from view, and the tub became filled with Chlamydomonas. During the summer a vast quantity of bacteria appeared and formed a white scum on the surface, which remained for a long time, to the almost entire exclusion both of Euglena and Chlamydomonas. Algæ of various kinds also appeared, and a large quantity of *Lamprocystis roseo-persicina.* Early in November, however, I noticed a greenish appearance in the white scum, and on examining it under the microscope, I found a number of large healthy Euglenæ mixed up with the zoogleæ of the bacteria. A few days later, during a heavy fog, this scum, together with the Euglenæ, disappeared; cold weather set in, with snow and frost, and no Euglenæ were again visible until (after several days of mild spring-like weather) January 22. They formed a faint green scum on the surface of the liquid mixed with Spirilla and Astasia. On January 26 the tub became frozen over to a depth of about half an inch, and remained so until February 4, when the ice began to melt. It was a cold damp morning, but at the edge of the tub there was a large number of Euglenæ just entering on the motile stage, together with a few Spirilla but no Astasia. In the course of the morning the ice had nearly all gone, and in the afternoon a faint green scum was again visible on the surface of the liquid. At night there was a sharp frost again, and nearly all the cells were frozen up in a layer of ice. On February 20 the ice began to melt, and the surface again became covered with a faint scum of motile cells. Then we had a good deal of rain, and the Euglenæ disappeared. On March 2 a slight green scum again appeared mixed with bacteria.

In the course of a few days the bacteria became very abundant, and only a few Euglenæ were left, but they soon became more prominent again, and the bacteria diminished in numbers.

The conditions were now apparently favourable for the development of Euglenæ, for during the next few weeks they appeared in great abundance, to the almost entire exclusion of other organisms. The surface became covered with a dense mass of motile cells. These gradually became encysted, and formed a thick scum of a yellowish-brown colour, which broke up into flakes and sank to the bottom of the water out of sight, and finally, towards the end of June, all the Euglenæ had disappeared and did not reappear again until the autumn.

2. *The Diurnal Movements of Euglena.*

In addition to the seasonal movements there are diurnal movements which are visible under natural conditions, but can be studied more easily in the laboratory than in the field.

A little mud containing Euglenæ is placed in a saucer of tap-water or rain-water in a good light. The mud soon settles in the water, and the Euglenæ in an hour or two come to the surface of the mud and there form a green layer of motile cells. They then rise through the water, generally in the direction of the source of light. In a diffuse or dull light the movement towards the surface is slower, and many of them reach it by a slow movement along the bottom and up the sides of the vessel. But, however they do it, they ultimately come to the surface, and are found there in a motile condition. If the light is sufficiently strong, a good proportion of them will make their way to that side of the saucer nearest the light, and there form a distinct green line at the edge of the water. As night comes on they gradually disappear from this place, and become again distributed through the water, and by the time it is dark the green line has completely vanished. Subsequently, the surface of the water is found to be covered with a green scum of rounded off cells; a considerable number are also found in the same condition on the surface of the mud, whilst a few remain swimming about in the water, but eventually most of these also settle down in the rounded condition. They remain like this all night, except that a certain number of them undergo division. The time at which this takes place varies, but it is generally in full swing at about 10 P.M., and is usually over by one or two the next morning. It may here be remarked as an interesting fact that if a portion of this non-motile scum is placed at any time during the night in a good light, such as that from an incandescent gas mantle concentrated by a condenser, the organisms in a very short time become motile again, and any divisions which have begun are finished whilst the organism is in this motile condition. So also, under these conditions, motile cells may start dividing and go through the whole process of division in the motile stage.

As soon as it becomes sufficiently light the next morning, the Euglenæ begin to pass into the motile condition again, and are seen moving towards the source of light and begin to accumulate in a dense green mass on that side of the saucer nearest the light.

Sometimes from this dense mass a number of streams will be observed to pass downwards into the deeper shaded regions below, from which they ascend again to the surface and once more move towards the light. This downward streaming into the shade and subsequent upward movement again into the light goes on continuously so long as the Euglenæ remain motile and the light is strong enough to attract them. If the light is intense, the Euglenæ may be repelled to the other side and into the deeper layers of the water, where they remain until the light becomes less intense and once more attracts them. As night approaches, they again spread themselves over the surface of the water and on the mud, to enter once more upon a resting stage during which further cell divisions take place. This cycle of daily changes may go on for several days, but a scum of thick-walled encysted cells is gradually formed; the number of motile cells decreases, and the culture slowly dries up.

If Euglenæ are exposed to a very intense light, they may become killed and their chlorophyll bleached. Sometimes a whole culture is destroyed by the attacks of parasites of various kinds, among which the most common are species belonging to the Chytridineæ, including occasionally an abundant formation of *Polyphagus euglenæ*, Nowak.

In cultures kept in the dark Euglenæ may remain in the motile condition for several weeks or until they have become deformed and degenerate. I have kept them in a motile condition in a corked bottle in the dark for as long as two months.

The fundamental power of movement possessed by *Euglena viridis* is capable of being influenced by the environment, and to this, no doubt, are partly due the seasonal and diurnal variations in its distribution which have been just described. Among the various factors which affect the movements of certain micro-organisms light, as is well known, is one of the most important. It is not so well known that, especially in the absence of light, a very definite directive influence is exerted upon their movements, under certain conditions, by gravity.

It is not proposed to consider here all the various factors which may have a share in the modification of the movements of micro-organisms. The main purpose of this paper is to give an account of a series of experimental observations which have been made to study the action of gravity upon *Euglena viridis*, together with a less detailed account of similar observations upon a few other forms—*Euglena deses*, Chlamydomonas, *Volvox globator*, bacteria and one of the fresh-water Peridineæ.

EXPERIMENTAL OBSERVATIONS ON *Euglena viridis.*

1. *Aggregation in Narrow Glass Tubes.*

The experiments described in the following pages are very easily performed, but a good supply of actively motile Euglenæ is necessary. These can be obtained fresh in the early spring and summer from almost any farmyard, and may be kept motile for several days if proper precautions are taken. The Euglenæ are usually collected along with a considerable quantity of mud, which should be placed in water in a flat open vessel, in a good light. In the course of a day the Euglenæ will be found accumulating at the surface. This surface film should be picked up on a glass slip, and the Euglenæ washed off into a very dilute starch solution, which is placed in a bottle tightly corked and kept in a dark cupboard. In this condition they will remain motile, especially if they are exposed to light for an hour or two daily and then replaced in the dark.

If a glass tube about 25 cm. long and 7 mm. in diameter is filled with water containing enough motile Euglenæ to give it a pronounced green colour and placed horizontally in the dark, the Euglenæ will be found, at the end of a few minutes, no longer evenly distributed through the tube, but aggregated into a series of more or less regular groups or bands (Plate 32, fig. 1). This aggregation begins immediately the tube is placed in the dark and indications of it are distinctly visible at the end of 30 seconds. If left for a longer time, the grouping becomes very regular and as the figure shows, the groups are nearly evenly spaced and separated by clear spaces (fig. 1, *a*, from the side, *b*, from above).

Seen from above, each separate group exhibits a dense central mass surrounded by a clearer region which extends all across the tube, and appears like a rectangle (fig. 1). In the earlier stages of the aggregation an irregular network with slightly more pronounced nodal points here and there (fig. 2) is observed.

The following are the details of one of the experiments made :—

11.55 A.M.—Tube filled with water containing enough Euglenæ to give it a distinct green colour, and put in the dark.
11.55½ A.M.—Aggregation just beginning.
11.56 A.M.—Irregular network visible (Plate 32, fig. 2, *a*, *b*).
11.58 A.M.—Network more distinct (Plate 32, fig. 2, *c*).
12.8 P.M.—Clear lines begin to appear across the tube, indicating the formation of separate groups (Plate 32, fig. 2, *d*).
12.27 P.M.—Groups visible, but not clearly marked off.
12.40 P.M.—Groups distinct, and more or less regularly spaced (Plate 32, fig. 2, *e*).
3.50 P.M.—In same condition.

If the tube contains only a few Euglenæ, it takes a much longer time for any aggregation to take place, in one case three minutes, in another, where there were very few, ten minutes, and then only slight streams here and there are formed, or irregular and distantly spaced groups (Plate 32, fig. 9).

If the Euglenæ are too much crowded together, or the tube is of much larger diameter than that given, the separate groups are not well differentiated, and the aggregation remains either in the network stage (Plate 32, fig. 2, *a–d*), or in the condition of irregular and less evenly spaced groups. The following experiment shows this :—

11.47 A.M.—Test-tube $\frac{3}{4}$-inch diameter filled and placed in the dark.
11.48 A.M.—Aggregation just beginning.
11.50 A.M.—Network visible.
11.57 A.M.—Irregular network ; tendency to form groups, but very irregular.
11.59 A.M.—Ditto.
12.8 P.M.—Ditto.
12.27 P.M.—Ditto.
3.50 P.M.—Ditto.
Practically no change in the aggregation took place.

Photographs showing some of the various stages in the aggregation of Euglenæ are given in Plate 32, fig. 3, from the side, and fig. 2, from above.

In a very shallow cell, such as is made by cementing strips of cover glass on the slide, or in thermometer tubing, no aggregation occurs.

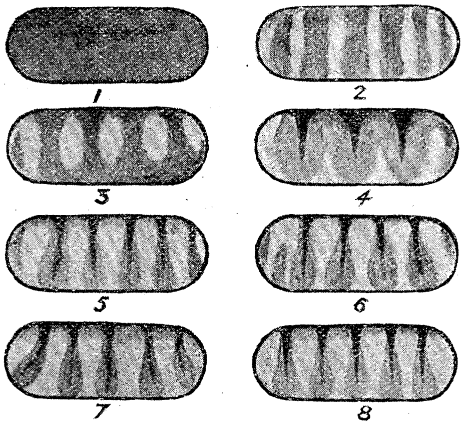
Exposure to bright light immediately causes the groups to disappear and the Euglenæ migrate towards that end of the tube nearest the light, where they form a dense accumulation, leaving the rest of the tube free. If the tube is now again placed in the dark, this dense mass at once begins to spread out towards the other end of the tube in the form of an irregular network, until regular groups extending throughout the tube are again produced. In this process the mass of Euglenæ first of all sink to the lower side of the tube and move for a short distance along it. Then they begin to rise again here and there, and gradually form the coarse network which is the forerunner of the regular groups.

An examination with a pocket lens shows that each separate group consists of two distinct parts, a densely aggregated central region tapering downwards to more or less of a point, and a lighter, less populated region around it (text-fig. 1, 4–8). Under a low power of the microscope, it can be seen that this dense central mass consists of cells apparently falling downwards ; the lighter region consists of cells swimming irregularly upwards (Plate 36, fig. 52).

If kept in the dark, the Euglenæ continue to exhibit this phenomenon of aggregation for several days, until they die, in fact. In one series of experiments I found that the length of time during which the aggregations persist varied from four to eight days, but during these periods a large number had become rounded off and settled to the lower side of the tube, and during the last two days the aggregations were faint and irregular. A few were usually found motile in the tube for many days after this, but not sufficient to produce any visible aggregation. The aggregation is so persistent that the rounded off cells are often found lying on the lower side of the tube in regular groups, having settled down in this position from the motile aggregations.

The aggregation takes place equally well whether the tubes are open at the end or not. A few cells usually accumulate at the surface of the water in contact with the air, but the general effect is not inhibited by the presence of the air.

The movements may be studied under the microscope in a shallow oblong cell. I have found it convenient to use a cell 3 cm. long by 1 cm. broad and 2 mm. deep; the aggregation is not quite so regular in a cell of this shape as in a tube, but it is more easily observed under the microscope and is essentially the same. The various stages of the aggregation from the homogeneous state during a period of about two hours are shown in text-fig. 1, and a diagram of the direction of the movements in Plate 36, fig. 52. In order to keep such a cell under observation with the microscope, the microscope should be placed in a dark box or under a black cloth and only red light allowed to pass through the cell. It was found that red light does not interfere appreciably with the phenomena of aggregation.



TEXT-FIG. 1.—Eight Stages in the Aggregation of *Euglena viridis* in a cell 1 in. × 3/8 in. × 1/16 in. Sketched at intervals of ½, 5, 4, 20, 16, 32, 42, and 25 minutes respectively. Stage 1 was sketched half a minute after the experiment was started.

The falling movement is a very pronounced one, the stream of Euglenæ starting from a dense mass on the upper side of the cell. In the great majority of cases the individual Euglenæ, in a steady downwards moving stream, have the posterior end downwards, but occasionally some of them, especially at the beginning of a streaming movement, have the anterior end downwards. These move at a more rapid rate than those which fall with the posterior end downwards. Towards the bottom of the stream, however, the majority are found with their posterior ends downwards; and they can be seen turning round into this position during the movement. None of them is quiescent during this downward streaming. It appears to an observer as if they try to swim upwards, but that some force tends to keep them in the downward stream. Sometimes an individual will be seen to sustain himself at the same level for some time against this force, and may make his way out laterally to join the upward stream. In this way, individuals may escape from the downward stream at various heights, especially near the bottom of the stream.

As they reach the bottom of the stream the Euglenæ move out laterally on all sides, and then begin to ascend, taking gradually a direction which brings them towards the main stream again, and finally as they approach the top they again become entangled in it and descend again. They become absorbed into the main stream at different heights, but most of them, in a tube 8 mm. in diameter, are able to get near the top before they are absorbed into it (Plate 36, fig. 52).

2. *Aggregation in Closed Shallow Vessels.*

Similar aggregations occur in flat shallow vessels which are so constructed that they can be covered with a glass plate and sealed with vaseline. Glass is the best material for the sides of the vessel, but I have made them of hard paraffin wax fastened to a glass plate by heat, or strips of wood or rubber cemented to the glass by any convenient cement which is not affected by water.

In a vessel 6 mm. deep filled with water containing enough Euglenæ to give it a green colour, and closed up so that no air could get in, the aggregation began in a few seconds after it was placed in the dark. A coarse network first of all appeared (Plate 33, fig. 10), consisting of dense well marked green lines of Euglenæ, with less deeply coloured regions adjacent to these, consisting of Euglenæ in a more or less regular movement upwards. In the figure, these lighter regions are clearly indicated as a lighter shading on both sides of the dark lines of the network. All the figures are from photographs taken at intervals during the progress of the experiment. In the course of a few minutes the network became broken up into a large number of more or less regular groups (Plate 33, figs. 11, 12).

Each group was more or less circular or oval in outline and exhibited similar movements to those previously described in the tube aggregations.

The following are the details of one experiment. The Euglenæ in a closed vessel were shaken up so as to become evenly distributed throughout the liquid, and at once placed in the dark at 10.54 A.M. :—

10.55 A.M.—Aggregation just beginning. Faint network visible.
10.56 A.M.—Network very clear (fig. 10).
10.58 A.M.—Separate, more or less regular rounded groups appear (fig. 11).
10.59 A.M.—Rounded groups more clearly marked and more evenly spaced.
11.0 A.M.—Groups now very evenly spaced and about equal in size (fig. 12).

In the course of a few minutes (6 to 12) these groups may become broken up still further into smaller and smaller groups as shown in figs. 13 and 14, and they remain like this, if they are not disturbed, for several days or until they die.

In vessels 10 mm. deep we get no definite separation into groups; a network only is formed (Plate 35, fig. 35), which gradually, however, becomes finer (fig. 36) and remains in this condition until the cells die. In a vessel which is only 4 mm. deep, on the other hand, the separation into groups is more pronounced than in the deeper ones, and takes place more quickly (Plate 34, figs. 25–28).

In a vessel two or more inches high, placed in the dark, the Euglenæ accumulate at the bottom and show a constant alternating movement up and down, with irregular aggregation (Plate 32, fig. 7). A few Euglenæ will be found swimming about freely in the upper layers of the liquid, and if the upper surface is exposed to the air, a few will be found there forming a thin green layer.

If a vessel containing motile Euglenæ is exposed to light in a window, the Euglenæ are all attracted to that side nearest the light and form there a dense green layer. If the vessel is now placed in the dark, an aggregation at once begins in this dense layer in the form of a twig-like branching (Plate 34, fig. 21), which gradually spreads until the Euglenæ are more or less evenly distributed in groups. This is illustrated in Plate 34, figs. 21–24 and 27, 28. Fig. 21 shows the state of aggregation two minutes after being placed in the dark, fig. 22 four minutes, fig. 23 six minutes, and fig. 24 eight minutes after being placed in the dark. In the course of about 20 minutes, a more or less regular grouping will be observed as in figs. 27 and 28, and at the expiration of about half an hour, very regular and evenly spaced small groups are produced similar to those in Plate 33, figs. 12 and 13.

3. *Aggregation in Shallow Vessels Exposed to the Air.*

So far, we have only considered the aggregation of Euglenæ in narrow vessels from which air is excluded. In a shallow vessel, such as an ordinary saucer, with the surface of the liquid exposed to the air, the Euglenæ aggregate, in the dark, into a coarse network in which the same streaming movements take place. In an ordinary saucer with sloping sides the network is more pronounced in the middle than at the edge of the liquid (Plate 35, fig. 39), but in a dish with upright sides the network is more regularly distributed. The network is more regular in a shallow layer of liquid than in a deeper one, and the meshes are smaller and separate groups may be produced.

The aggregation in the presence of air is not so persistent as when air is excluded. This is due to the fact that, in contact with air, the Euglenæ gradually become encysted and form a scum of non-motile cells at the surface of the liquid. If, instead of air, the surface is in contact with carbon dioxide, the Euglenæ remain motile, and the aggregation persists for a much longer time. This tends to support KHAWKINE'S suggestion ('86) that the persistent motility of the organism under certain conditions is due to its excitation by irritant vapours produced by the putrefactive substances in the liquid in which it normally lives, and among these may, perhaps, be reckoned carbon dioxide.

But the presence of air has also the effect of exerting some attraction upon the Euglenæ by which the aggregation is slightly modified in deep cells. In an upright vessel 2 inches high by $2\frac{1}{2}$ inches long by $\frac{3}{16}$ inch deep, sealed up with vaseline, so that no air was present, the Euglenæ sank to the bottom of the cell and there remained, with an irregular streaming motion up and down, up to a height of $\frac{1}{2}$ inch to 1 inch (Plate 32, fig. 7). This was kept up for about a week, but at the end of that time nearly all the Euglenæ had rounded themselves off and sunk to the bottom. There were none at the upper surface. In a similar cell, with the upper surface exposed to the air, the Euglenæ soon began to aggregate with irregular streams, starting at various heights, but many of them from the surface of the liquid, the oxygen attraction being sufficient to carry them upwards, above the height to which they would ordinarily rise (Plate 32, figs. 4–6, photographed at intervals of five minutes). This continued for about three days, and, at the end of that time, about half of them were found rounded off and lying at the bottom of the cell, the remainder having accumulated at the surface in contact with the air, some of them on the surface of the water, others forming a bright green scum on the glass sides of the vessel, and all of them rounded off.

AGGREGATION OF OTHER FORMS.

1. *Colourless Forms of* Euglena viridis.

Under certain conditions the chloroplasts of *Euglena viridis* may become pushed back into the extreme posterior end of the body, whilst the anterior end remains colourless and filled with paramylum grains. I have not been able to determine the exact conditions under which these forms can be produced, but they may be found in nature during the spring of the year, and can usually, but not always, be produced in greater or less abundance in a potato starch solution kept in the light or in obscurity. ZUMSTEIN ('99) finds that in *Euglena gracilis* completely colourless forms are produced in a good nutrient solution, either in the light or in darkness. I have not been able to obtain the colourless forms of *Euglena viridis* when kept absolutely in the dark, either in starch or albumen solution. Some small amount of light seems to be necessary. Neither have I been able to obtain completely colourless forms. The amount of chlorophyll may have become much reduced, but a slight green colour is always visible at the extreme posterior end of the organism.

The forms with reduced chlorophyll have been obtained by me as follows :—

1. In a dilute starch solution kept in the light, a scum of encysted cells was soon found at the surface of the liquid, which to some extent prevented the penetration of light to the deeper parts of the vessel. In this region a large number of motile, nearly colourless, forms was found.

2. In a dilute starch solution, kept in a saucer, covered with a sheet of cardboard so that a small quantity of light could penetrate between the cardboard and the edge of the saucer, a large number of these forms was found in a heap at the bottom of the saucer, with very few encysted at the surface.
3. A few were produced on the underside of a small quantity of mud, which had been placed on a piece of glass and inverted over a damp chamber, where they were only in a diffused light.

In 1 and 2, all stages between the colourless forms and normal Euglena could be seen, so that there is no reason to doubt their being *Euglena viridis*.

Euglenæ kept in the dark in starch solution, albumen solution, or tap water, showed no colourless forms, even after 15 days. At the end of that time, the chloroplasts were abundant, and distinctly seen more or less completely rounded off; signs of degeneration were observable, as was shown by the large number of rusty red spots in the cytoplan and the deformed appearance of the cells.

The colourless forms are always full of paramylum grains, and their specific gravity appears to be greater than that of normal cells. They sink at once in the water, either in the light or in darkness, and form a layer or mass at the bottom, where they remain in an active motile condition. They appear to be unable to rise to any great height in the water, and are not very sensitive to light.

I have not observed any distinct aggregation of them in normal cultures in an open saucer, but in a long tube placed horizontally in the dark they fall to the bottom and form a green band all along the lower side of the tube, which gradually becomes broken up into irregular green patches.

In a small flat cell they at once sank to the bottom and soon formed irregular groups here and there, which constantly but slowly changed their position and size, finally accumulating into two or three (sometimes only one) groups, consisting of masses of motile cells. This probably explains the absence of aggregation visible in the normal cultures. They all tend to aggregate into a single mass.

If the colourless forms are kept in a closed cell in water, they show at first little or no aggregation, but in the course of 24 hours they begin to use up the stored food substance, the paramylum grains; the chlorophyll takes up its normal position, and the Euglenæ recover their motility and become capable of aggregation again.

In a mixture of coloured with colourless forms, the former show a distinct aggregation, the latter sink to the bottom of the vessel and exhibit no aggregation. If the supernatant liquid containing the aggregated coloured forms is removed by means of a pipette, the two types can be completely separated from one another.

2. Euglena deses, *Ehrb.*

This is a long, cylindrical form commonly found creeping over the surface of mud containing much organic matter in solution. It is very flexible and contractile, but, according to DUJARDIN, does not swim freely in the water. I have collected it several times in a pure culture on mud containing sewage sediment, and I have found it mixed with other forms from various localities. When it occurs in large quantities, it forms a bright green layer on the surface of the mud. This species is very variable in size and structure, and KLEBS has separated one form of it as a distinct species under the name of *E. Ehrenbergii*, and another as a distinct variety, *E. deses*, var. *intermedia*. According to DANGEARD ('94), none of the characteristics by which they are separated is constant, and there seems to be no reason to consider that they are more than different forms of one and the same species. In my own cultures there seemed to be no distinct line of demarcation between the three forms described, and I have not attempted to differentiate them from one another in their behaviour.

In the typical form of *Euglena deses* the body is long and cylindrical, pointed or slightly rounded at the posterior end, and with a short flagellum at the anterior end. The nucleus is in the middle of the cell, and the cytoplasm around it is filled with ovoid chlorophyll corpuscles and oval or elongate paramylum grains of variable size. In cultures kept in the dark the paramylum soon disappears, but the long cylindrical form of the body, with its pointed posterior extremity, remains very distinct. In a good light and abundant food supply the organism becomes filled with large paramylum grains and contracts into a more or less irregular shape, often with a rounded posterior end.

By means of its flagellum, *Euglena deses* can swim slowly and not very vigorously in water. This is more apparent, however, in specimens kept in the dark or in diffuse light, and depends partly on the temperature. In a good light, with abundant organic food material in the water, the flagellum is frequently absent, and the Euglenæ move over the surface of the mud or the sides of the vessel in which they are contained by a creeping movement, which is brought about chiefly by a more or less regular amœboid contraction of the body, and partly, also, when it is present, by a slight quivering vibration of the flagellum. Motile individuals may be attracted or repelled by the light, or may remain indifferent to it. Their behaviour towards the light seems to depend partly upon the temperature, partly upon the intensity of the light, and partly upon their physiological state, such as may be induced by leaving them in the dark for a few days. At low temperatures, *Euglena deses* becomes very sluggish in its movements and does not respond readily to the action of light. Thus a culture in water at a temperature of 6°–8° C. was placed in a good light. The Euglenæ were moving slowly; a few were repelled by the light, but the majority were not affected by it. At 12° C., however, their movements became more vigorous and the majority moved towards the light, a few away from it. At 15° C. they were all attracted. Specimens which had been kept in the dark for four days, when exposed to the light, the water in which they were swimming being at a temperature of 11°–12° C., were all repelled at first, but in a short time their active movements ceased: they began to coil up and contract into irregular shapes, and at the same time sank slowly in the water to the bottom of the vessel. In a short time they apparently recovered from this light paralysis, and at a temperature of 15° C. became very active and were all attracted by the light.

In the actively motile condition the Euglenæ, when placed under suitable conditions, become aggregated into networks and groups similar to those of *Euglena viridis*. Those which respond freely to the light aggregate much more quickly and definitely than those which are in a sluggish condition. In shallow vessels or narrow tubes placed in a horizontal position, the grouping was just as definite and clear as in *Euglena viridis*, and persisted for several days. The sluggish forms, containing large quantities of paramylum grains, behaved very much like the sluggish colourless forms of *Euglena viridis*. When placed in a shallow vessel or tube, in the dark or in a dull light, they at once sank to the bottom in a nearly homogeneous layer, in which, after a time, an irregular network-like aggregation became visible. Those forms which are only able to creep about on the surface of the mud also showed, when kept in the dark or exposed only to a dull light, such as gaslight or diffuse daylight, an irregular network-like grouping on the surface of the mud.

Observation under the microscope showed that, during the aggregation of the freely motile forms, the individuals were nearly always in a vertical or slightly oblique position, with their pointed posterior ends downwards, either falling in the downward stream or moving upwards at its periphery.

3. *Chlamydomonas.*

Experiments were made with motile cells which appeared in abundance in one of my cultures of Euglena. The cells were slightly oval, and varied in size from 9μ by 12μ to 16μ by 20μ; a single pyrenoid was present and the chromatophore was cup-shaped.

The motile cells were attracted by a dull light, but repelled by a strong one. When placed in a shallow open vessel in the window in a dull light, they were attracted to the side nearest the light and formed a dense layer at the edge of the liquid, from which, when a certain stage of congestion had been reached, falling streams were set up. In the dark, this streaming became more prominent, as with Euglenæ.

In a bright light the cells sank to the bottom of the water and moved away from the light. In a shallow dish, 1 cm. deep, which was sealed up, they also sank to the bottom in a good light. In dull light they at once moved up to the surface, and a network-like aggregation was produced in three or four minutes, which disappeared again in a short time.

In the dark, a network-like aggregation was at once produced, which gradually became resolved into groups, similar to those of Euglenæ.

In a tube 20 cm. long by 8 mm. in diameter, placed horizontally in the dark, an aggregation similar to that of Euglenæ was obtained (see Plate 32, fig. 1). The same effect was obtained in a dull light, but in a good light they all sank to the lower side of the tube. The light appears in this case to inhibit the upward movement.

Observations under the microscope in a shallow vessel showed that, in falling, the cells have their posterior ends directed downwards in most cases, but all positions were observed, and in cases where the flagellum was directed downwards, the movement was a very rapid one. As they reach the bottom, they escape from the downward stream and swim upwards, but as they reach the top they become absorbed into the downward stream again, and then a regular cycle of movements is kept up, just as with Euglenæ.

Unless a large number are present in the water, the aggregation does not take place so readily as with Euglena, and may be absent altogether. In some very minute forms with a low specific gravity, I was unable to obtain any aggregation at all. In the larger forms the tendency to aggregation was more pronounced after they had been kept in darkness for some time.

A tube filled with vigorously motile cells which had been kept in the light was placed horizontally in the dark at 10 A.M. No aggregation became visible until 3 o'clock in the afternoon. The same tube was kept in the dark until 10 o'clock the next morning; it was then shaken up to get rid of the aggregation and was again placed in the dark. In the course of an hour a distinct aggregation again became visible. The organisms appear to be less active after being kept in darkness for some time, and this may to some extent account for the difference in the time taken to aggregate. When they are in a vigorous motile condition they seem to be able to overcome the forces which tend to bring about the aggregation.

4. *Peridineæ.* (Glenodinium Cinctum.)

In one of the fresh-water Peridineæ, probably *Glenodinium cinctum,* which appeared in abundance in one of my cultures in the spring of 1904, a similar phenomenon of aggregation was observed, differing, however, in some details, owing to its behaviour towards light. A sealed-up cell containing a large number of Glenodinium was placed upright in the dark. The organisms at once sank to the bottom, but in a few minutes indications of an aggregation were visible, and at the end of half-an-hour the aggregation, consisting of delicate streams moving downwards (Plate 36, fig. 50), was very distinct. On placing the cell flat in the dark an aggregation into groups was obtained in half-an-hour. This was not so regular as with Euglenæ, but was quite distinct (Plate 36, fig. 51).

In a horizontal tube (25 cm. by 8 mm.) a regular aggregation was obtained in about an hour, similar to that obtained with Euglenæ.

In an upright cell with the upper surface of the liquid exposed to the air, the organisms accumulated in a dense layer at the surface, from which falling streams were observed to start (fig. 45). These became at first slightly irregular (fig. 46) and then nearly straight and parallel to one another (fig. 47).

Glenodinium appears to be very sensitive to changes in the intensity of the light. In fairly strong light in the middle of the day (February, 1904), they collected on the sides of a vessel away from the light. In dull light, or light from an incandescent gas mantle, they collected on the side nearest the light. In both cases the aggregation and the streaming movement were maintained for some time. In a strong light, however, they gradually all sank to the bottom of the vessel, where they remained until the light became less intense, when the streaming and aggregation were again set up.

In an open vessel exposed to a fairly good light during the morning (February, 1904), the organisms were repelled to the bottom of the vessel on the side away from the light, and accumulated in a dense circular patch, about 2 inches in diameter, in which a regular aggregation appeared in the form of nearly equally spaced circular groups, each one showing a dense brownish red spot in the middle and surrounded by a lighter brown area. In the course of the day, as the light became weaker, they gradually moved towards it, still retaining their aggregation, and were finally attracted by the light to the edge of the vessel, where they formed at the surface a dense narrow band, from which downward streams were observed to fall, with here and there a tendency to a network-like aggregation.

If a vessel such as shown in fig. 47 is taken when the streaming is in full swing and placed in the middle of the room, so that the weak light strikes it on one side, the streams are bent in the direction of the source of light (fig. 48), and gradually become broken up and disappear, some of the organisms being attracted towards the light, others sinking to the bottom.

If the light from an incandescent gas mantle is focussed on one of these falling streams (fig. 49, *a*), a slight repulsion is set up as shown in the diagram, which causes the stream to become expanded in the region of most intense illumination (fig. 49, *b*). If the light is allowed to act for two or three minutes, the lower part of the stream may be destroyed, and only the upper part left (fig. 49, *c*).

5. NÄGELI'S *Observations on some Green Swarmers.*

The results obtained by me with Chlamydomonas and Glenodinium resemble to some extent those obtained by NÄGELI with an unknown swarmer which he received from A. BRAUN in 1848. These were placed in a flat plate in the window. Here they formed a fairly broad zone about one-third the diameter of the plate, on the side near the light, but denser at the edge of the plate nearest the light. Inside this denser ring the green zone was dotted with circular groups, each dark in the middle and surrounded by a lighter green ring. These groups were smaller and more crowded at the periphery, larger and less crowded towards the centre, ranging from about 6 mm. to 12 mm.

When the water was agitated the groups disappeared, but re-formed again in two to three minutes; the groups consist of actively moving swarm cells densely accumulated in the centre. The groups extended downwards into the water like an inverted cone. During the night they settled down to form a layer on the bottom of the plate, in the morning they came to the surface and gradually accumulated at the edge, from which twig-like branches were formed, which altered much in shape but became simpler in form and less branched. They were not only on the surface but extended some depth into the water.

In some further experiments on swarm-cells of *Tetraspora lubrica*, he found that when the water was filled thick with the cells, so as to give a distinct green colour, groups with a dark green centre were found; but, if the water were light green, he found a twig-like branching or a network extending downwards into the water 5 to 10 mm. deep.

6. *Bacteria.* (Spirillum.)

Experiments were made with vigorously motile Spirilla obtained from a liquid containing much organic matter in solution. The motility of these organisms depends upon a supply of oxygen, so that in closed vessels the absence of oxygen soon causes the Spirilla to come to rest, and consequently no aggregation is observable. The influence of oxygen upon the movements of bacteria is well known, and has often been studied since its discovery by ENGELMANN.

Some interesting phenomena of aggregation are observable when the Spirilla are much crowded together, which resemble, in some respects, the aggregations and streaming of Euglena, but are not due to the same causes. They appear to be mainly due to oxygen chemotaxis. I have not yet fully investigated them, and propose to deal with them in a later paper.

Under certain conditions, however, a downward streaming movement can be set up, which appears to be similar to that obtained with Euglena and other organisms. If the Spirilla are placed in an upright cell with the upper surface of the liquid in contact with air, they immediately begin to accumulate in a zone a short distance below the surface. (*Cf.* MASSART, 1891.)

If a large number of Spirilla are present this zone becomes very dense, and, after a time streams of motile individuals begin to fall from it (Plate 34, fig. 31). As they reach the lower side of the cell, they spread out and make their way to the surface again and become absorbed into the dense zone from which streams are constantly falling. In this way a constant cycle of movement is kept up which prevents a too dense accumulation, and, at the same time, gives all the Spirilla an opportunity to share in the oxygen available.

7. *Volvox Globator.*

Very few observations have been made upon this form. Water containing sufficient Volvox to give it a pronounced green colour was placed in a glass tube supported in a horizontal position in the dark. The Volvox sank almost immediately to form a more or less regular green layer on the lower side of the tube, leaving very few swimming freely in the tube. They remained for some time moving about slowly and irregularly, but with a tendency to a more or less pronounced up and down motion, and gradually became aggregated into indistinct groups.

In a shallow vessel in the dark the Volvox also sank to the bottom to form a homogeneous layer, in which, after a short time, a faint network-like aggregation became visible. They were then exposed to oblique one-sided illumination, and at once began to move towards the light. A distinct network-like grouping at once became visible, which persisted for a short time, as the Volvox were making their way to the edge of the vessel, but disappeared as soon as they reached it. As the accumulation of the Volvox at the edge of the vessel gradually became denser, however, an aggregation again became visible, due to a downward streaming of the organisms from the shaded layer on the underside of the dense mass, just as takes place with Euglenæ under similar conditions. But in none of these experiments was the aggregation as definite or as regular as with Euglenæ; it was sufficient to show, however, that the same forces are concerned, and that the aggregation is brought about in a similar way.

INFLUENCE OF EXTERNAL CONDITIONS ON THE AGGREGATION.

1. *Effect of Light.*

If Euglenæ in a state of aggregation in a closed cell are exposed to a strong light, they are at once attracted towards it, and the aggregation sooner or later disappears. If the light is vertical the Euglenæ move directly upwards and become more or less evenly distributed in a thin layer on the surface; if it comes in a slanting direction, the Euglenæ move to that side of the cell nearest the light, and accumulate there in a dense narrow zone.

The following experiment is typical of the normal effect due to the action of a fairly strong light upon the aggregation. A closed cell in which the Euglenæ were evenly spaced in groups, as in Plate 33, fig. 12, was placed in the window in a bright light, but not exposed to the direct rays of the sun, at 11.18 a.m., on July 1. The following table shows how this grouping became affected :—

11.18 A.M.—Regular groups.

11.19 A.M.—One minute after exposure to light : groups still clearly visible, but central dark mass in middle of each group is less clear.

11.20 A.M.—Groups a little less distinct : not so clearly circumscribed. Movement in direction of light just visible.

11.21 A.M.—Pronounced movement towards light. Groups smaller and more irregular, and not so clear in that half of the cell remote from the light.

11.24 A.M.—A few irregular groups still visible in that half of the vessel nearest the light: none in the other half. Green homogeneous layer of Euglenæ now accumulating at the edge of the vessel nearest the light.

11.26 A.M.—Green layer at edge of cell very distinct. A few faint groups still visible near it.

11.29 A.M.—Aggregation entirely disappeared. Most of the Euglenæ at edge of vessel, and some beginning to settle down in rounded off condition. A few individuals were still found in other parts of the vessel, mostly swimming towards the light, but a few away from it.

In a weak light the grouping becomes irregular, but the aggregation does not disappear. The light, although it may be sufficiently strong to attract the Euglenæ, is not able to overcome altogether the tendency to aggregation. The Euglenæ move more slowly towards the light and an irregular network may persist until they have reached the side of the cell. If the weak light is vertical, the Euglenæ tend to accumulate at the surface, but the aggregation persists as long as the Euglenæ remain in a motile condition.

In open vessels similar results are obtained, but the phenomena are not so clearly marked, owing to the formation of a scum of contracted and rounded off cells at the surface in contact with the air. This scum can be easily picked up for microscopic examination, by bringing a glass slip gently in contact with it. In a good light the scum forms very quickly and the aggregation at once disappears. On being placed in the dark again, the scum disappears and the aggregation is once more produced. If, however, the Euglenæ are exposed to a strong light for any length of time at normal summer temperature, they tend to remain in their encysted condition even when placed again in darkness and kept there.

These experiments show, therefore, that light tends to inhibit the aggregation by altering the direction of movement of the Euglenæ, and that the extent to which this takes place depends upon the strength of light. If the light is weak, the aggregation may persist for a short time, but gradually disappears. If the light is strong it disappears at once.

Experiments with coloured glasses show that the blue end of the spectrum is most effective in modifying the aggregation; this power is greatly diminished as we reach the less refrangible rays, and at the red end of the spectrum ceases altogether. Under red glass, the aggregation persists as in the dark; under orange or green glass the Euglenæ behave as in a weak light, and under blue glass, as in a good white light.

Not only is a weak light unable to overcome the aggregation of Euglenæ, it is also unable to prevent the aggregation taking place. If the Euglenæ are well shaken up so as to cause an even distribution of them through the liquid, and are then placed in a weak light which falls vertically, an aggregation at once takes place just as in the dark (Plate 35, fig. 43). Even in a strong light some indications of it may be observed, and under certain conditions a very pronounced aggregation may take place. If a shallow vessel, exposed to one-sided illumination, in which the Euglenæ have accumulated at one side in a narrow dark green zone, is turned round horizontally through 180°, the Euglenæ at once begin to move back again towards the light. They are first of all attracted to the surface and then move along it in a series of ripple-like aggregations (Plate 35, fig. 41), which persist until the opposite side of the cell is again reached.

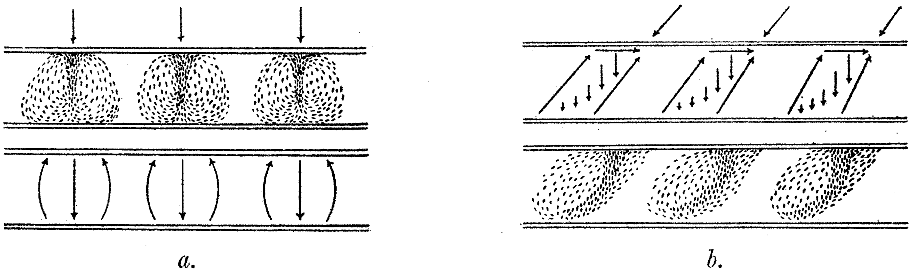
The following are the details of an experiment made to show this, a continuation of the one described on pp. 350, 351.

11.29½ A.M.—The vessel containing the dense mass of Euglenæ on one side was rotated through 180° so that the organisms were on the side away from the light.
11.30 A.M.—The Euglenæ began to move towards the light, and an aggregation became visible.
11.31 A.M.—Aggregation clear: green streaks with light green area.
11.32 A.M.—Irregular network-like grouping, spreading out towards the light.
11.33 A.M.—Aggregation a quarter of the way across the vessel.
11.34 A.M.—Aggregation about one-third the way across.
11.36 A.M.—Aggregation nearly half-way across. Groups began to disappear on that side of the vessel away from the light.
11.38 A.M.—Groups in the middle third of the vessel only in the form of streaks or ripples transverse to the direction of the light rays and moving rapidly towards the light (Plate 35, fig. 41).
11.40 A.M.—Ripples now in the half of the vessel nearest the light.
11.43 A.M.—Ripples very near the edge nearest the light.
11.45 A.M.—Accumulation of Euglenæ at edge of vessel in dense green layer: the ripples nearly gone.
11.47 A.M.—Aggregation entirely disappeared.

If a long narrow tube is used instead of a cell a similar effect is produced. The Euglenæ must first of all be brought to one end of the tube, either by placing it upright in the dark, or by exposing one end of it to the light. It is then placed horizontally with the end in which the Euglenæ are massed together away from the light. The Euglenæ at once begin to move towards the light, and at the same time a downward streaming movement is observed which soon results in the production of the characteristic aggregations.

In the normal aggregations which take place in the dark, or in a dull light which falls in a vertical direction, two distinct movements are to be seen, one downwards, the other upwards (Plate 36, fig. 52, and text-fig. 2, *a*). If the light is strong the downward movement may disappear entirely and the organisms accumulate at the surface

In the aggregations which take place when the light falls obliquely three distinct directions of movement can be seen, (1) a movement downwards in the denser part of an aggregation or ripple, (2) a slanting, more or less irregular movement in the direction of the light, and (3) a movement along the surface horizontally towards the light (text-fig. 2, *b*). In a weak light the movements (2) and (3) towards the light are not so pronounced as in a stronger light, and the aggregation is consequently more distinct. In a strong light the downward movement (1) may be very slight or may disappear entirely, in which case no aggregation would be visible ; the Euglenæ would accumulate at the surface and move briskly along it in the direction of the light, or under certain conditions, if the light were strong enough, away from it. In other words the aggregations depend upon conditions which favour the possibility of a downward movement. Thus if a vessel in which a ripple-like aggregation is taking place is shaded by interposing a sheet of white paper or a piece of green glass, the downward movement becomes more strongly marked, and the ripples become clearer (Plate 35, fig. 42). If the vessel is covered with a sheet of cardboard, so as to exclude the light altogether, the downward movement becomes still more pronounced; the ripples soon disappear, and in their place is produced the ordinary network or group aggregation.



TEXT-FIG. 2.—Diagrams showing the Direction of the Movements of *Euglena viridis* during Aggregation in the Light. *a*, when the light is vertical; *b*, when the light is oblique. The arrows above the figures show the direction of the light rays.

The ripple-like aggregation appears to be, therefore, the resultant of the tendency of Euglenæ when crowded together, to fall through the water, and their attraction by the light, which tends to keep them at the surface. This is illustrated diagrammatically in text-fig. 2, *b*. As the Euglenæ in the densest part of the ripple sink in the water, their place is taken by others, partly by attraction along the surface of the water, partly by attraction from below, upwards towards the light. In this way the persistence of the ripples is maintained all the time the Euglenæ are moving across the surface towards the light.

If the reaction of the Euglenæ to a strong light is negative they may show no tendency to aggregation, but between the two extremes of strong attraction and repulsion there is a stage at which they are neither strongly attracted nor repelled, and in this condition they may exhibit a very pronounced aggregation. The following experiments illustrate this. A number of motile Euglenæ were placed in a shallow vessel in the window in a dull light. They at first sank to the bottom of the vessel, but soon rose in cloudy aggregations, and gradually accumulated on that side nearest the light. As the light increased in intensity, however, a large number of them were repelled and accumulated at the bottom on the opposite side of the vessel. About an equal number remained on the light side. Between these two there gradually appeared about half-way across a circular patch of cells in which a beautiful network-like aggregation was produced. Experiments made with some of these cells in a drop of water on a glass slip showed that they were almost indifferent in their reaction towards the light, but with a slight tendency to attraction. In the absence of any very pronounced light reaction, therefore, Euglenæ, although exposed to light, are capable of an aggregation similar to that which occurs when light is excluded.

If a large number of Euglenæ is present in the water contained either in a closed or open vessel, and they are allowed to accumulate in a dense layer at the edge of the vessel, a number of falling streams will, after a time, be observed to start from those regions where the Euglenæ are densest, and to pass downwards in close contact with the side of the vessel. As they reach the bottom they spread out, and soon begin to move upwards again towards the source of light, and into the marginal layer, from which a constant downward streaming is now taking place. A regular cycle of movement is thus kept up, and the Euglenæ are prevented accumulating in such a dense mass as would interfere with the effect of light in promoting assimilation.

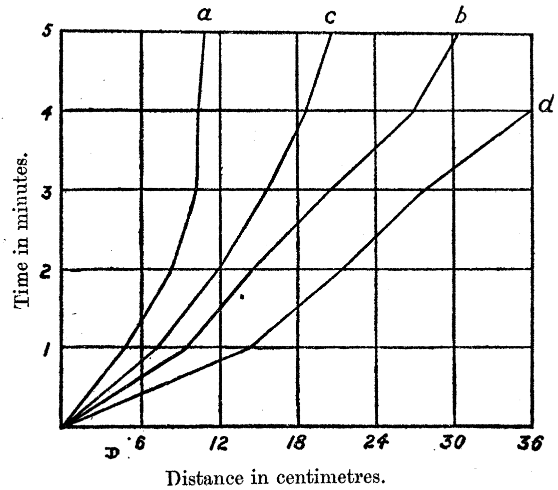
Here it will be observed that the downward movement does not begin until the Euglenæ have accumulated in the strong light, and that when the Euglenæ reach the deeper layers of the liquid, where the light is less intense, they begin to move upwards again. It therefore seemed to me that, in this case, the downward movement might be due to a repulsive action exerted by a too intense light, especially as it only takes place after the light has been acting for some time and is usually more pronounced towards the middle of the day, when the light is strongest. I found, however, that if only a few Euglenæ are present, no such streaming is produced, that if a number of these Euglenæ were placed in a drop of water on a microscope slide, and exposed to the light, there was no indication of repulsion, that the streaming movement is only set up when the Euglenæ are much crowded together, and that this takes place whether the light is strong or weak. Further, the downward movement is increased if the vessel is shaded, and it becomes very pronounced if light is entirely excluded, or if a sheet of red glass is interposed between the vessel and the light.

Whether the downward streaming movement shall take place or not seems to depend, therefore, not upon the repulsive action exerted by the intense light, but simply upon the accumulation of a sufficiently dense layer of the Euglenæ. Moreover, since the streaming is intensified when the light is reduced, and still more when the light is altogether excluded, it suggests the explanation that the thick layer of Euglenæ may act as a shade for those individuals that are not directly on the surface, and may thus bring about the condition necessary for aggregation, viz., a reduction in the light which is allowed to act upon them, similar to the effect produced when a sheet of green glass is interposed. In other words, so long as the individual Euglenæ are in such a position that they can be acted upon by light, no streaming takes place. But as soon as they become so congested that a certain number of them are obscured or placed in the shade by the others, they are no longer attracted or held up by the light, and a downward streaming at once begins. It appears to be, in fact, a manifestation of precisely the same phenomenon as occurs in the process of aggregation in closed tubes and cells in the dark.

The following experiment illustrates this clearly : A small number of motile cells in a white saucer is placed in a window. In a short time they will all be attracted to the light side, and in the form of a distinct green layer at the edge of the water. As the number is small, however, all of them remain exposed to the light, no shading effect is produced, and no streaming takes place. If the saucer is now covered with a piece of cardboard so as to exclude the light, there will be formed, in about 30 seconds, a fringe of falling streams. This only takes place, however, if the organisms remain motile. If the light is very strong, or if they have been exposed to a moderate light too long, they become rounded off and form a scum which remains closely attached to the edge of the saucer and does not sink when placed in the dark. Consequently the phenomenon does not take place so readily in the summer as in the spring.

The shading effect produced by a layer of closely packed Euglena cells can also be shown in another way. A test tube, filled with water containing a large number of Euglenæ distributed evenly through it, is tightly corked and placed upright in a good light. A large number are at once attracted to that side of the tube nearest to the light, and there form a dense layer through which only a little light can pass. The remainder of the Euglenæ being thus placed in obscurity fall to the bottom of the tube just as if they were in the dark. If there are fewer Euglenæ in the tube they all accumulate on the side of the tube, in the light, with a tendency in the course of a day or two to congregate more towards the top and bottom of the tube than in the middle. If such a tube is placed in the dark, while the Euglenæ are in the motile condition, they at once fall to the bottom.

The effect of light of different strengths upon the fall of Euglenæ can be shown very clearly by the following experiments : If a long tube in which the Euglenæ are aggregated at one end is placed upright in the dark with the Euglenæ upwards they at once begin to fall in a dense stream just as a precipitate would do under like conditions. The rate of fall can be measured. If the same tube, when the Euglenæ have all fallen to the bottom, is turned upside down in the light the Euglenæ begin to fall as before, but in a very short time they curve towards the light side of the tube ; the downward movement becomes slower and slower, and finally ceases altogether, and the Euglenæ come to rest on the side of the tube. In diffuse light the Euglenæ are not brought to rest so soon, and the downward movement is not so strongly retarded; and in very diffuse light or in gaslight they may fall quite to the bottom of the tube, but at a slower rate than in the dark. In red light the motile Euglenæ fall as in the dark and at about the same rate. If a few drops of osmic acid solution be added to the tube the Euglenæ are killed in the expanded condition, and it is then found that they fall at about the same rate, or a little faster, than do the motile forms in the dark. This seems to show that the fall of living Euglenæ in the dark is very little, if at all, dependent upon or accelerated by their own movements. The variation in the rate of fall can be plotted, and the results of a typical series of measurements are shown in text-fig. 3.



TEXT-FIG. 3.—Diagram showing the Rate of Fall of *Euglena viridis* in an upright Glass Tube. *a*, in bright light; *b*, in darkness; *c*, in diffuse light; *d*, when killed in osmic acid solution.

Another interesting experiment to show the effect of light of different strengths upon the fall of Euglenæ is the following: A tube in which the organisms have accumulated at one end is placed upright in a good light with the Euglenæ uppermost. They at once begin to fall, but as shown in the previous experiment, they are attracted by the light and soon come to rest on the side of the tube in the light. If the tube is now turned about its longitudinal axis, whilst still in the upright position, through 180°, the Euglenæ will at once begin to move back again towards the light, but in so doing they will fall a short distance through the water, so that when they reach the other side they will be in a position a little lower than they were before. If the tube is again turned round as before they will fall a little more, and the process may be repeated until they have all reached the bottom of the tube. The number of times the tube has to be turned round before they reach the bottom is a rough measure of the intensity of the light. If the light is very strong the downward movement is slight, and the number of steps downwards will be correspondingly large. If the light is weak the downward movement will be more pronounced; the Euglenæ will fall a longer distance during each passage across the tube, and the number of steps downwards will be less.

The general conclusion as to the effect of light arrived at from these experiments is that, whilst the light tends to inhibit the aggregation, traces of it can still be observed if the Euglenæ are free to move, and are sufficiently congested for gravity to act upon them, and that a streaming movement is at once set up in a congested mass of Euglenæ so soon as they are sufficiently dense to partially shade a proportion of them,

2. *The Effect of Temperature Changes.*

The general effect of a rise in temperature within certain limits is to increase the activity of Euglenæ; at a lower temperature they are less active—at 2°–3° C. their movements are very sluggish, and at 1° C. they practically cease, except for a very slight quivering motion. At the freezing point they become quiescent, and tend to round themselves off. They can be frozen up completely in ice, however, without coming to any harm, and when the ice melts begin to swim about freely again as soon as a temperature of 3°–5° C. is reached. If exposed to still lower temperatures, −2° and −5° C., they do not revive so quickly, and some more slowly than others. At a temperature of −9° many of them are killed.

Teodoresco (1909) has shown that the movements of various micro-organisms are maintained at temperatures below the freezing point of water if solidification of the water is prevented. Thus *Euglena viridis* remains in a motile condition at as low a temperature as −10° C. In one experiment the movements continued for 45 minutes between −4°·8 C. and −11°·8 C.; at a temperature of −11°·8 C., however, the cells showed only metabolic movements. Similar results were obtained with other organisms. Among the many organisms studied it was found that *Euglena pisciformis* remained motile at −7°·7 C., *Peridinium tabulatum* at −8°·8 C., *Chlamydomonas pertyi* at −10°·7 C., and *Hæmatoccus pluvialis* at −12°·6 C. In species of Dunaliella, which live in salt water, the movements ceased totally only between −17° C. and −22°·5 C. In all these cases the movements become feebler as the temperature is reduced, and generally a few individuals only remain motile; many pass into a condition of rigidity and a certain number is killed.

I have not been able to make any observations upon the resistance of encysted cells to reduced temperatures, but it is probable that in this condition they may withstand a very low temperature.

At normal temperatures Euglenæ respond freely to light; even at temperatures as low as 3°–5° C. the response is very distinct, but at 2° C., although they are still motile, they do not respond to light. The effect of a reduced temperature upon their aggregation is very striking. Motile cells put, in a horizontal tube or flat cell, in the dark at a temperature of 2° C. at once sank to the bottom; there was a slight aggregation visible at the end of half-an-hour, but this was not very definite, and at a still lower temperature there was no aggregation at all. On increasing the temperature to 3° C. a more pronounced aggregation was visible, which was very distinct at 5° C., but on lowering the temperature again the aggregation became more irregular and again disappeared. On alternately raising and lowering the temperature between 5° C. and 1° C. the aggregation appeared and disappeared.

The tendency to aggregate varies therefore with the motility of the organism. When it is quiescent, as at a temperature of 1° C., there is no aggregation at all, but it begins as soon as the organism begins to move, however slowly, as occurs at 2° C. So long as even a slight movement is maintained, visible only as a gentle quivering of the cell, the Euglenæ exhibited an aggregation into groups or a network at the bottom of the cell or tube, provided they were sufficiently crowded together.

It appears therefore that a reduced temperature tends to inhibit the aggregation by reducing the motility of the organism. The upward movement is stopped, and they all sink to the bottom. This may possibly explain Miss MOORE's observations on Paramœcia. She finds that at a low temperature, 1° C., the positive geotropism of these organisms is very marked, whilst within certain limits negative geotropism is induced by a higher temperature.

So long as oxygen (air) is absent the cells remain in an expanded condition, even at as low a temperature as 1° C., but if they come into contact with air at the surface of the water, either in the dark or in the light, they tend to contract and become rounded off.

At the ordinary summer temperature, 60°–70° F. (15°·5–21°·1 C.), the Euglenæ are very active, and in the dark very quickly aggregate. At a temperature of 30° C., however, their movements become sluggish, the aggregation tends to disappear, and the cells become contracted and rounded off; at 35° C. many of them are killed. If one end of a tube containing Euglena is at a higher temperature than the other the aggregation is still visible, but a convection current is set up which causes a movement, on the lower side of the tube, towards the heat, and, on the upper side of the tube, away from it. If the temperature is not too high, 18° C. or 19° C., at one end, and 10° C. at the other, the aggregation remains visible for some time, but the separate groups are bent slightly out of the perpendicular in the direction of the warm end of the tube. A certain number of the motile cells are carried along by the convection current. If the temperature is raised above 20° C. at the warmer end of the tube the Euglenæ tend to become rounded off and the aggregation disappears. The cells then sink to the lower side of the tube and are carried along in the convection current and accumulate in a dense green layer at the hot end of the tube. This accumulation seems to depend entirely upon convection currents, and not, as stated by FRANZÉ ('93) and WILDEMAN ('94), to an attraction exerted upon the Euglenæ by heat. FRANZÉ pointed out that in a thin glass tube, one end of which was at a temperature of 55° C., the Euglenæ became immobile from the effects of heat, but at a lower temperature, 30°–40° C., they mostly approached the source of heat. This is quite in accordance with the results obtained by me, but which, as I have shown, are due to convection currents, acting upon rounded off cells.

WILDEMAN placed Euglenæ in wet sand to avoid convection currents, and found that they accumulated at the warm end of the tube at 30° C. This, as I have pointed out, is a temperature at which they become rounded off. On repeating WILDEMAN's experiment I could not get any definite evidence that there is such an attraction even at 30° C., provided the sand is not too wet. If too much water is present and the tube is placed horizontally the sand sinks to the lower side, leaving a layer of water on the upper side, in which the Euglenæ accumulate, and in which convection currents can be set up. I filled a tube 5 inches long by $\frac{1}{2}$ inch broad with rather stiff mud containing motile Euglenæ diffused through it. On placing this horizontally in the dark with one end at a temperature of 30° C., the other at 12° C., the Euglenæ came to the upper side of the mud, and formed a green layer all along the upper surface of the tube, but there was no special accumulation at the warmer end.

We find, therefore, that so long as the temperature is not high enough to destroy the motility of Euglena, the aggregation is not inhibited. When the heat is at one end of a horizontal tube the streams are, as we have seen, bent out of the perpendicular (if the heat is strong enough), and the Euglenæ gradually move, still in the condition of aggregation, towards the warmer end of the tube. The *continued* effect of the higher temperature, 20° C. or more, which is necessary for this, tends, however, to destroy the motility of the cells by rounding them off; the aggregation disappears and the Euglenæ accumulate in a dense green layer at the hot end of the tube on its lower surface.

Both Glenodinium and Chlamydomonas are thermotactic. In a tube 30 cm. long, placed horizontally with one end at a temperature of 22° C., the other at a temperature of 17° C., the Chlamydomonas accumulated at the warmer end of the tube, the Glenodinium at the other. In a mixture of the two organisms, it was possible to separate them from one another in this way. The Chlamydomonas collected on the lower side of the tube at the warmer end, and showed no aggregation : the Glenodinium aggregated into two or three irregular groups at the cooler end of the tube.

The same results were obtained when the temperatures were 18° C. and 12° C., or 17° C. and 11° C.

In an upright tube, with the upper end at a slightly higher temperature than the lower end, the Chlamydomonas rose in the water and accumulated at the warmer end of the tube, whilst Glenodinium sank to the bottom or cooler end.

3. *Temperature Changes are not the Cause of the Aggregation.*

To what extent the aggregation of Euglena may be due to heat will now be considered. The conditions under which the experiments were made when the aggregation was most clearly defined seemed to preclude the existence of convection currents, either strong enough to maintain the Euglenæ in such a constant movement, or regular enough to cause so definite an arrangement into groups. SACHS, however, considered the groupings of swarm spores which he observed ('76) to be due entirely to currents set up in the water by differences of temperature. In his paper he does not figure any of the appearances seen, but compares them to the emulsion figures obtained when minute drops of oil are allowed to settle in a mixture of alcohol and water, or, if the solution allows them to float, the figures formed at the surface, of of which he gives six illustrations in Plate 10.

I have repeated SACHS' oil emulsion experiments, and find that, whilst the figures produced resemble in some respects the aggregations of living organisms, they are not produced under the same conditions, and do not admit of the explanation given by SACHS.

A mixture of alcohol and water (35 per cent. alcohol) is made in which olive oil will just float. A small quantity of oil, coloured black with osmic acid or red by alkanin, is poured into it. The mixture is then shaken up until a fine emulsion is obtained, and this is poured quickly into a shallow cell. The oil particles at once begin to rise to the surface of the liquid, and for a few seconds remain evenly distributed, but as soon as they reach the surface, where evaporation of the alcohol is taking place, a network-like aggregation is at once produced. This is formed entirely at the surface, and does not extend into the deeper parts of the liquid.

These reticulations are more regular and perfect when the vessel in which they are formed is kept at an equable temperature all round, where, in fact, it is not possible, even by the use of extremely minute and light particles, to obtain any indication of convection currents in a vessel of water placed in the same position.

The application of heat to one side of the vessel causes a more rapid movement of the oil particles, and, if the heat is sufficient, a convection current is set up, which produces a complete modification in the aggregation, resulting in the appearance of polar figures (*cf.* DETMER, 1898) which do not at all resemble the aggregation figures of the living organisms.

If the evaporation is checked by covering the dish with a glass plate, so that there is a very small air space between it and the surface of the alcohol mixture, the aggregation is not so clear, and may be prevented altogether if the evaporation is reduced to a minimum.

It is quite clear from these experiments, therefore, that the aggregation of the oil particles is not so much due to currents set up by changes of temperature, which probably play but a small part, as to surface tension changes and diffusion currents (*cf.* PFEFFER, 1906, p. 324, and ERRERA, 1907, p. 50). But neither of these explanations is sufficient to account for the aggregations described in this paper. In the case of Euglena, the aggregation takes place when the environment is such that the vessel is at an equable temperature all round, and I have not found that heat on one side of it makes any appreciable difference on the aggregation until it is sufficient to set up diffusion currents, and then the aggregation tends to disappear. Nor is evaporation at the surface of the liquid a possible explanation; for the aggregation of Euglena is more pronounced and perfect in a sealed up cell, in which no evaporation is possible, than in an open one.

If the aggregation were due to currents in the water set up by heat, we ought to get some indication of these currents when particles of a precipitate are slowly sinking or in suspension in the water. If a finely divided precipitate, such as osmic dioxide, manganese dioxide, or sediments such as starch grains and dead cells of Euglena or Chlamydomonas are thoroughly shaken up, so as to become equably disseminated through the water, they settle down slowly in straight lines without any signs of aggregation. Even with some very small dead cells of a green organism which remain suspended in the water for hours, and even days, no aggregation was obtained, and STRASBURGER ('78) found the same with minute particles of amorphous Boron, which remained suspended in water for a month. In all these cases, if currents are set up by warming one side of the vessel sufficiently strongly, the particles tend gradually to accumulate at the bottom of the vessel on the warm side, but with no definite aggregation into networks or groups.

EXPLANATION OF THE AGGREGATION.

We have already seen that the cause of the peculiar aggregation with which we have been concerned is not explainable as due to currents set up in the water by the unequal distribution of heat or by evaporation from the surface. Nor, as we shall see later, is the phenomenon the result of the purposeful movements of the organisms themselves, but appears to be due mainly to the action of gravity combined with the effects of cohesion phenomena, which are brought into play under the conditions of the aggregation. The explanation offered is based mainly upon the observations which have been made on the aggregation of *Euglena viridis.* Two distinct phenomena have to be accounted for: (1) the cyclic up and down movements, and (2) the attraction of the motile organisms into networks and groups. Both are concerned in the aggregation, but (2) is dependent on (1), and both are primarily dependent upon (*a*) the obvious fact that Euglena is capable of autonomous movements, and (*b*), which is perhaps not so obvious, that it is distinctly heavier than water. The first problem we have to consider, therefore, is the density of Euglena.

1. *The Density of* Euglena viridis.

If a tube about half an inch in diameter is filled with water containing a sufficient number of Euglenæ to give it a distinct green colour, and placed upright in the dark, the majority of the organisms will, in the course of a few minutes, sink in the water and form a dense green mass at the bottom of the tube. On turning the tube upside down, still keeping it in the dark, the green mass of motile cells will begin to sink again, and the downward movement is so regular that the rate of fall can be easily observed. Experiments show that this varies with the number of Euglenæ in the falling mass, and if comparisons are made with the same or similar quantities of dead Euglenæ which have been killed in the elongate condition, either by osmic acid or heat, it will be found that the rate of fall of the living cells is practically the same as that of the dead cells. This indicates that the specific gravity of living Euglenæ is greater than water, and that it varies very little from that of dead Euglenæ when observed under similar conditions. The experiments can be very easily made in a tube about 15 cm. long and 7 or 8 mm. diameter, to which a centimetre scale is attached and the results plotted on squared paper (text-fig. 3).

My first attempts to determine the actual specific gravity of Euglena were made with solutions of common salt. I found that they floated at the surface of a solution of a density of 1·025 to 1·030, but sank in solutions of a density below this, thus indicating a specific gravity of about 1·025. This does not agree with the result obtained for Paramœcium by JENSEN ('93, (2)), who used solution of potassium carbonate, and found that Paramœcia just floated in a solution of a density of 1·25. DEVONPORT considered this method a bad one, however, because the potassium carbonate acts so powerfully in withdrawing water and causing the organism to shrink, thus increasing its relative weight. This objection also holds good for solutions of common salt, which cause a considerable contraction and distortion of the Euglenæ. I therefore made another series of experiments with solutions of gum arabic, which, as DEVONPORT ('97) points out, have so slight an osmotic action that micro-organisms live in them for hours.

I found that in solutions of gum arabic whose density is as great as 1·025 Euglenæ will remain living for many days. The first effect of the gum arabic is to paralyse them; but they recover from this in a short time and become actively motile again. Solutions with densities varying from 1·012 to 1·025 were used, and the experiments were conducted in narrow test tubes. A small quantity of motile Euglenæ, deprived of as much superfluous water as possible, was intimately mixed with a few drops of the solution in which they were to be tested, and the mixture was at once gently delivered on to the surface of the solution in the tube. The Euglenæ, although paralysed, remain in the expanded condition for some time.

In solutions of a density below 1·014 they at once begin to sink, and the majority soon reach the bottom of the tube, leaving very few floating about in the upper layers of the solution. In solutions of density 1·014 to 1·015, they also begin to sink at once, but fall more slowly, and a large number remain for a longer time floating about near the surface. In a solution of specific gravity 1·016 very few of the Euglenæ begin to fall at once; the majority form a distinct green layer at the surface, which persists for some time, but ultimately begins to sink. In solutions of specific gravity 1·017 and 1·018 the majority float at the surface, a very small number sink at once, whilst in a 1·019 and stronger solutions they all, so far as could be observed with the naked eye or a pocket lens, remained floating at the surface in a green layer clearly marked off from the underlying liquid.

The specific gravity of living Euglenæ therefore appears to lie between 1·013 and 1·019, but as the majority float at the surface of a solution whose density is 1·016, and sink in a solution of a density of 1·015, we may conclude that the density of the majority of these organisms is about 1·016. This agrees on the whole with the figures given by Miss PLATT for living Paramœcia and Spirostoma, which neither sank nor rose when the specific gravity was between 1·016 and 1·019: the specific gravity lying, therefore, probably near 1·017. (DAVENPORT, '97.)

The density appears to vary slightly, however, during the day. Experiments made in the afternoon upon the same culture of Euglena gave results which indicated a density rather lower, 1·013–1·015, than those experimented with in the morning. It seems quite reasonable to suppose that the density of an organism whose metabolic activity is so varied as that of Euglena may differ according to the conditions of its food supply. This is a problem which may be worth careful investigation. In the case of the colourless forms of *Euglena viridis*, for example, although I had no opportunity to determine their density, they appear to be considerably heavier than the ordinary forms, probably owing to the much larger quantity of food material in the shape of paramylum grains which they contain. OSTWALD has pointed out also (quoted by LOEB, '06) that with increasing temperature the internal friction of the water diminishes rapidly, in consequence of which organisms would tend to sink more easily or would be prevented moving upwards.

Dead cells which had been killed in the elongate condition in warm water or osmic acid and kept for some time in iodine solution gave a slightly lower density than the living cells, about 1·014, but specimens killed in a 1-per-cent. solution of potassium bichromate had a density between 1·014 and 1·016. Cells which had died naturally in some of my cultures and had sunk to the bottom of the vessel in a rounded off condition sank at once in ordinary tap water but remained floating at the surface of a solutions of a density of 1·010 to 1·012, thus indicating a density much lower than that of the ordinary cells.

From some rough experiments made with other organisms the density appears to be very variable. A small species of Chlamydomonas, for example, was very slightly heavier than water and remained floating at the surface of a gum solution with a density of 1·005.

2. *The Action of Gravity.*

It is a well-known fact that Euglena, in common with many other micro-organisms, exhibits an active orientation and movement under the influence of gravity, by which it is caused to move in a definite direction. These phenomena have been studied by various observers, especially SCHWARZ ('84), ADERHOLD ('88), VERWORN ('89), MASSART ('91), JENSEN ('93), JENNINGS ('97), DAVENPORT ('97), SOSNOWSKI ('99), PARKER ('01), MOORE ('03), LYON ('05), and ESTERLEY ('07).

If a mixture of motile Euglenæ and mud are shaken up and then allowed to settle in water, the Euglenæ after a short time, even in the dark, come to the surface of the mud and form a green scum upon it.

SCHWARZ ('84), who investigated this, found that the Euglenæ were not carried upwards by currents in the water, but that it is entirely due to their own locomotion. Hence he concludes that the phenomenon is a geotropic one and the Euglenæ must be regarded as negatively geotactic organisms. SCHWARZ also stated that when the

Euglenæ were free to move in water, they acted as in the mud or sand, that is, they moved upwards to the surface of the water. Therefore he concluded, after eliminating by experiment various possible sources of the causes of the movement, such as low specific gravity of the organism, currents in the water or convection currents, presence of the oxygen at the surface, that the organisms, even in the water, move against the attraction of gravity and are therefore negatively geotactic. He also found that the attractive force of gravity can be replaced by centrifugal force, and that when this was below 8·5 *g* the organisms moved in opposition to it, a further proof that they exhibit negatively geotactic phenomena.

ADERHOLD ('88) also, in studying Euglena, came to similar conclusions, but pointed out that the organism is strongly positively aerotropic.

MASSART ('91) found that various unicellular organisms are geotactic, bacteria, Ciliata and others, and that individuals of the same genus but of closely allied species may be either positively or negatively geotactic. Two species of Spirillum gave different geotactic reactions, one being positive, the other negative. In the case of *Chromulina woroniniana* the geotactic phenomena appear to depend upon the temperature; at 5° to 7° C. they were positively geotactic, at 15° to 25° C. they were negatively geotactic.

Miss MOORE ('03) states that the geotaxis of Paramœcium varies with the temperature and food supply. Positive geotaxis is brought about by mechanical shock, such as shaking the tube in which they are contained, reduced temperature (very marked at 1° C.), increase in concentration of surrounding medium (not so constant as other factors, however, and not so well marked), and lack of food. Negative geotaxis may be induced by a plentiful supply of food and by an increase of temperature within certain limits. She points out that these variations in geotactic response are of use to the Paramœcia in that they are carried downwards away from the surface of the liquid, under conditions which would be unfavourable to them, such as the formation of ice, surface agitations and failure of surface food supply.

ESTERLEY ('07) points out that in the case of Cyclops, both light and gravity are responsible for the diurnal movements of these organisms. Females of *Cyclops albidus* are normally positively geotactic. In a tall cylinder they all, as a rule, fall to the bottom. A certain number make excursions to the top and then fall again, but it is rare to find the animals anywhere, except in a section two or three centimetres deep at the bottom of the vessel. On exposure to light they tend to become negatively geotactic in darkness. Negatively geotactic specimens of Cyclops become positively geotactic again on exposure to light, even if exposed to such intense illumination from below that if the light were acting alone, they would be negative to it.

Various explanations have been given of these phenomena of geotaxis. VERWORN ('89, p. 122) came to the conclusion that the orientation of a flagellate must be a purely mechanical phenomenon due to the action of gravity. He gave this up later, however, in favour of the pressure stimulus hypothesis put forward by JENSEN.

From his experiments with various organisms under all sorts of different conditions, JENSEN ('93) came to the conclusion that a mechanical explanation will not suffice. He states that in the upward movement of Euglena, its orientation is exactly opposite to what would be the case if gravity acted mechanically. He considers that the mechanical action of gravity would bring it into a vertical position with its anterior end downwards. The explanation he offers is that the reaction is due to a stimulus brought about by the difference in pressure of the water at different levels acting upon the organism and causing it to move upwards constantly to a region of lower pressure.

DAVENPORT ('97, I, p. 122) put forward the hypothesis that the stimulus is due to the resistance of the water. The organism, being heavier than water, experiences greater resistance in going upwards than in going downwards. RADL (quoted by JENNINGS, '06, p. 77) has pointed out, however, that it is not apparent how, under the uniform action of gravity, any such difference of resistance could be perceived.

LYON ('05) found that living Paramœcia are precipitated by the centrifugal machine, anterior end first. He concludes, therefore, that negative geotaxis cannot be mechanical, but must be an active process on the part of the organism. He does not accept JENSEN'S pressure theory. Geotaxis is directly dependent on gravity and not on pressure. Paramœcia are geotropic in solutions of the same density as themselves, in which, therefore, the resistance is the same going up or down. By the use of gum solutions and the centrifugal machine, he finds that the specific gravity of Paramœcia varies from 1·042 to 1·054, or an average of about 1·048 to 1·049. He concludes that gravity must act directly on the inner constitution of the cell, involving pressures or stresses within, which could only come about in a system with substances of different densities. Such differences actually exist in the body of Paramœcium. The theory which best explains the facts is, therefore, practically the same as the statocyst theory developed for higher animals, except that instead of a complicated sense organ and reflexes, we have the whole mechanism of stimulation and response in a single cell.

Whether we accept LYON'S explanation or not, it seems to be quite clear from his observations, and others previously quoted, that neither JENSEN'S "perception of pressure" hypothesis nor DAVENPORT'S "reaction to resistance" hypothesis is necessary to account for the facts observed. All that we can conclude from these investigations is, that the effect of gravity brings about a movement of motile organisms either upwards or downwards, that, as LOEB ('06) points out, the precise nature of the determining factor in the reaction is very obscure, but that the direction of the movement varies under the varying conditions of temperature (MASSART, MOORE), food supply (MOORE), mechanical shock (MOORE), and light (ESTERLEY).

To what extent the aggregation of Euglena and other micro-organisms described in this paper may be due to gravity has now to be considered. The upward and downward movements which take place during such an aggregation differ from the normal geotactic movements described by the observers just quoted, in that the downward movement is immediately followed by an upward movement, and that a constant cycle is thus kept up, which may persist, as we have seen, for several days. As the organisms are capable of movement in any direction under certain conditions, their movements in any given direction or directions must be brought about by some definite controlling force.

In the case of Euglenæ, for example, examination under the microscope shows that the downward movement is evidently not due to their own active exertions. They are carried down in a stream, in opposition to their normal direction of movement, in a vertical or slightly oblique position. As soon as they reach the bottom they at once change their direction of movement and swim out laterally away from the downward stream, but are almost immediately oriented again into a vertical position, and, although they are now able to use their flagella effectively, they are kept in this position and are compelled to move upwards.

That these movements are due to the action of gravity, there seems to be no doubt, but whether they can be described as geotactic, that is, whether the organisms possess some sensitivity associated with geotropism, appears very problematical (JOST, '07), and demands further consideration.

3. *The Orientation of Euglena under the Influence of Gravity.*

In discussing the orientation of a motile organism under the influence of gravity we must first consider the purely mechanical possibilities due to its density, its structure and shape, and the position of its centre of gravity. If it is heavier than the liquid in which it lives; if the position which it takes up in moving downwards is the same as that which it assumes when dead; and if this is also in opposition to its normal direction of movement, it is fair to conclude that the movement is a purely mechanical one and not geotactic. As LOEB points out ('06, p. 149), one must be careful not to mistake the passive sinking or rising of such forms for geotropic reaction.

In a tropic or tactic* stimulation the orientation must be due to two forces, one external, such as gravity, heat, light, etc., the other internal, by which the movement is brought about. Tropic movements are distinguished from ordinary mechanical phenomena of orientation (such as falling bodies) in that they are always accompanied by the exercise of some internal force.

Light, for example, exerts a very marked tropic stimulation upon Euglena, which results in a definite orientation and movement of the organism. This appears to

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* I use these terms simply as convenient ones to denote the reaction to a stimulus by some internal change in an organism, and not as implying any special tropism theory.

depend upon a responsive action of the motor apparatus. **Engelmann** has shown that it does not take place until the light strikes upon the colourless anterior end of the organism, and I have shown ('99) that it appears to be due to the absorption of light by the eye-spot which is in close contact with a swelling upon the flagellum.

The reaction of Euglena to gravity does not appear to be of this nature. There is no evidence that the orientation produced is caused by a gravitational stimulus which sets up a responsive action on the part of Euglena; it appears to be a purely mechanical result of the action of gravity upon an organism heavier than water, and therefore capable of sinking naturally when from any cause its own active movements are not sufficiently powerful to prevent it. (*Cf.* **Strasburger**, '78, p. 558).

In determining this question much of course depends upon the behaviour of dead Euglenæ when sinking through the water in response to gravity. The observations which have been made upon this point are conflicting. **Schwarz** ('84) states that they take up no definite position in falling, whilst **Jensen** ('93) states that when killed in the elongate condition Euglena invariably falls with its anterior end downwards. My own experiments, on the other hand, show that when killed by warm water, osmic acid, or iodine solution, Euglena almost invariably falls in a vertical or slightly oblique position, with its posterior end downwards. This is what one would naturally expect from its structure, for, although it is pointed at its posterior end whilst its anterior end is rounded or slightly flattened, and therefore slightly larger, the density of the middle and posterior regions is probably greater, through the inclusion of them of the chlorophyll bodies, paramylum grains and nucleus, than the anterior end, which contains a vacuolar space—the pharyngeal cavity—and a pulsating vacuole.

If the Euglenæ are in a dense mass, they may assume all possible positions in falling, but this is due to their being entangled with one another; this is especially the case when they have been kept for a long time in the fixing fluid. When precautions are taken to prevent overcrowding and entanglement, it is rare to find an individual which falls with its anterior end downwards. The orientation is, in fact, precisely similar to that observed in the downward moving streams of the living cells during aggregation, and is in opposition to their normal direction of movement. We thus see that the conditions for a purely mechanical explanation of the downward movement are fulfilled, and that an appeal to geotaxis is unnecessary. The following experiment supports this conclusion. A horizontal tube in which Euglenæ were aggregating, as shown in fig. 3, *a*, was gently moved into a slanting position so that the streams were at an angle to the direction of the gravitational force. The streams at once became bent into a vertical or nearly vertical direction (Plate 33, fig. 19).

The effect of gravity is observed only under certain conditions. If they are not crowded together, and in the absence of light and oxygen, the Euglenæ do not appear to be constrained to move in any particular direction; they swim about freely in the fluid in all directions, but with a tendency to move upwards. They keep themselves from sinking by their own active exertions.

If we observe Euglena carefully under the microscope we shall see that it always swims in the direction of its longitudinal axis along a more or less spiral path. Owing to its weight being greater than that of water, and the posterior end heavier than the anterior end, gravity tends to bring it into a vertical position. By its own active exertions it is able to resist this gravitational pull, but if for any reason its movements are interfered with or become slower, the anterior end at once swerves upwards, and the organism becomes oriented into a more vertical position. As JENNINGS points out ('06, p. 112), "when stimulated by coming in contact with a weak chemical, by a mechanical shock, or by a change in the intensity of light, Euglena responds by an avoiding reaction similar to that of Paramœcium and Chilomonas. The forward motion becomes slower, ceases, or (more rarely) is transformed into a backward motion. Then the organism swerves more strongly than usual towards the larger lip. Thus the spiral becomes wider, and the organism becomes pointed successively in many directions. In one of these directions it finally swims forward, repeating the reaction if again stimulated." The strong swerving of the organism, and the consequent widening of the spiral, are no doubt caused partly by the downward pull exerted by gravity. This can be very easily seen if Euglenæ which are swimming more or less in a horizontal direction towards a source of light are suddenly shaded by bringing some opaque body between them and the light. They at once become oriented into a more vertical position with a kind of swinging motion, which is, I have no doubt, the same as that described by JENNINGS ('06, p. 136), who observed the reaction from above and not from the side.

These movements were very clearly seen in some experiments made with *Euglena deses*, a long cylindrical form which appears to be distinctly heavier at the posterior end. The specimens used had been kept in water at a temperature of 10° C. for some hours and were in a motile condition and sensitive to light. On exposing them to fairly bright daylight the majority moved slowly towards it, a few moved away from it, and some remained indifferent. On shading them by placing the hand between them and the light, those which were moving either towards or away from the light at once swerved downwards and either began to sink, if they were crowded, or to move upwards, if they were sufficiently isolated to allow of free movement. On removing the hand they at once moved back again into their original direction. If, instead of the hand or other opaque body, a piece of green glass was used, they at once swerved downwards as before, but recovered almost immediately and moved on again in the direction of the light. By interposing white paper or frosted glass between them and the light a slight downward movement only was produced and the organisms recovered their original direction of movement at once. When the temperature of the water was raised to 12° C. the Euglenæ moved more rapidly towards the light and the swerving reaction was more rapid and striking.

In all these experiments the downward swerving of the posterior end is due to gravity, which becomes effective as soon as the movements of the organism are affected by a change in the intensity of the light. Similar results are produced when the Euglenæ become crowded, especially in the dark or a diffuse light; their active movements are slowed and at once the directive force of gravity is seen. If the organisms are much crowded they become oriented into a vertical position; their freedom of motion is to a great extent lost and they begin to sink. If the crowding is not so great, they may retain their power of movement, but, being oriented into a vertical position with their anterior ends upwards, they are compelled to move in that direction. This can be shown by introducing into an upright tube full of water, by means of a pipette, a drop of water containing only a few Euglenæ. No fall will be observed. If now a drop of water containing a large number of Euglenæ be introduced, the mass at once begins to fall and continues to fall so long as any of them remain at all crowded together. A drop of water containing a large number of Euglenæ, when placed in clear water, behaves as a fluid of greater density than water would do, such, for example, as a drop of ink.

In a long upright tube containing motile Euglenæ a downward streaming can be observed to start here and there in various parts of the tube, whenever a sufficient number are congregated together; they descend for a certain distance, which depends upon the density of the stream, and then gradually disappear by the gradual spreading out and diffusion of the Euglenæ again in the water. If the tube contains a large number of Euglenæ, the streaming movements are very pronounced and result in the large majority of them being carried to the bottom of the tube, where they remain in a constantly streaming state, up and down, within a distance of from $\frac{3}{4}$ of an inch to $1\frac{1}{2}$ inches, but if only very few are present no streaming movements may be observed at all. CARL NÄGELI ('60), to whose observations I have previously referred, had already noticed this in connection with the aggregation of swarmers in shallow vessels. He states that he did not know the cause of this remarkable appearance; it was apparently not due to any movement or streaming in the water, but he suggested that it might be the result of certain hitherto unknown peculiarities of the swarm cells which depend upon the greater or less number of them present.

That the downward pull of gravity is not effective until the organisms have become congested into a heap, is beautifully demonstrated by the following experiment: A small number of motile Euglenæ are placed in a shallow cell which is completely filled with liquid and sealed up with a cover glass and vaseline. This is now placed under the low power of a microscope placed in a horizontal position. On examination, the organisms will be seen moving about freely, if the light is not too strong or if red light is used, and no special tendency to move either upwards or downwards will be seen. If a bright spot of light is concentrated upon the middle of the cell from the source of illumination, which can be most easily done by manipulating the substage condenser, the Euglenæ will be attracted by it and will in a very short time become congested in the light space. If now the condenser be moved so as to take away the bright spot, leaving the field in a diffuse light only, the congested mass will be at once acted upon by gravity and begin to fall; but this will not take place until a certain stage of congestion is reached. The minimum congestion appears to be when the cells obscure not quite half the light, or, roughly, when they cover about one third to half the area of the clear light space. When they have begun to fall their downward movement can be stopped almost at once, by bringing back the bright spot of light into a position just below the falling stream; a few will pass beyond it at first, but in a very short time the streaming will be stopped, and they will again begin to congregate in the light space.

If the light is strong, gravity may play but little part in controlling the movements; if the light is weak or absent, gravity appears to be the sole determining factor. Between these two extremes both may act together, in varying degrees, according to the intensity of the light.

We may I think conclude, therefore, that the downward streaming movement of the Euglenæ is an effect due to gravity acting upon groups or masses of cells, in which the free movement is to some extent prevented by the crowding together. These form a more or less compact mass, upon which gravity can act just in the same way as upon a mass of particles forming a precipitate. If the particles of a precipitate are closely crowded together, they sink more easily than when more widely spaced.

The upward movement which always regularly follows the downward one is caused by the active movements of the organisms themselves. As soon as the congested streaming mass of cells approaches the bottom of the vessel, it begins to spread out, and the Euglenæ become diffused in the water. The crowding becomes diminished and the result is that the Euglenæ are now once more free to move. But they are still too crowded for entire freedom of motion; their axis orientation is still more or less vertical with the anterior ends upwards; consequently they are compelled to move upwards. That they are not able to move in any other direction is due to the action of gravity. Freedom of motion in all directions is only possible when the Euglenæ are few in number and more or less isolated from each other. The force of gravity always tends to bring them into a vertical position, and anything which impedes, even to a slight degree, their movements, allows this action of gravity to be brought into play.

Under the microscope it was extremely interesting to watch this orientation into a vertical position. An individual, moving actively in a more or less horizontal direction, would be observed to come almost into contact with another individual and immediately the movement became slower, the hinder end swerved downwards and the animal started off again and continued for a time its movement in a more vertical direction than before.

The upward movement is also helped in another way. The downward streaming of the Euglenæ through the water tends to set up a movement in the opposite direction. The friction between the Euglenæ stream and the water sets up a vortical motion by which many of the Euglenæ become separated from the main mass; and, being free, but still with a vertical orientation, they swim upwards again, being assisted to some extent by the impetus given to them by the vortical motion.

When they reach the surface the Euglenæ, if they are present in large numbers in the water, as is the case when the conditions for aggregation are fulfilled, soon become massed together; their freedom of movement gradually becomes more and more impeded and very soon they find themselves sinking downwards again under the gravitational influence. It is in this way that the cyclic downward and upward movements which persist during an aggregation are kept up.

If there are few Euglenæ present in the liquid they may turn into a more or less horizontal position in contact with the surface film or, if the vessel is closed, with the solid body, cork, glass plate, etc., which may be used for that purpose. This contact reaction interferes, as Loeb ('06, p. 76) points out in the case of Paramœcium, with the reaction to gravity. So long as the Euglenæ remain in this position, the action of gravity may be prevented. But if they are disturbed in any way, such as by agitating the surface of the liquid with a glass rod or by shaking the vessel, they become once more oriented into a vertical position, and if a sufficient number has accumulated, they may sink downwards again. This, no doubt, partly explains the disappearance of the motile Euglenæ from the surface of water, which is observed in Nature during rain or wind.

4. *The Formation of Networks and Groups.*

A. *The Action of Oxygen and Carbon Dioxide.*—So far, we have only dealt with the cause of the movements which take place during the aggregation. We have next to consider how it is that, as soon as gravity begins to act upon a mass of the motile cells, they are at once attracted into networks or more or less regularly defined groups. It occurred to me, at first, that it might be a chemotactic phenomenon brought about by a variation in the amount of oxygen in the liquid, such as brings about a less complex but definite aggregation of some bacteria. Euglenæ are certainly attracted to some extent by oxygen, as Aderhold ('88) has shown; they tend to accumulate around bubbles of air in the water, and their accumulation at the free surface of water in contact with air is, no doubt, partly due to this. I found, however, that the aggregation takes place just as well and as quickly in water which has been thoroughly boiled to get rid of the air, as in water that has been well aërated, so that it could not be due to variation in the oxygen contained in the water. Nor is it due to air at the surface, for the aggregation is more perfect in closed vessels from which all air is excluded than in open vessels exposed to the air. That the oxygen given off by the organisms themselves is not the controlling cause seems to be proved by the fact that the aggregation takes place best in the dark, when we may suppose the Euglena are not giving off oxygen.

The presence or absence of carbon dioxide in the liquid appears to make no difference in the aggregation. JENNINGS states ('97, p. 318) that the crowding of Paramœcium is due to the chemotaxis exerted by the excreted carbon dioxide. I have not been able to obtain any evidence that this is the case with Euglena. Various experiments tried with it gave purely negative results. Bubbles of carbon dioxide introduced into the tubes or vessels containing motile Euglenæ seemed to have no effect; there was no accumulation of Euglenæ around them as in the case of the oxygen bubbles, and no modification in the aggregation. The only effect of the carbon dioxide that I have been able to detect is that, in its presence, the Euglenæ remain motile for a longer time than in ordinary air or oxygen, in which they tend to become rounded off.

It is possible that there may be some kind of secretion or secretions from the Euglenæ, other than oxygen or carbon dioxide, which might bring about an aggregation; or the phenomena may be related to that of agglutination in the bacteria, but there is no evidence of this, and it is difficult to see how these explanations would account for the regular grouping which takes place, and the persistence of the cycle of movement upwards and downwards.

Under certain conditions Euglenæ may become agglutinated or clumped together in small masses, but the phenomena are quite different from those which are here under consideration. If motile Euglenæ are exposed in a shallow vessel to light, they gradually come to the surface and form a thin scum in contact with the air. If the liquid is then shaken or stirred up, the Euglenæ at the surface become clumped together into small granular masses, and an appearance of curdling is produced. This only takes place among those Euglenæ which have reached the surface, and is due, no doubt, to the secretion of a slime which, as KHAWKINE ('86) points out, is always produced around Euglenæ in the presence of oxygen in a good light. If the Euglenæ are prevented from coming into contact with air no agglutination is visible, however much they may be shaken or stirred up.

B. *The Action of Molecular Forces. Cohesion Figures.*—The fact that the organism is heavier than water and that the downward movements are due to the mechanical action of gravity suggested the possibility that the network-like grouping and aggregation might also be a purely physical phenomenon due to surface tension and cohesion, and it occurred to me that the behaviour of chemical precipitates and other fine sediments of various kinds might afford some clue to an explanation of the phenomena. I accordingly made a few preliminary experiments, and at once obtained such results as tended to confirm this view.

It was necessary, of course, to obtain a precipitate as nearly like the Euglenæ cells as possible as regards density, and absence of a tendency to become flocculent. Various precipitates were tried, as well as sediments of different kinds, such as starch grains, dead cells of yeast and Chlamydomonas, fine coal dust, manganese dioxide, etc., all of which were useful; but the best results were obtained with dead cells of Chlamydomonas killed in osmic acid, and a precipitate of osmic dioxide (OsO_2), produced by adding a dilute solution of ferrous sulphate to the ordinary 1 per cent. solution of osmic acid used for fixing. This precipitate does not become flocculent, does not stick, but always remains in a fine granular condition, and has, moreover, the advantage of being black, so that the figures formed by it can be easily seen. I got good results when this precipitate was allowed to settle in water, but much better when it was allowed to settle in a denser liquid, such as a solution of one part of glycerine in four parts of water, by which its relative density to the glycerine solution was nearly equal to that of the Euglenæ to water.

If a tube 6 mm. in diameter, filled with water containing such a sediment disseminated through it, is placed in a horizontal position, the particles settle to the lower side of the tube in straight lines without any visible aggregation, to form a homogeneous layer. If it is turned round gently, so that this homogeneous layer is uppermost, the particles now, instead of falling in straight lines, become aggregated together and fall in a series of irregular streams. If the density of the liquid is increased by the addition of glycerine or gum arabic, so that the sediment falls more slowly, these streams become more regular, and an appearance is presented which, to some extent, resembles the early stages in the aggregation of Euglenæ (Plate 32, fig. 3, *a*, *b*). The phenomena are, however, more clearly marked in flat shallow vessels than in tubes.

If a flat glass dish, which can be covered with a glass plate to exclude air, similar to that used in experiments with Euglenæ, is filled with water containing a sediment similar to that used in the last experiment, the separate particles settle down in straight lines and form a thin film on the bottom. If the dish is now gently turned upside down, this film of particles, in a second or two, again begins to sink in the water, but directly gravity begins to act upon them, they become attracted into a network (Plate 33, fig. 17) almost exactly resembling the network produced during the Euglena aggregations under similar conditions (Plate 33, fig. 10).

If the downward movement is retarded by the use of a denser solution, such as glycerine and water, the network which is at first formed (figs. 29 and 30) is not only much clearer but, under such conditions, tends to break up into separate masses or groups of particles, exactly resembling a later stage in the aggregation of Euglena (Plate 34, fig. 27; *cf.* figs. 28, 29). The resemblance extends even to the darker, denser central stream surrounded by a less dense peripheral layer (fig. 29). This less dense peripheral layer consists of a more or less well formed vortex ring produced by the friction of the water. In the case of Euglena, the lighter area around the denser central mass is also of the nature of a vortex ring, but is not so clearly marked owing to the upward movements of the organism.

Except for the fact that the cells of Euglena are capable of free movement, the aggregation of the particles of a precipitate appears to be similar to the aggregation of living Euglenæ.

Figs. 15 and 16 show the aggregations formed when dead cells of Euglena are treated as precipitates; in fig. 15 the Euglenæ are aggregating from a rather loose surface film, in fig. 16 from a firmer and more coherent film. These figures, and figs. 17 (manganese dioxide) and 30 (osmic dioxide), resemble very closely the aggregation of living Euglenæ in fig. 10. In fig. 44 is shown the aggregation of Chlamydomonas killed in a solution of osmic acid.

The aggregation of these various sediments appears to depend upon the fact that a thin film of particles, together with the surface layer of water in which they are contained, acts as a liquid heavier than water. The aggregation is brought about therefore not by the action of gravity upon the separate particles but upon the mixture of particles and water as a whole, just as would be the case if we were dealing with a homogeneous liquid heavier than water. Thus if we fill a shallow vessel with water and then drop into it a small quantity of ink the ink sinks in the water and spreads out to form a layer over the bottom of the vessel. If this is now carefully closed with a glass plate so that no air bubbles are left in the water and then turned gently upside down, the layer of ink will at once begin to sink and a network like aggregation will be produced resembling very closely that shown in figs. 16, 17, 30, and 32, or if a circular vessel be used those shown in figs. 34, 38, and 40. Instead of ink a mixture of one of the sediments mentioned above and water may be used. If a drop of such a mixture is placed in the water it sinks and spreads out in a thin layer on the bottom of the vessel and behaves when turned upside down in precisely the same way as the ink.

The aggregation thus produced is simply a modification, due to the use of a shallow vessel and a thin layer of the heavier liquid, of that described by TOMLINSON ('64) and now so well known, which takes place when a drop of coloured liquid such as ink, a solution of an aniline dye in water, or a mixture of small solid particles and water, is placed gently in a jar of water. The drop at once begins to sink, with, at first, very little if any diffusion in the surrounding water. The surface tension reaction between it and the water causes it to assume the form of a more or less perfect vortex ring. As it sinks the ring gradually becomes broken up into a number of ramifications which branch in all directions, and breaking up at the same time into numerous vortex rings until the liquid gradually becomes diffused through the water and the vortex motion ceases. The cohesive force in the coloured liquid or in the mixture of sediment and water is greater than the adhesive force between the mixture and the pure water in which it is placed, consequently the mixture in its passage through the water resists more or less completely the disintegrating effect of the friction between it and the water. The physical forces which are called into play during the aggregation of a layer of sediment in a closed shallow vessel thus appear to be (1) gravity, (2) the adhesion of the sedimentary layer to the upper surface of the vessel, (3) the cohesion in the sedimentary layer, (4) the surface tension reaction between it and the water, and (5) the friction of the water.

In addition, there is the movement set up in the liquid by turning the cell upside down, which is more apparent in a deep cell than in a shallow one; it always produces a slight modification of the network pattern, but does not prevent the discrimination of the aggregation due to the forces above mentioned.

The aggregation is therefore to be regarded as the resultant of these forces, and appears to be explainable as follows :—

When the film is turned upside down the force of gravity immediately begins to act upon it, and the first effect is that the adhesion to the glass plate or surface is weakened. As soon as this takes place the cohesion of the sedimentary layer asserts itself, and the particles come closer together in some places, and separate in others, the result being an irregular network. If the downward movement due to the fall of gravity is slow, as when a denser solution than water is used, the network is more regular in character, and as the particles descend in the liquid slowly, they are gradually attracted into more or less regular and equally spaced groups (Plate 34, fig. 29). These aggregations, therefore, appear to be cohesion phenomena, and the patterns produced are cases of cohesion figures. We can obtain similar cohesion figures in a variety of ways. Thus if a layer of vaseline is evenly distributed between two glass plates and the plates are then forcibly separated a cohesion figure in the form of an irregular network is found, which is the resultant of the adhesion of the vaseline to the glass, the cohesion of the vaseline, and the force acting to separate the two plates. The network-like cohesion figures described by TOMLINSON ('61) which are produced when a drop of oil of lavender, olive oil, or oil of almonds is placed on the surface of the water are somewhat similar. In the case of oil of lavender, for example, "the adhesion of the water will cause it to spread out into a film; but the cohesion of the oil immediately begins to reassert itself; the film opens in a number of places, forming long irregular arms or processes resembling the pattern assumed by wood when it has been much worm eaten. These processes tend to gather up into separate discs or lenticules; the adhesion of the water spreads them out; the cohesion of the oil struggles to prevent this, and soon prevails; the almost immediate issue being the formation of the original drop into a number of discs with sharp, well-defined outlines and convex surfaces." This "cohesion figure" "may be regarded as the resultant of the cohesive force of the substance, its density, and the adhesion of the surface on which it is placed."

When oil is used the cohesion figures are confined entirely to the surface, but as we have previously seen (p. 374) if a heavier liquid, such as ink or a solution of some colouring matter, is placed on water it will sink and produce figures of great variety and beauty.

I find that a combination of a surface cohesion figure, such as is produced by a drop of oil, with the figure produced in the deeper layers of the water by a heavier liquid can be obtained if we have a liquid which is heavier than the water, but is capable of spreading over it by surface tension. If a drop of ordinary black ink, or better, Indian ink, is gently brought into contact with the surface of water in a clean shallow vessel, it is at once, if the conditions are favourable, drawn out into a thin film. But the cohesion of the ink immediately begins to reassert itself, probably assisted by gravity, as it is heavier than water; the film opens in a number of places and the ink begins to collect, still on the surface, along certain lines, often more or less concentric curves, and gradually forms a more or less regular network. It then begins to sink slowly in the water; the network becomes more and more distinct as more of the film becomes drawn into it, and finally becomes more or less broken up by the greater accumulation of the ink at the nodes of the network, and its consequent more rapid sinking, with the formation, if the water is deep enough, of small vortex rings.

The forces operative here are: (1) the cohesion of the ink, (2) the adhesion of the surface on which it is placed, (3) gravity, which causes it to sink, (4) diffusion, which causes it to expand (into a ring), (5) the friction of the water, which retards (*a*) the descent of the ink (or ring of ink) and (*b*) its diffusion. A very complex reticulate cohesion figure is thus formed, which may be regarded as the resultant of all these forces. The appearances produced resemble almost exactly, but on a smaller scale, the reticulate figures in the earlier stages of the aggregation of Euglena.

Somewhat similar, but not such clearly marked, figures are produced when we bring a drop of a dilute solution of silver nitrate into contact with the surface of a dilute solution of common salt. The silver nitrate spreads out into a thin film, and at the same time a reaction between the two takes place resulting in the formation of a thin layer of silver chloride which behaves in a similar manner to the ink film. Similar results can be obtained with ammonium hydrate on stannous chloride or on cupric sulphate, and possibly numerous other combinations would be found effective in the production of such cohesion figures.

Cohesion figures can also be produced by coating a glass plate with ink or a solution of fuchsin or other aniline dye which is allowed to dry, and is then placed in contact with the surface layer of water contained in a shallow dish. As the water penetrates into it the film separates from the glass plate and becomes aggregated into a more or less regular network, which sinks slowly in the water and becomes gradually broken up with the production of vortex rings.

But one of the most interesting, and one of the most easily observed cases, in which a network-like cohesion figure is produced by the sinking of a thin film heavier than water, is that which is brought about by the oxidation, at its surface, of a solution of some easily oxidisable substance, such as a mixture of pyrogallol with an alkali, or any ordinary photographic developer which turns brown or black on exposure to air. All that is required is to leave the developer standing in a suitable dish, —preferably of glass, in order that it may be observed by transmitted light—to oxidise. As soon as the surface begins to turn brown, it will be observed that a slight irregularity appears, and very soon the whole layer becomes aggregated into a most beautiful network, closely resembling that obtained during the aggregation of the living organisms described in this paper (Plate 34, fig. 32, and Plate 35, fig. 37).

One of the most satisfactory methods of obtaining a cohesion figure in this way is by means of a solution of pyrogallol mixed with rodinal. Two solutions must be made :—

1.

Water	95 c.c.
Rodinal	3 ,,
Ammonia . . .	2·5 ,,

2.

Water	100 c.c.
Pyrogallol . . .	1 grm.

To 30 c.c. of 1 add 2 c.c. of 2 and pour the mixture into a shallow dish. In about two minutes the exposed surface of the solution will have become slightly brown, and at the same time a beautiful cohesion figure is formed which gradually becomes more clearly defined as the solution becomes more oxidised and of a deeper colour. If diluted with about four times its bulk of water, before exposure to the air, the reaction is slower and the network somewhat coarser.

The aggregation is modified according to the depth of the solution employed. In some experiments made with Bothamley's standard developer without ammonium bromide, I found that in a very shallow layer $\frac{1}{16}$ inch deep, a delicate network was formed in two minutes which gradually became resolved into separate groups; in a layer $\frac{1}{4}$ inch deep, a coarser network was formed without separate groups, whilst in an ordinary saucer with the solution about $\frac{5}{8}$ inch deep in the middle, a coarser network appeared, but with finer meshes at the periphery.

The following solutions also gave satisfactory results.

Watalu developer.—After a Watalu plate has been developed the solution is allowed to stand in a shallow dish exposed to the air. At the expiration of about an hour sufficient oxidation takes place to give a visible reticulation (figs. 32, 37).

Pyro-soda and hydroquinone developers made up from tabloids according to the directions given by Messrs. Burroughs Wellcome and Co., show reticulation only after some hours.

A mixture of pyrogallol with sodium hydrate gives an aggregation very rapidly, as also does an ordinary pyro-ammonia solution.*

The action which takes place in the formation of these oxidation cohesion figures, is probably (1) the production of a thin film of the oxidised substance on the surface, which is heavier than the unoxidised liquid underneath; (2) this film, being different in composition from the liquid below it, acts now as a thin film of a different liquid would do, that is, there is an adhesion between it and the liquid below it, and cohesion in the film itself; (3) the cohesion of the film begins to assert itself as against the adhesion of the surface on which it stands; it opens out and begins to collect along certain lines and a cohesion figure is the result; (4) then it begins to sink in the water, and becomes further broken up to form a very complex, but more or less regular, reticulation with formation of vortex rings.

All these observations seem to show that network-like cohesion figures are always produced, whenever we have a more or less homogeneous film placed under such conditions that a force or forces acting in opposition to the cohesive force can be brought into play.

If we now consider the aggregations of living Euglenæ and other micro-organisms described in this paper, in the light of these experiments, we shall see that they also are in reality cohesion figures, produced in the same way as those just described, and directly comparable with those which are formed by precipitates or fine sediments in water.

A drop of water which contains a large number of motile Euglenæ or other micro-organisms behaves just as a liquid heavier than water, such as a drop of ink or of a solution of cochineal. If it is placed on the surface of clean water, it sinks as a whole beneath the surface, expands into a vortex ring, which gradually becomes larger, but is slowly broken up by the friction of the water, and finally becomes diffused in it, just as a drop of cochineal would do or a vortex ring produced by a mixture containing a fine sediment.

So also, if the Euglenæ in a dish of water become crowded together in any place, the congested mass becomes subject to the action of gravity and cohesive forces, and behaves just like a liquid heavier than water.

If the Euglenæ are much crowded, but evenly distributed, either in an open or closed shallow vessel, the first effect of gravity is to cause them to sink. This brings them at once into closer contact with one another, and a dense layer is produced, in which phenomena of cohesion are apparent in the formation of a well marked reticulation (Plate 35, figs. 35, 36).

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* The formation of these cohesion figures probably explains the reticular markings which sometimes appear on photographic plates when the development is prolonged for a considerable time.

If the number of Euglenæ in the water is only sufficient just to give it a green colour, this downward movement does not take place, but the effect of gravity is, first of all, to cause an orientation of the cells in a vertical direction, with their anterior ends upwards. Their direction of movement is thus definitely determined, and as they are not sufficiently crowded for the *downward* pull of gravity to be effective, they begin to move more or less directly upwards. As this takes place simultaneously in all parts of the dish, they soon become congested as they reach the upper layers; they lose more and more their freedom of movement, and now the conditions are such that the downward pull of gravity becomes operative and the Euglenæ begin to sink. At the same moment the cohesive force comes into play, and a network-like cohesion figure is formed.

If the cell is a shallow one, the aggregation is more regular at the beginning, and takes place more quickly than in a deeper one (*Cf.* figs. 10–12, 35, and 36). This is explained by the fact that the upward movement brings them more quickly to the upper surface, and that they form here, before cohesion has had time to take effect, a more continuous layer than is possible in a deeper cell, where the upward movement is more irregular, and cohesion begins to take effect before they have formed a homogeneous layer.

As we have seen previously, the aggregation is never so regular or perfect in a deep vessel as in a shallow one. In a deep cell the movements of the Euglenæ are not so regular, owing to the longer distances to be covered, and the fact that the upward movement is never a perfectly upright one, but always along a more or less spiral path, which varies according to the intensity with which the movements of the organism are directed. Consequently, their accumulation into masses or layers, and their subsequent production of cohesion figures is more irregular (Plate 35, figs. 35, 36).

If the Euglenæ are very few in number in a shallow cell, they may aggregate at once into rather large groups without the intervention of a definite network (Plate 34, figs. 25–28). If there are very few of them and they are unable to accumulate in masses or layers sufficiently dense for the cohesion to be called into play, there is no aggregation.

A very beautiful and regular aggregation, which exactly resembles that of a precipitate, takes place if the Euglenæ are placed under such conditions as allow of the formation of a more or less homogeneous layer in contact with the upper surface and therefore all in the same plane. This is brought about naturally in a shallow dish, as we have previously shown, by the upward movement of the Euglenæ. But it can be brought about artificially in a vessel of any depth, by allowing light to fall upon it vertically. The Euglenæ are all attracted to the upper surface, and form there a thin homogeneous layer. The condition is now exactly similar to that obtained when a vessel containing a sediment in a thin layer on its lower surface is turned upside down. On covering the cell with a piece of card or red glass, the attractive force of the light is removed, gravity and cohesion begin at once to act, and the result is the formation of a cohesion figure exactly resembling that of a precipitate (Plate 32, fig. 8; *cf.* Plate 33, fig. 15), the only difference being that, in the case of the motile micro-organism, the particles are capable of rising up to the surface again by their own exertions; in the case of the inert sediment, the particles as they settle down adhere closely to the lower surface and are unable to move upwards again. The result of this is, that the aggregation of the motile cells continues as long as they are capable of movement, with the formation of groups as in a normal aggregation; the aggregation of the inert particles ceases as soon as they have reached the bottom of the vessel.

But the resemblance is almost exactly parallel if (instead of allowing the Euglenæ to move upwards again), on placing the card on the upper surface, we at the same time, or shortly afterwards, allow light to impinge vertically from below on the lower surface. The attractive force of light will prevent the upward movement of the Euglenæ and will keep them in a thin layer on the lower surface of the vessel. We can still further emphasise the resemblance to the precipitate by now, in the dark or a weak light, turning the vessel upside down, so that the film lies uppermost, when the aggregation figure is at once again produced (Plate 33, fig. 18). These experiments can be repeated any number of times, so long as the Euglenæ remain motile. In these experiments we might, in fact, regard the living Euglenæ as a precipitate consisting of motile particles.

In the last experiment, if, instead of turning the vessel upside down, we simply remove the light, the Euglenæ will at once begin to move upwards, and almost at the same time a delicate reticulation will be observed in the film. Examination under a low power of the microscope shows that here also we have a slight movement upwards and downwards, as in the ordinary aggregation, and it is no doubt explainable in the same way. A somewhat similar effect is produced in a thin layer of sediment if the vessel in which it is contained is gently tapped on its under surface. A slight movement of the particles is set up and, as they settle down, a reticulate pattern is produced.

In Plate 35 are shown some of the aggregation effects produced in a circular cell half an inch deep by living and dead cells of *Euglena viridis* and precipitates. Fig. 33 shows living Euglena aggregating from a surface film which had been obtained by allowing light to impinge upon the lower surface of the cell from below. The Euglenæ were attracted downwards by the light and formed a homogeneous layer on the lower surface of the cell, which was then turned upside down. Fig. 34 shows the same Euglenæ killed in osmic acid solution and allowed to settle to the lower surface of the cell, which was then turned upside down as before. It will be noticed that the aggregations are very similar. Fig. 38 shows the aggregation of a film of manganese dioxide, and fig. 40 the aggregation of osmium dioxide. The results obtained in all these cases resemble each other sufficiently to show that they have in the main been brought about in the same way.

The conclusion at which we arrive, therefore, as the result of these investigations, is that the aggregations of motile organisms, such as Euglena, Chlamydomonas, and others, is not due to the purposeful movement of the organisms themselves, but is due to the action of gravity combined with cohesive forces; that the networks and groups which are formed are of the nature of cohesion figures, similar to those obtained with fine sediments of various kinds or liquids heavier than water, and that the movements of these organisms under these conditions, and in the absence of light, are controlled in a purely mechanical fashion by the action of gravity, and are not in any sense geotactic or tropistic.

General Conclusions.

The action of the physical forces, gravity and molecular attraction, over which Euglenæ have little or no control appears, therefore, to play an important part in their life history, and, whilst not inhibiting their power to move, compels them to limit the sphere of their activity to certain definite areas in such a way as to promote a more or less regular dissemination of them through the liquid, and thus prevent any undesirable congestion of the organisms in one place.

It is obviously an advantage to such motile organisms as Euglenæ that they should not become too much crowded either at the surface of the water in the light or in the deeper layers of the liquid on the mud.

Their respiratory functions, assimilating activity, and food absorption would be interfered with, and it is extremely probable that under such conditions they would more easily succumb to the attacks of those numerous parasites which prey upon them.

Many of the so-called cases of geotaxis, which have been described for various organisms, may be found to be explainable in the light of these observations as purely mechanical phenomena and not true cases of geotactic response, and would account in part for the very conflicting results which have so far been obtained.

So also in respect of the complex phenomena of plankton distribution, it may be found that the purely mechanical action of gravity and cohesion may play an important part.

Summary.

(1) When kept in shallow vessels or tubes in the dark, *Euglena viridis* exhibits peculiar aggregations into networks or more or less regularly defined groups of cells.

(2) The appearance and the regularity of the aggregation depend upon the depth of the cell and the number of organisms present. It is more regular and more pronounced in a shallow vessel than in a deep one, and is more perfect in a closed vessel than in an open one. In a narrow tube placed horizontally, the aggregation

takes the form of a series of nearly regular groups, equally spaced from one end of the tube to the other.

(3) On shaking or otherwise disturbing the liquid in the vessel, the aggregation disappears, but reappears again in a few seconds after the disturbance has ceased.

(4) Exposure to a strong light causes the aggregation to disappear, and the Euglenæ move in the direction of the light and accumulate in a dense mass on that side of the vessel nearest the light. On placing the vessel in the dark again the aggregation begins again in this dense mass and the Euglenæ become more or less evenly distributed through the liquid again in the form of a network.

(5) Experiments were also made with colourless forms of *Euglena viridis*, *Euglena deses,* species of Chlamydomonas, one of the fresh-water Peridineæ (*Glenodinium cinctum*), *Volvox globator,* and Spirillum, in all of which somewhat similar phenomena of aggregations were observed.

(6) In a weak light the aggregation of Euglenæ may persist for some time, but ultimately disappears. In the dark it persists for several days or until the death ot the organism takes place.

(7) Under red glass it takes place or persists as in the dark. Under green glass the Euglenæ behave as in a weak light.

(8) The general effect of an increase of temperature within certain limits is to increase the motility of the organism, but not to inhibit the aggregation. Their movements become enfeebled at a temperature of 30° C., and at 35° C. death may ensue.

(9) The ease and rapidity with which the aggregation takes place depend upon the activity of the Euglenæ. In the cold weather their movements are more sluggish than in warm weather, and they do not aggregate so quickly or so definitely, and at very low temperatures they may not show any aggregation at all.

(10) The aggregation is not due to currents set up in the water, either by heat or evaporation.

(11) The aggregation does not appear to be dependent upon the presence or absence of oxygen or carbon dioxide. The characteristic networks and groups are more clearly displayed in a shallow vessel which is completely filled with liquid, and covered with a sheet of glass so that air is excluded, than in an open one.

(12) During the aggregation there is a constant cyclic movement downwards and upwards. The downward movement is a passive one, the Euglenæ being orientated into a vertical position with their anterior ends upwards. This is due apparently to the greater density of the posterior half of the cell. If Euglenæ are killed in the elongate condition, or if they are inactive, they sink in the water in the same position. This shows that the posterior half is heavier than the anterior, and this is borne out by their structure.

(13) The specific gravity of *Euglena viridis* was determined for living cells by

means of solutions of gum of different strengths. It varies from about 1·013 to 1·019, but the majority have a density of 1·016.

(14) Since, therefore, the specific gravity of the living Euglenæ is greater than that of water, and in the downward moving stream they assume a more or less vertical position with their posterior ends directed downwards, exactly similar to the position taken up by dead elongate cells, it is clear that the downward movement is a purely mechanical one, dependent upon the specific gravity of the organism, and is not due to a stimulus which evokes a physiological response as in cases of geotropism and geotaxis.

(15) If this downward movement took place only in the light it would be possible to explain it as due, partly, at any rate, to an increase in the specific gravity of the organism brought about by the storing up of the products of the assimilative activity of its chlorophyll in the light, but as this movement is more pronounced when the organism is kept in the dark, this explanation is precluded.

(16) The upward movement depends upon the active movements of the organisms themselves, controlled, so far as their orientation is concerned, by the action of gravity.

(17) The attraction exerted by gravity is not effective unless the Euglenæ are present in large numbers. If they are few in number, they are capable of moving in any direction, but always with a tendency to move upwards. The aggregation into networks or groups can only take place when the Euglenæ are sufficiently crowded together for the downward pull of gravity to be effective.

(18) The examination of fine sediments of various kinds shows that under certain conditions, when they are allowed to settle down in water or solutions of various kinds, they form networks and groups similar in appearance to those produced in the aggregation of Euglenæ. They are brought about by the action of gravity combined with cohesion, and are of the nature of cohesion figures.

(19) The aggregations of living organisms are probably brought about in the same way, and the general conclusion is arrived at that these also are "cohesion figures," brought about in a purely mechanical fashion by the action of gravity combined with the cohesive force which comes into play as soon as the organisms are in a sufficiently crowded condition to allow gravity to act.

In other words, the forces of gravity and cohesion, combined with the friction of the water, which sets up a vortical motion as soon as the Euglenæ begin to fall, are sufficient to account for all the phenomena observed.

(20) This aggregation appears to be of benefit to Euglenæ in that it results in a constant dissemination of them through the liquid and prevents their accumulation in such dense masses as would be detrimental to their existence by interfering with their assimilative and respiratory functions.

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DESCRIPTION OF PLATES 32–36.

Plates 32–35.—Photographs; in all cases, unless otherwise stated, natural size.
Plate 36.—Freehand drawings.

PLATE 32.

Fig. 1.—*Euglena viridis* in a tube placed horizontally; A, seen from the side, B, from above.

Fig. 2.—Five stages in the grouping of *E. viridis* in a horizontal tube, kept in the dark, photographed from above.

Fig. 3.—Three stages photographed from the side.

Figs. 4–6.—Stages in the movements of Euglena in an upright cell with the upper surface of the water in contact with air, photographed at intervals of five minutes.

Fig. 7.—Hermetically sealed cell, showing Euglena at the base. The vertical streaks are the downward-moving streams of Euglena. The upper part of the liquid contains very few of the organisms.

Fig. 8.—Hermetically sealed cell, showing Euglenæ aggregating from a homogeneous surface film, brought to the upper surface of the liquid in the cell by allowing light to fall perpendicularly upon it. As soon as the Euglenæ had accumulated at the surface in a homogeneous layer, the cell was

placed in the dark, and immediately the aggregation began as shown in the figure.

Fig. 9.—Two stages showing the beginning of the aggregation in a horizontal tube with very few Euglenæ, photographed from the side.

Plate 33.

Figs. 10–14.—Aggregation of living Euglenæ in a flat hermetically sealed cell, $\frac{5}{16}$-inch deep, placed horizontally in the dark.

Fig. 10.—Two and a half minutes after being placed in the dark. Network grouping.

Fig. 11.—Four minutes after being placed in the dark. Separate groups beginning to form.

Fig. 12.—Six minutes after being placed in the dark. Separate groups.

Fig. 13.—Seven minutes after being placed in the dark. The groups are beginning to break up into smaller ones.

Fig. 14.—Ten minutes after being placed in the dark. Small groups. The Euglenæ remained in this condition for several days.

Fig. 15.—Dead Euglenæ aggregating from a somewhat loose surface film.

Fig. 16.—Dead Euglenæ aggregating from a firmer and more coherent film.

Fig. 17.—Manganese dioxide falling from a surface film formed by the settling of the precipitate to form a layer on the bottom of the vessel, which was then turned gently upside down.

Fig. 18.—Living Euglenæ falling from a surface film, obtained by attracting the Euglenæ to the bottom of the vessel, by means of an appropriate arrangement of the light, to form a homogeneous layer, which was then turned upside down.

Fig. 19.—Euglenæ in a slanting tube. The tube was first placed horizontally in a dark chamber. As soon as the grouping had begun the tube was tilted, and it was then seen that the streams of Euglenæ became bent into a vertical direction, thus showing that the streams are under the influence of gravity.

Fig. 20.—Shallow upright cell in which Euglenæ are just beginning to fall from an upper layer. Magnified about 4 diameters.

Plate 34.

Fig. 21.—Cell in which Euglenæ were brought to one side by allowing light from one side to fall upon them. They formed there a dense mass. Two minutes after being placed in the dark they began to aggregate, as shown in the figure, photographed from above. The following figures (22–24) show successive stages.

Fig. 22.—At the end of four minutes.

Fig. 23.—At the end of six minutes.

Fig. 24.—At the end of eight minutes. The Euglenæ, at the end of 20 minutes, had spread themselves all over the cell in separate groups, as shown in fig. 28, and ultimately into smaller groups as in fig. 14 (Plate 33).

Figs. 25–28.—These figures show the aggregation of Euglenæ from a diffused state in a shallow cell, when fewer are present than in Plate 33, fig. 10. The separate groups are formed almost at once, and are much larger, breaking up later into smaller ones, as shown in the figures.

Fig. 29.—Osmic dioxide precipitate, in dilute glycerine, to retard the downward movement. A netlike arrangement of the precipitate is first of all formed, which soon breaks up into more or less separate groups as shown in the figure. *Cf.* figs. 26–28.

Fig. 30.—Osmic dioxide in dilute glycerine. Grouping formed from a film not quite so coherent as in fig. 29, that is, it had not been allowed to stand quite so long before it was turned upside down, and was photographed at a slightly earlier stage than fig. 29.

Fig. 31.—Spirilla in a very shallow cell, in which, by capillary attraction, the surface of the water had become much curved. The bacteria formed a dense layer just beneath the surface of the water, at the level of the optimum oxygen layer, from which downward moving streams were set up. Magnified about three or four times.

Fig. 32.—Figure produced by the oxidation in air of a solution of Watalu developer. Later stage than fig. 37.

PLATE 35.

Fig. 33.—Living Euglenæ falling from a film which had been obtained by allowing light to impinge upon the lower surface of a cell $\frac{1}{2}$ inch deep, which was then turned upside down.

Fig. 34.—The same Euglenæ killed in osmic acid solution and allowed to settle to the lower side of the cell to form a homogeneous film which was then turned upside down. The Euglenæ are just beginning to fall again and an aggregation is visible.

Fig. 35.—Living Euglenæ aggregating from the diffuse state in a cell $\frac{1}{2}$ inch deep. A large number of Euglenæ were present.

Fig. 36.—A later stage than fig. 35. The meshes of the network have become smaller, and there is some indication of a separation into groups, but the cell is too deep and the number of Euglenæ too large to allow a definite separation of the groups.

Fig. 37.—Figure produced by the oxidation in air of a solution of Watalu developer in a shallow cell.

Fig. 38.—Precipitate of manganese dioxide falling from a film in a cell $\frac{1}{2}$ inch deep. This cell is much deeper than those used in most of the experiments previously figured, and the movement of the water as the cell is turned upside down is sufficient to give a definite direction of movement to the surface film of precipitate. The main lines of the grouping are definitely due to this. Possibly the circular shape of the cell has something to do with it also. A similar arrangement is observed in figs. 33, 34, and 40.

Fig. 39.—Aggregation of Euglenæ in a shallow cell with sloping sides. The grouping is denser towards the middle and radiates more or less equally from the centre.

Fig. 40.—Aggregation of a precipitate of osmic dioxide (OsO_2) from a surface film in a shallow, hermetically sealed cell. The cell is smaller than in the other figures, but the same general result is obtained.

Fig. 41.—Euglenæ moving across from one side of the cell to the opposite side under the influence of light. The upper side of the cell in the figure was the light side. The Euglenæ move across in a series of ripple-like aggregations.

Fig. 42.—Cell as in fig. 41, but with a dark screen placed over it for two seconds. The Euglenæ in each group at once begin to sink and become drawn together into denser masses, which is clearly shown in the figure. The same effect would have been produced by a red glass.

Fig. 43.—Euglenæ aggregating into a network in diffuse light from above. The same effect would have been produced under green glass. Under red glass the Euglenæ aggregate as they do in the dark.

Fig. 44.—Chlamydomonas killed in osmic acid solution, just beginning to fall from a film in a shallow cell turned upside down.

PLATE 36.

Figs. 45–50.—Aggregation figures of *Glenodinium cinctum.*

Fig. 45.—Downward streaming just beginning in an upright cell with the upper surface of the water exposed to the air. Diffuse light.

Fig. 46.—A slightly later stage than fig. 45. The streams have in most cases reached the bottom of the cell.

Fig. 47.—A still later stage showing the streaming downward in lines. As soon as they reach the bottom, the organisms swim irregularly upwards again to reach the oxygen layer.

Fig. 48.—The effect of a stronger light falling on the cell from one side. The streams are bent towards the light, but for a time gravity still acts; the angles formed represent the resultant of the two forces; the

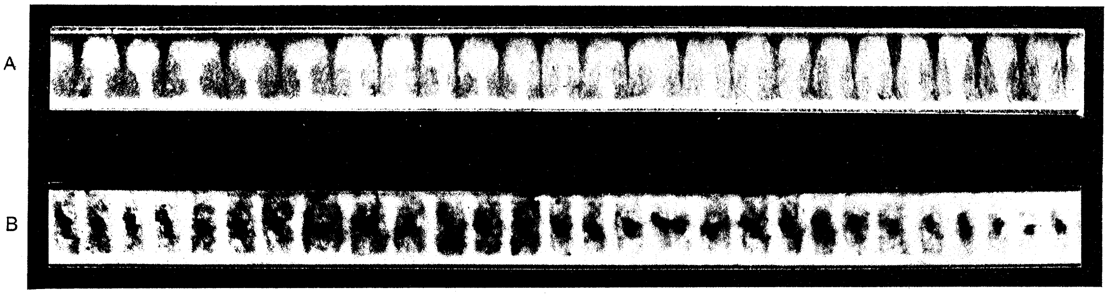
aggregation gradually disappears as the organisms move towards the light.

Fig. 49.—This figure shows the effect of a strong beam of light concentrated by a condenser upon one of the moving streams. The organisms move away from the immediate vicinity of the light, which thus has the effect of causing an expansion of the stream (see the middle one of the three streams). If the light continues to act, the upper portion of the stream persists for some time, but the lower part disappears; the organisms are repelled too far by the light for them to become aggregated again below it (see the left hand figure). On the right hand of the figure is seen a stream on which the light is not falling.

Fig. 50.—*Glenodinium cinctum* in a hermetically sealed, upright cell. A very regular streaming up and down takes place at the bottom of the cell; a few of the organisms rise nearly to the upper surface, and descend in very delicate lines. The majority move up and down within a distance of about a quarter of an inch.

Fig. 51.—The grouping of *Glenodinium cinctum* in a shallow cell placed flat. The groups are well spaced.

Fig. 52.—Diagram showing the movements of *E. viridis* in two of the separate groups seen in fig. 1. There is a constant cyclic movement downwards and upwards in the general direction indicated by the arrows.



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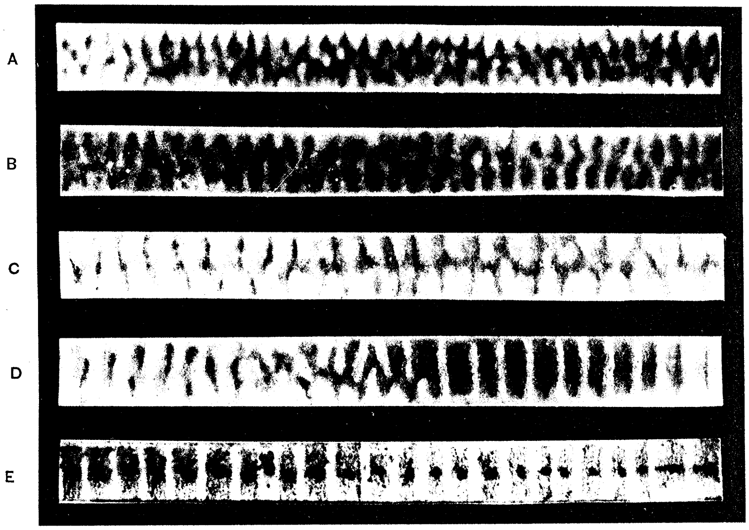

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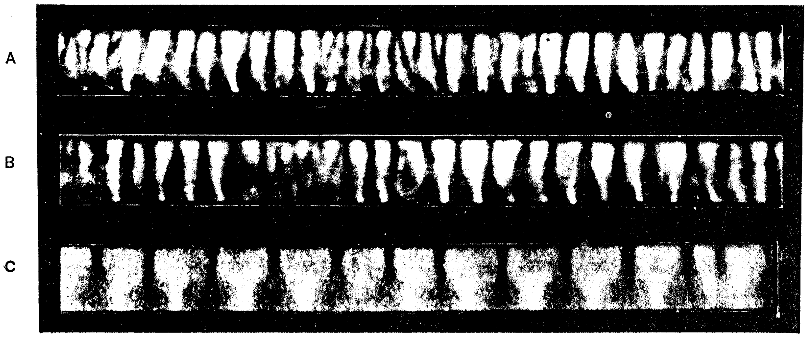

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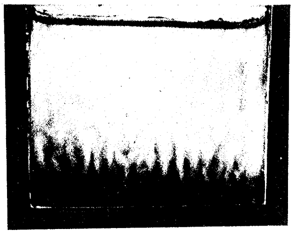

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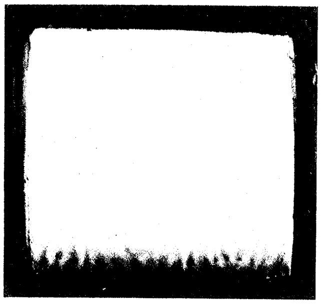

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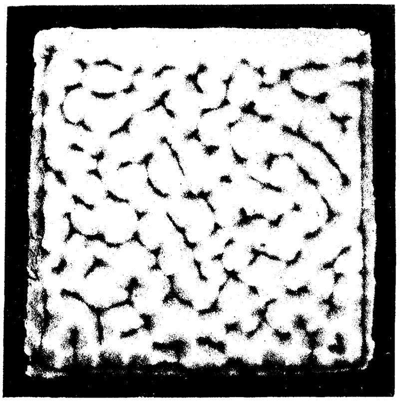

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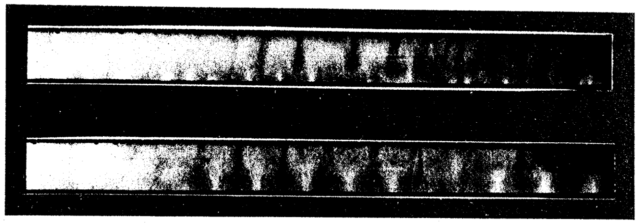

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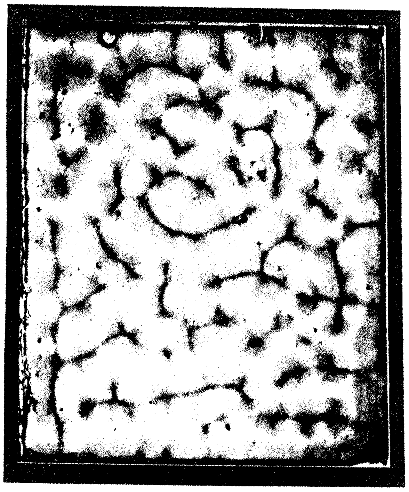

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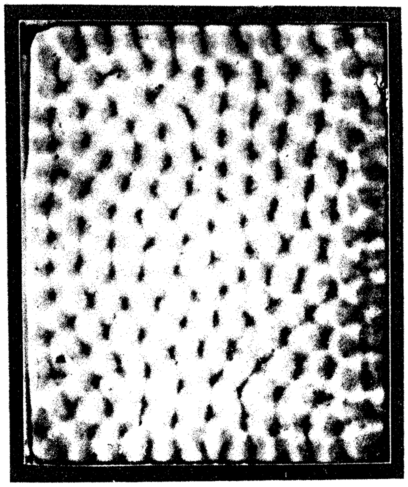

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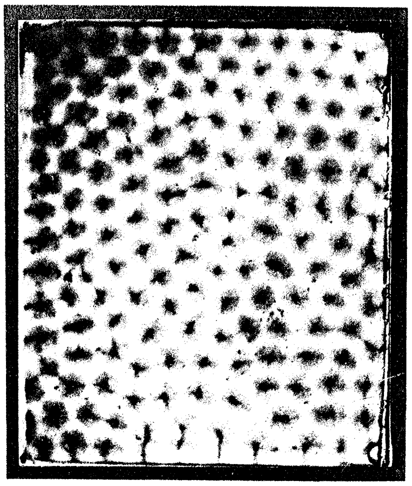

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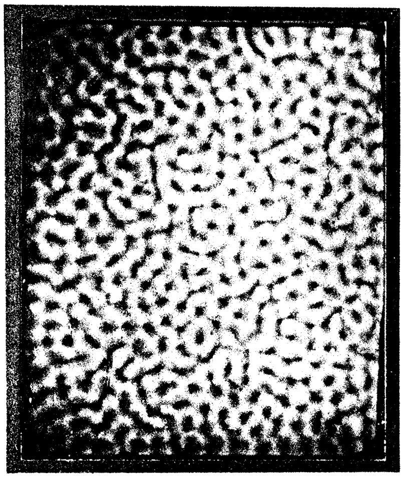

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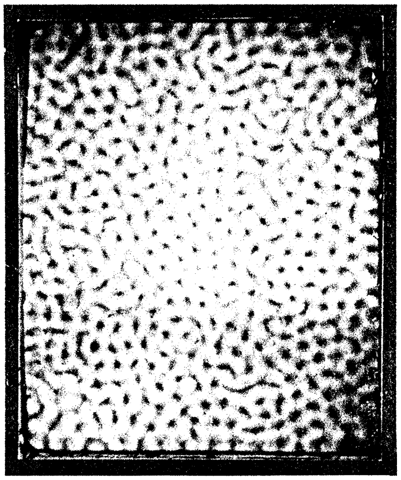

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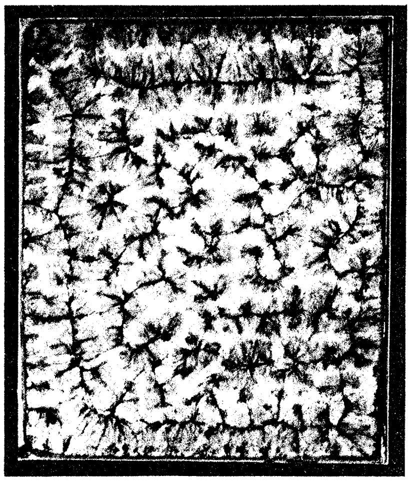

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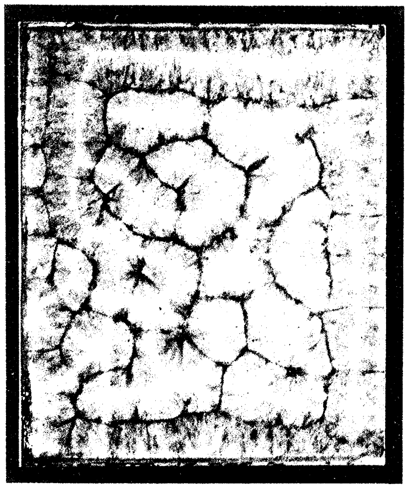

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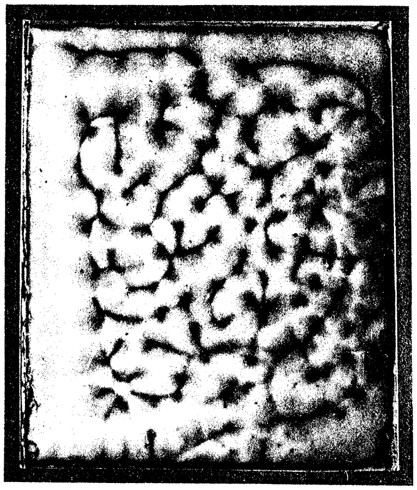

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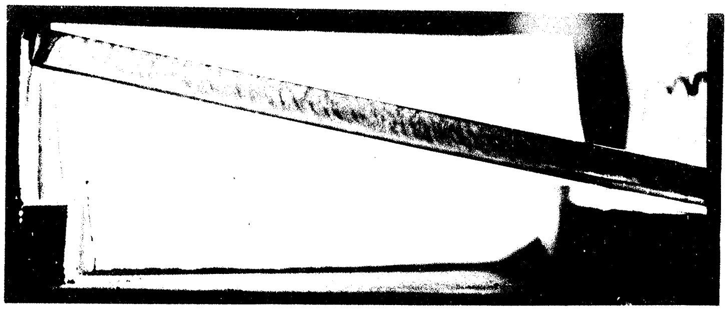

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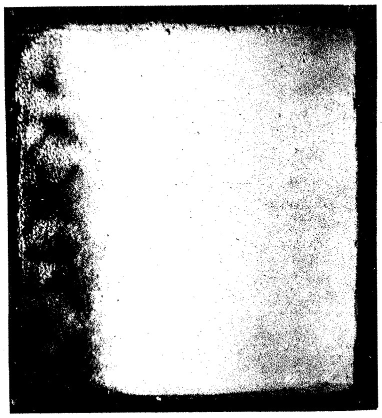

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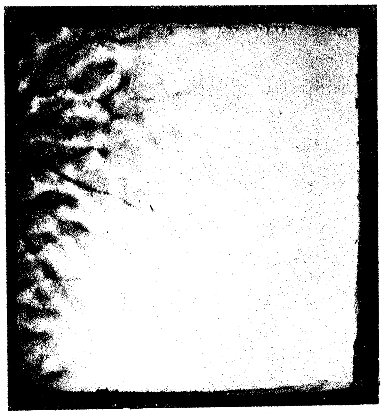

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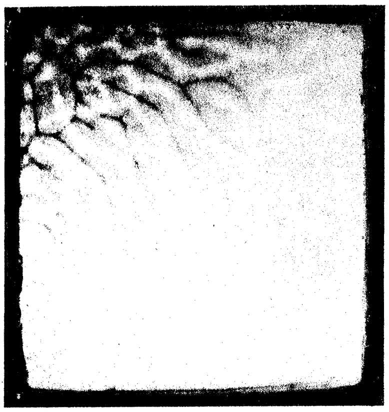

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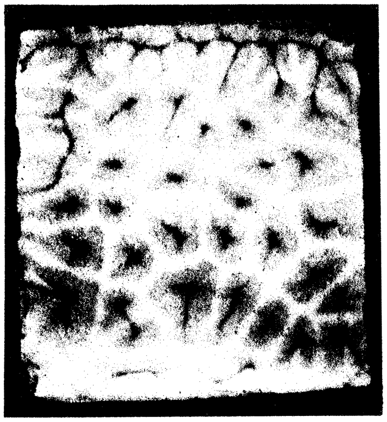

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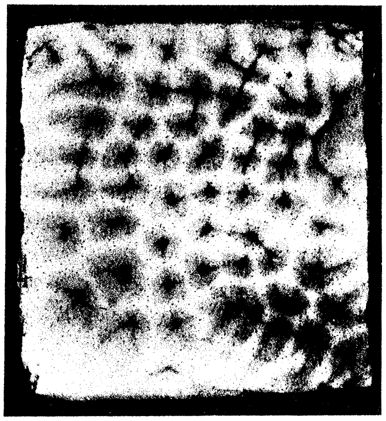

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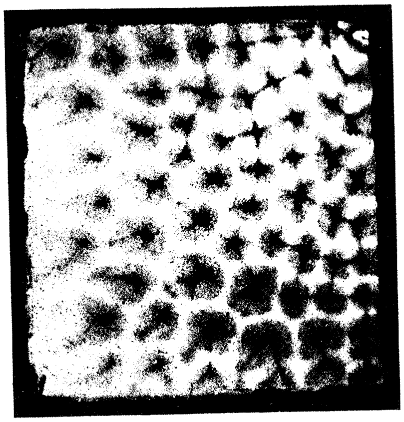

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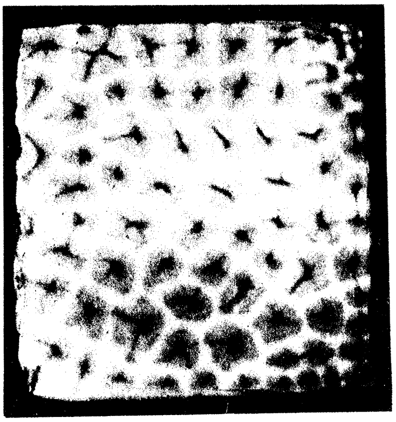

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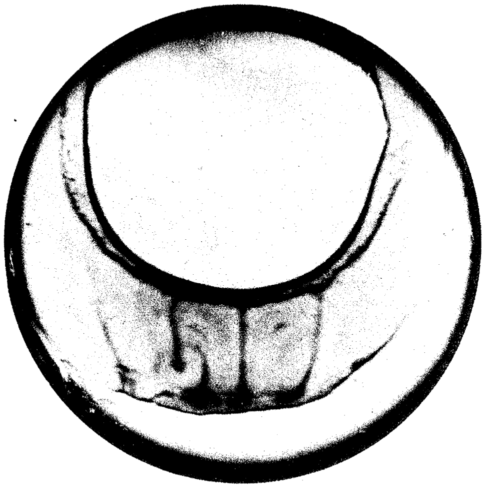

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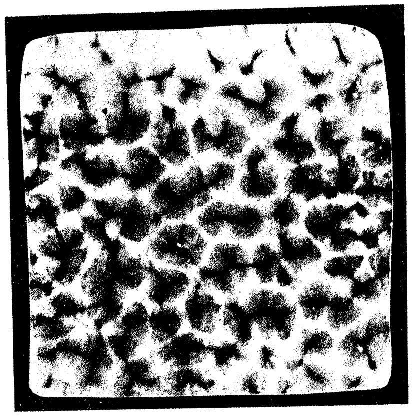

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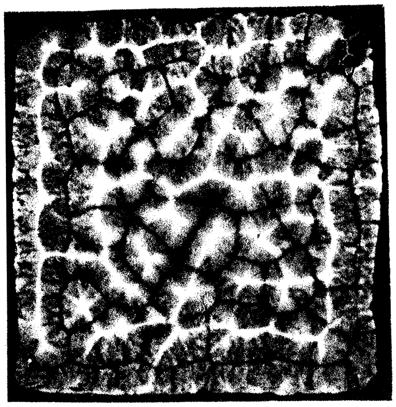

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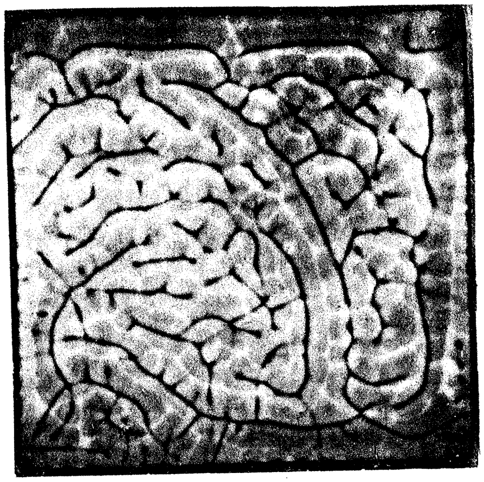

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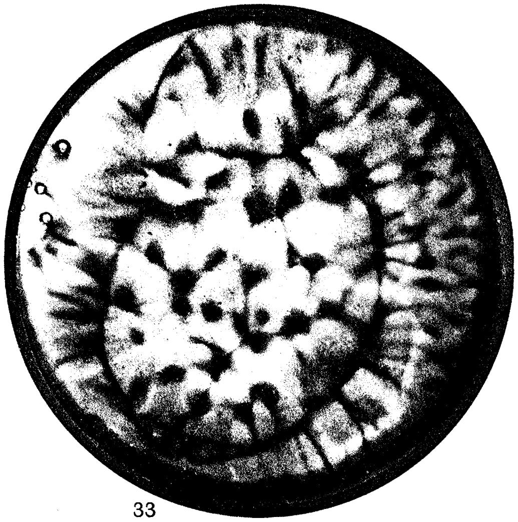
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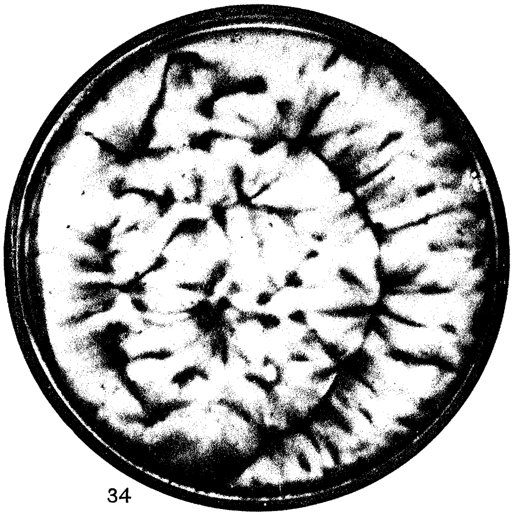
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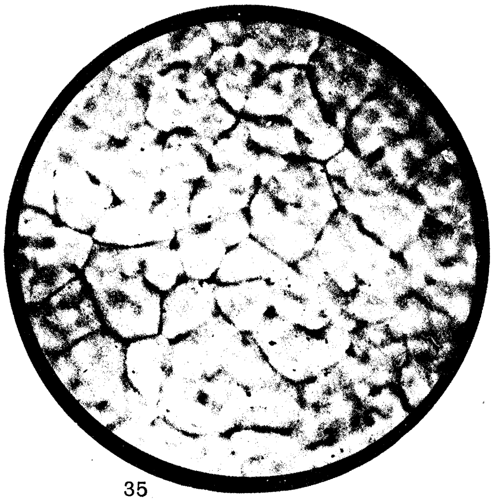
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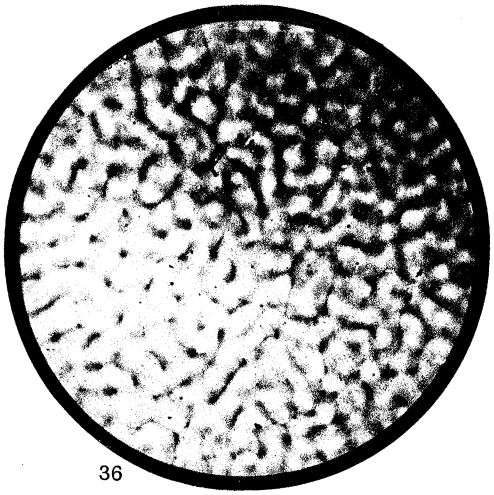
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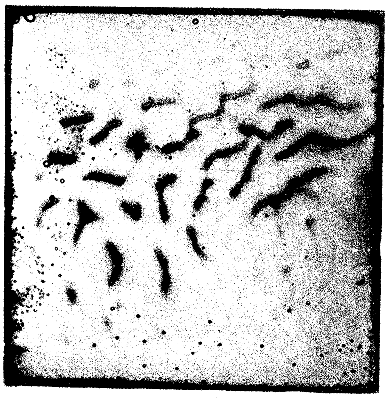
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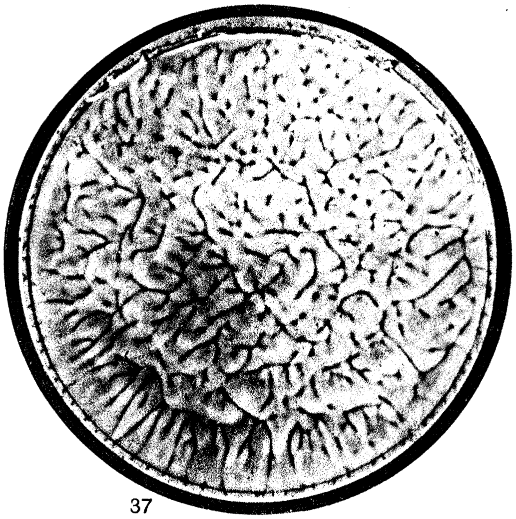
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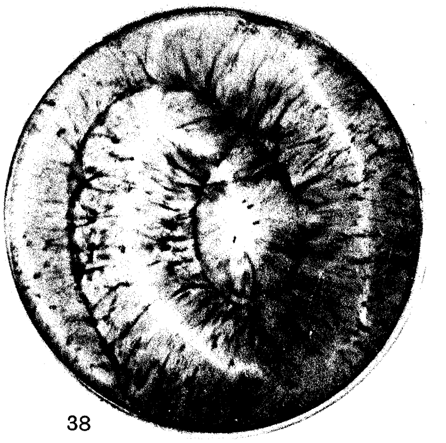
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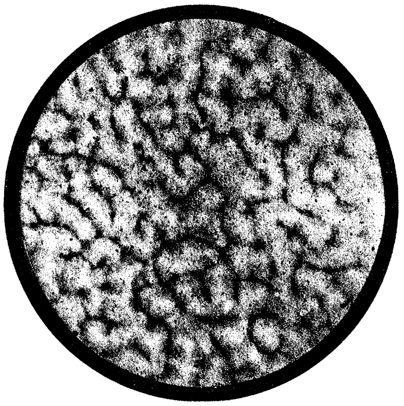
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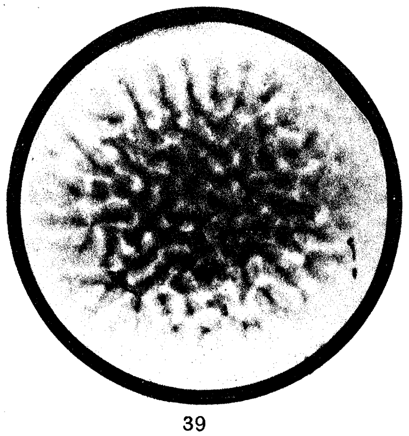
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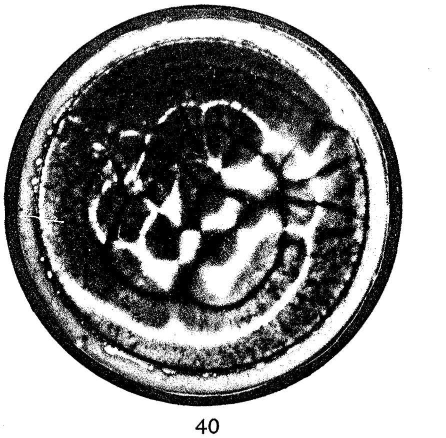
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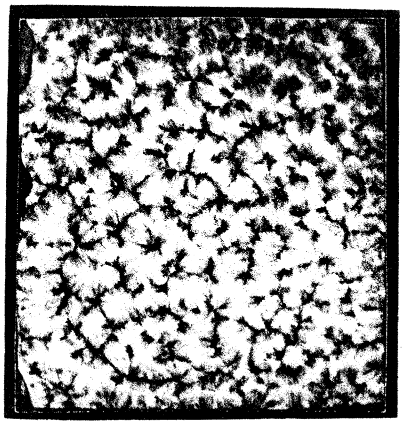
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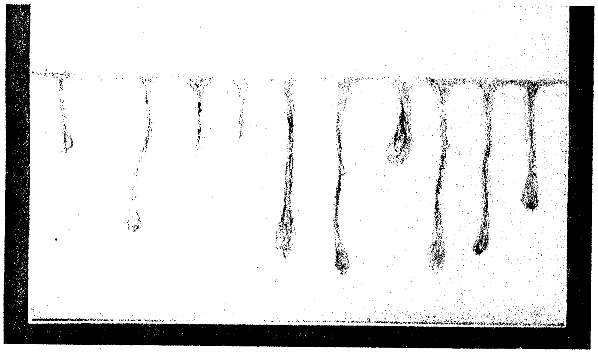

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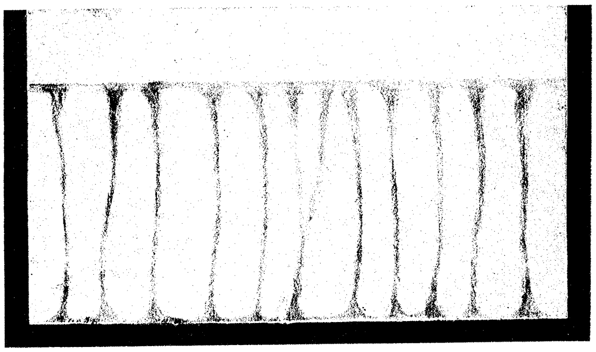

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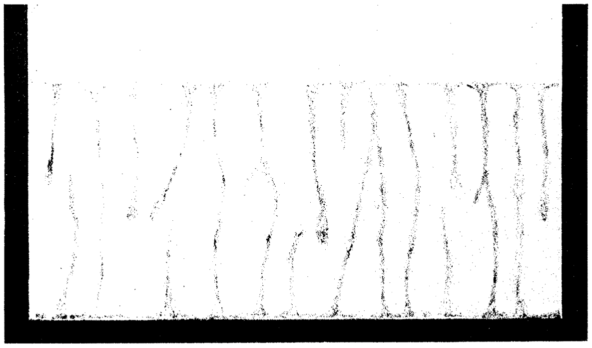

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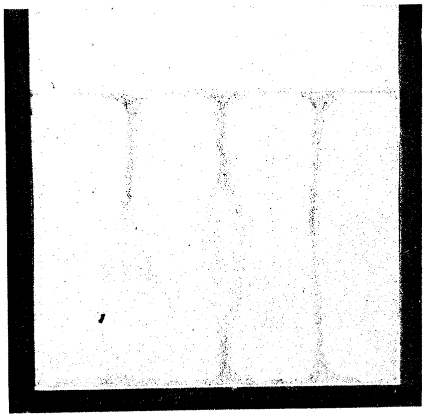

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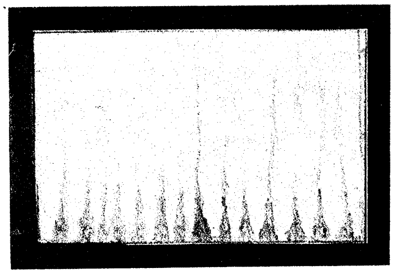

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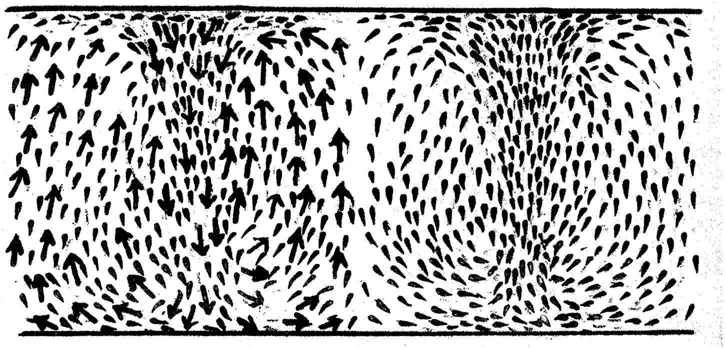

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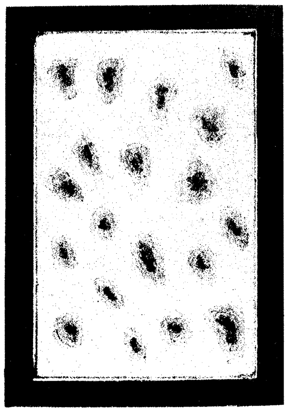

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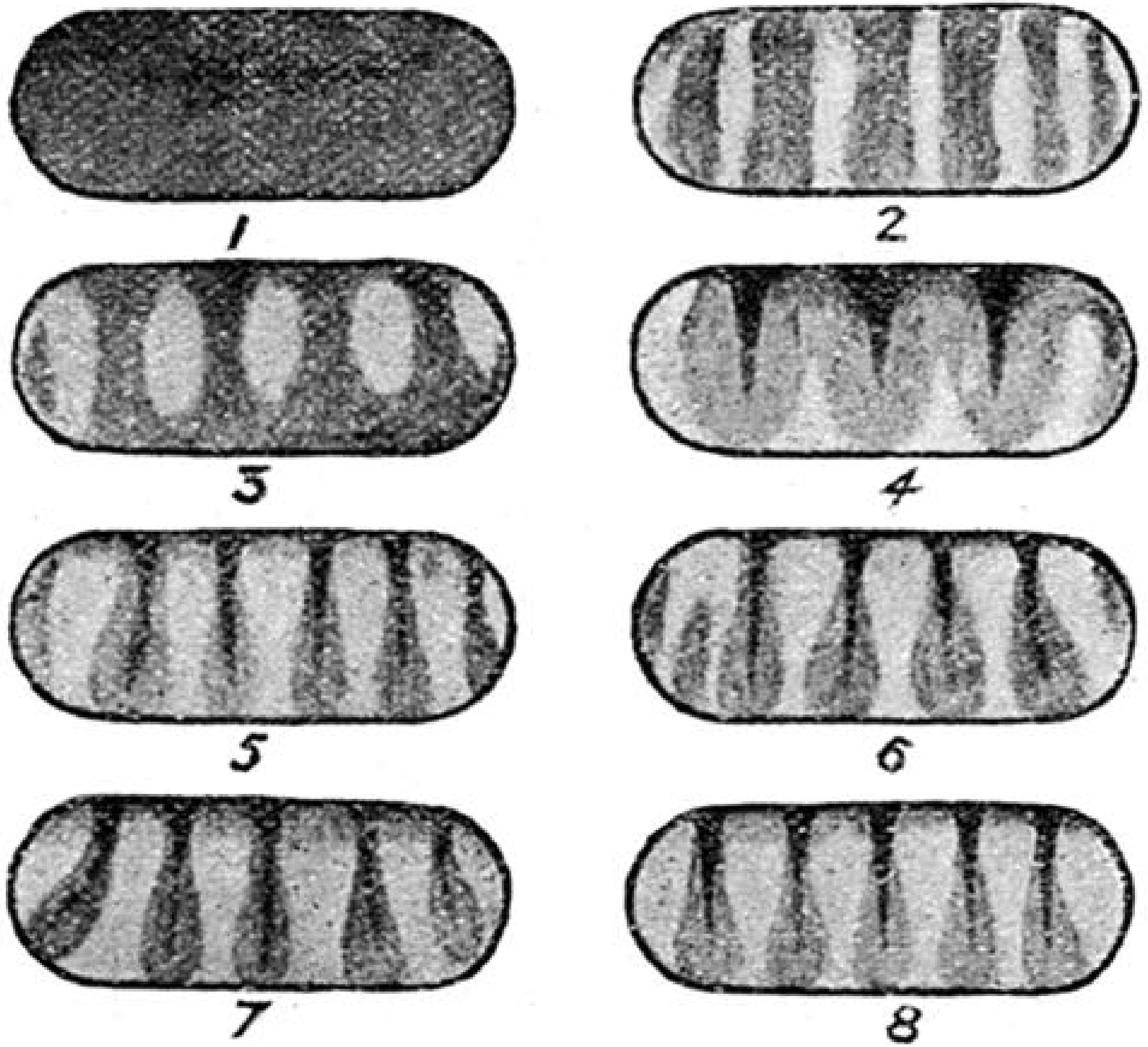
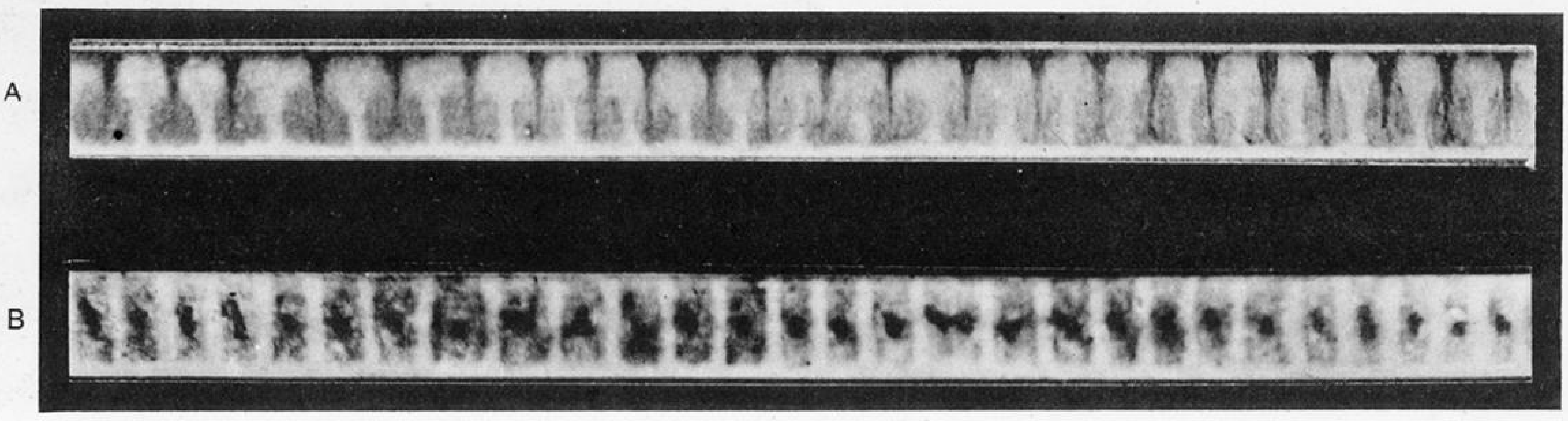


TEXT-FIG. 1.—Eight Stages in the Aggregation of *Euglena viridis* in a cell 1 in. × 3/8 in. × 1/16 in. Sketched at intervals of $\frac{1}{2}$, 5, 4, 20, 16, 32, 42, and 25 minutes respectively. Stage 1 was sketched half a minute after the experiment was started.



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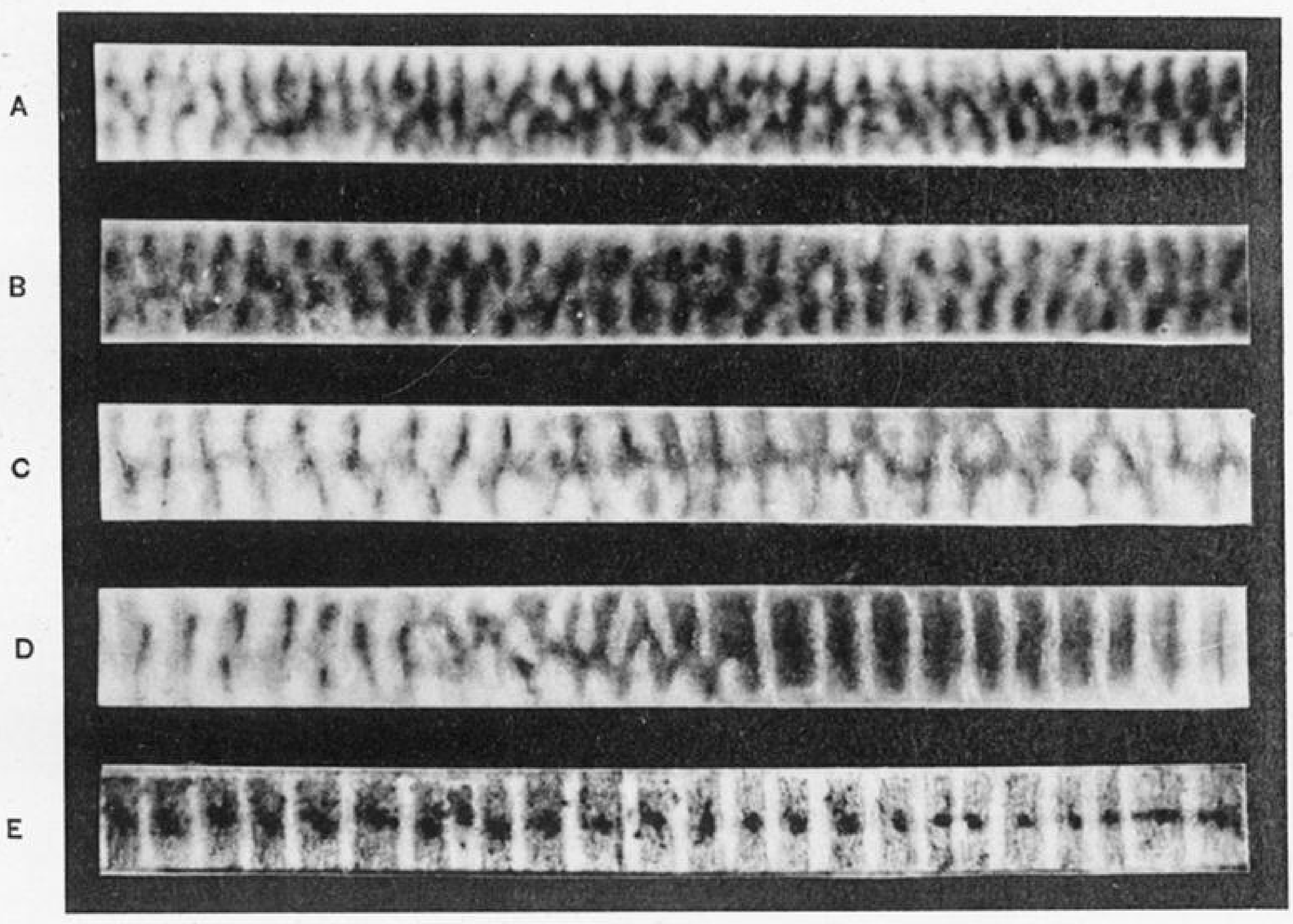

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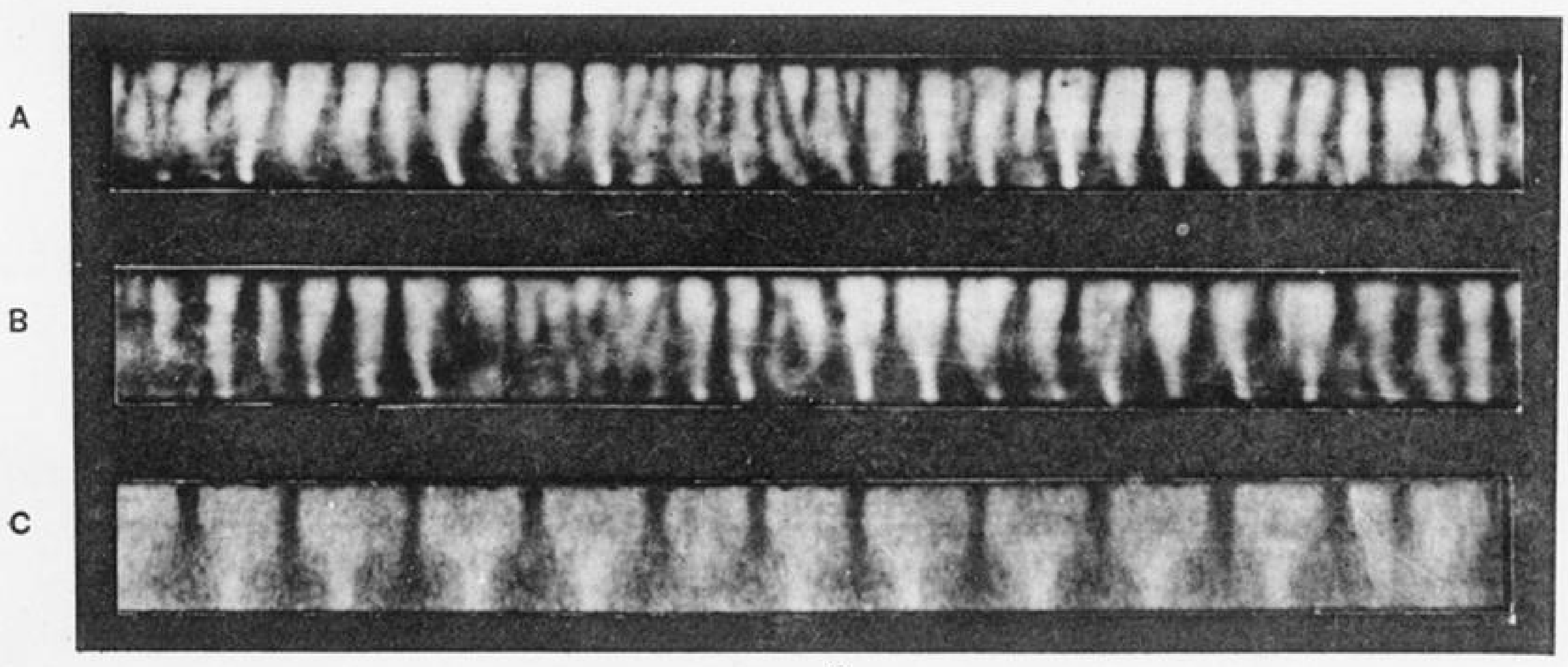

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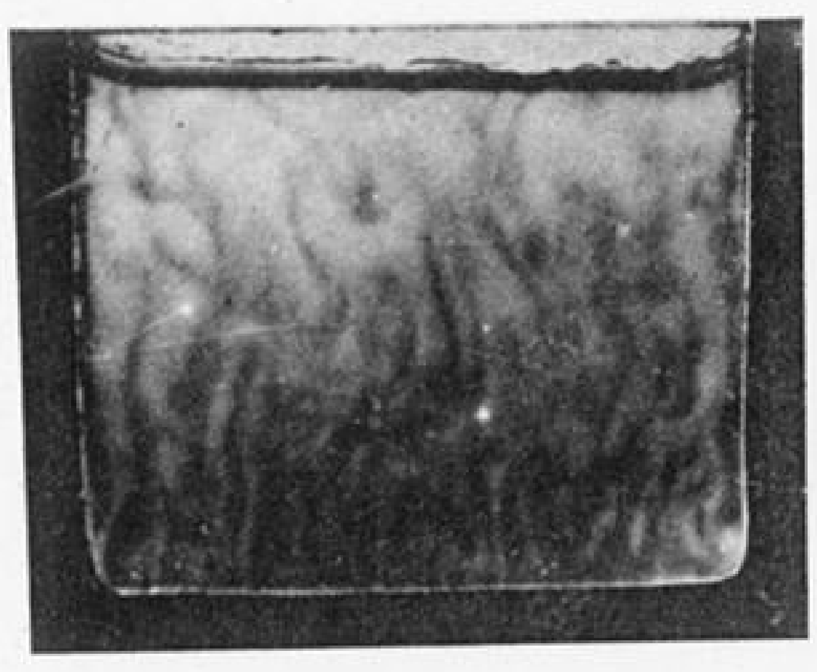

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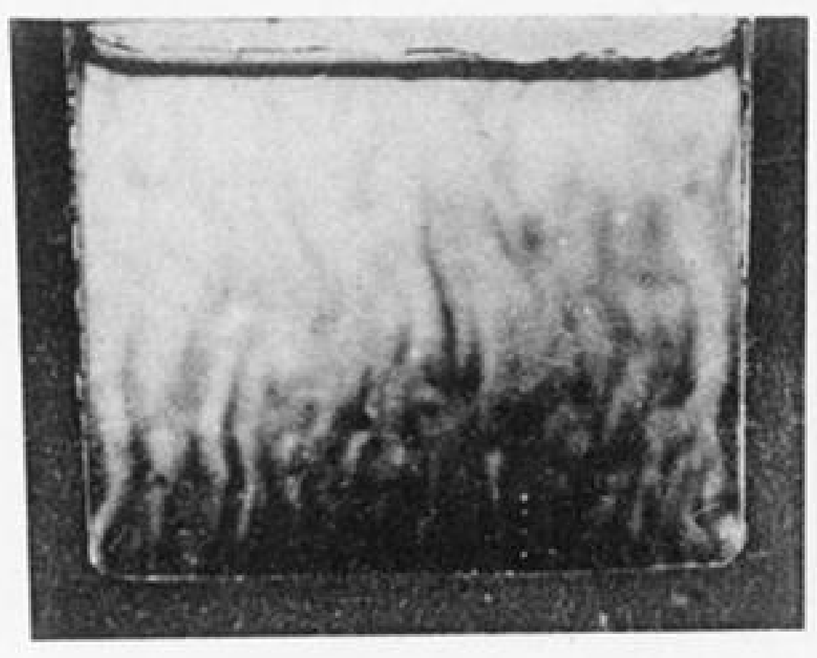

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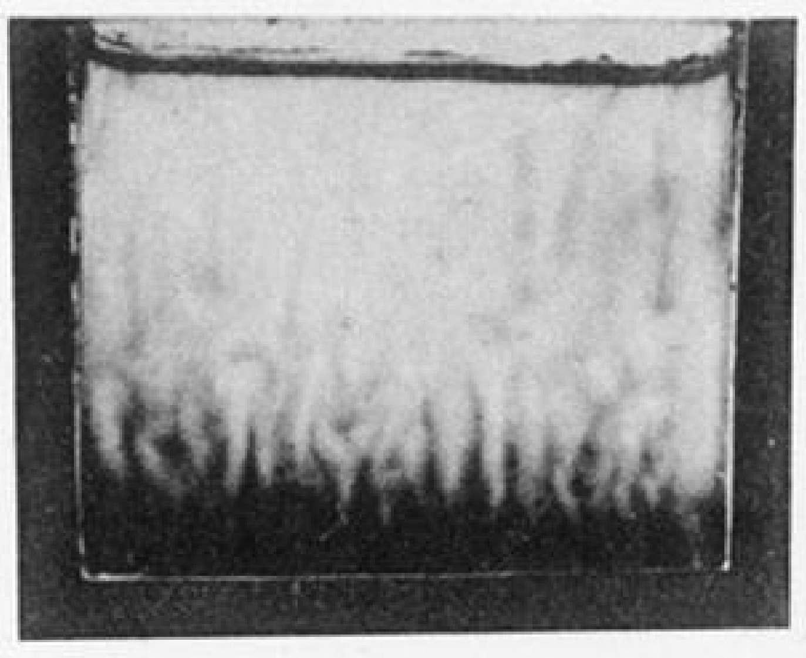

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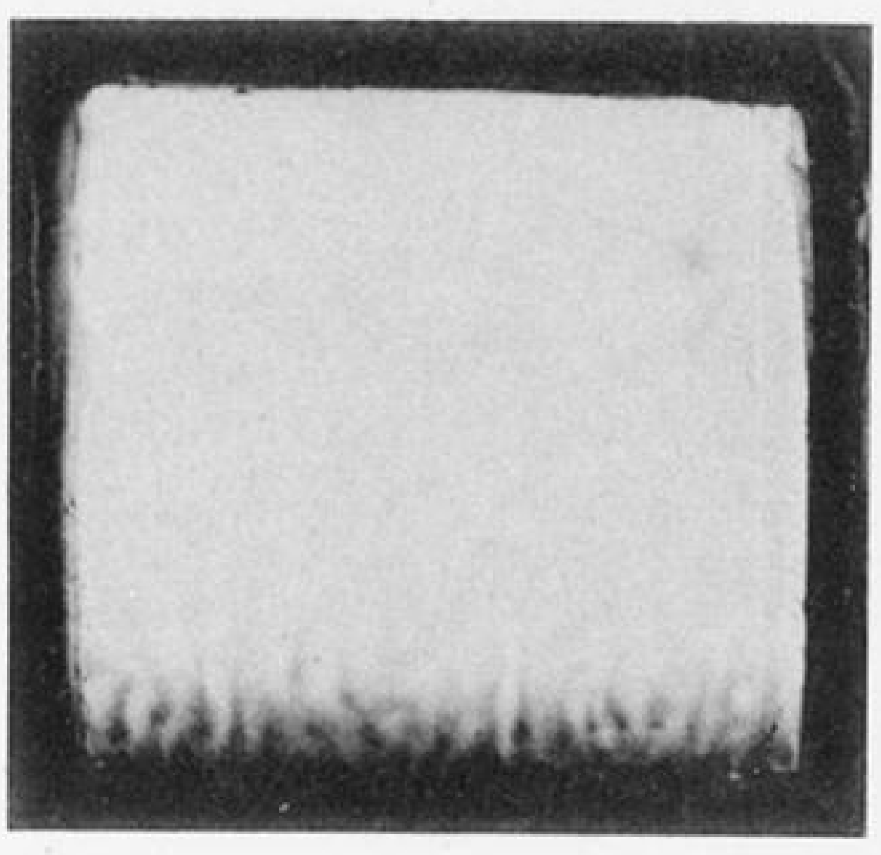

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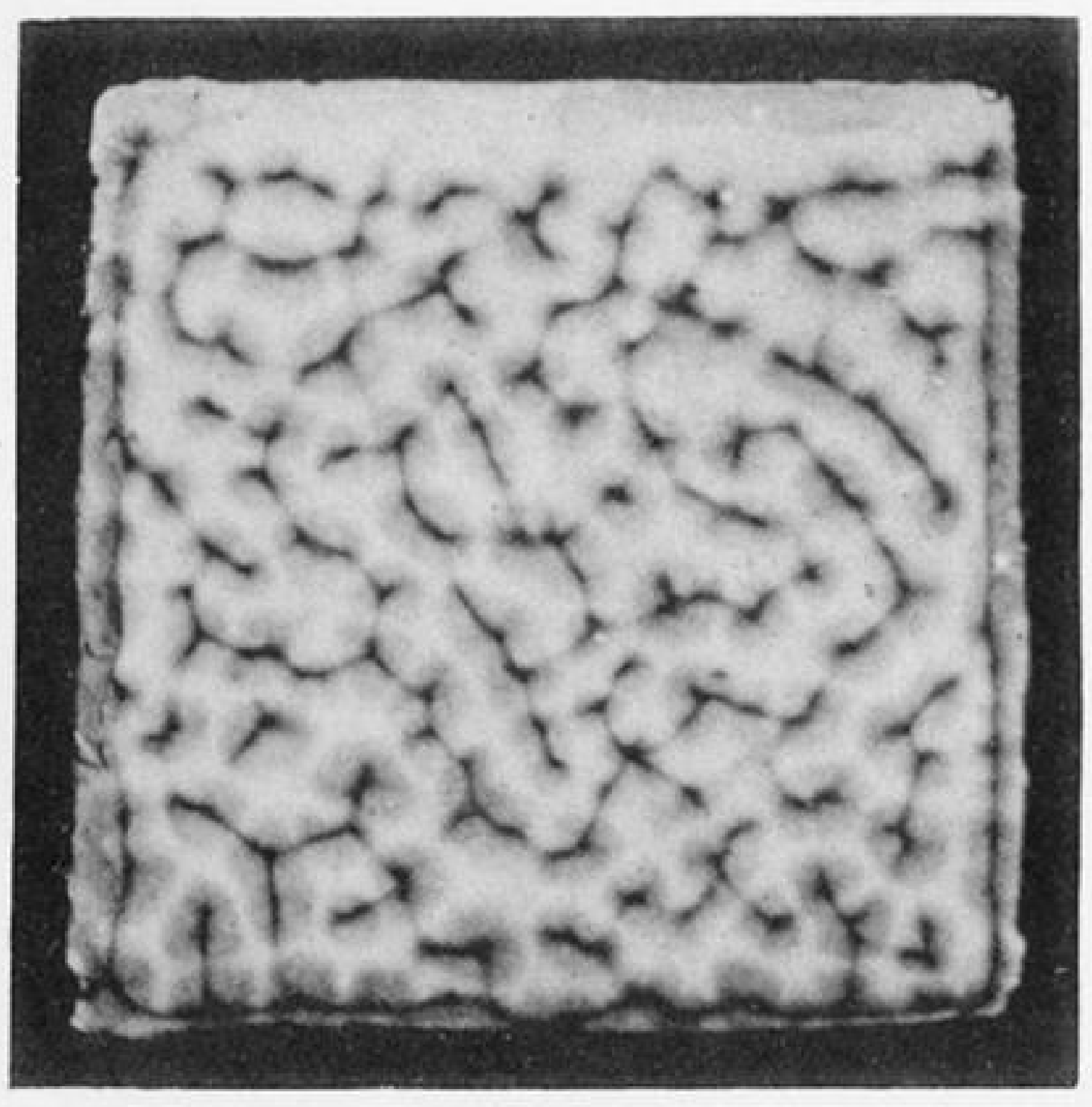

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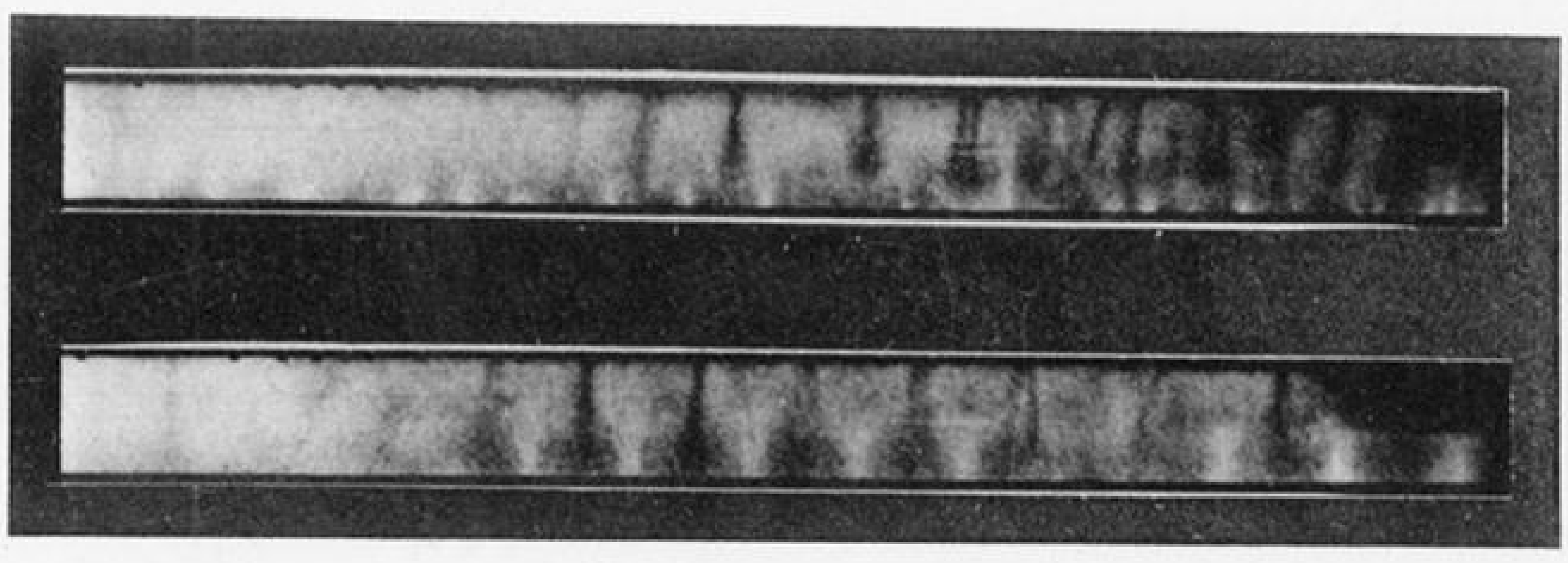

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PLATE 32.

Fig. 1.—*Euglena viridis* in a tube placed horizontally; A, seen from the side, B, from above.

Fig. 2.—Five stages in the grouping of *E. viridis* in a horizontal tube, kept in the dark, photographed from above.

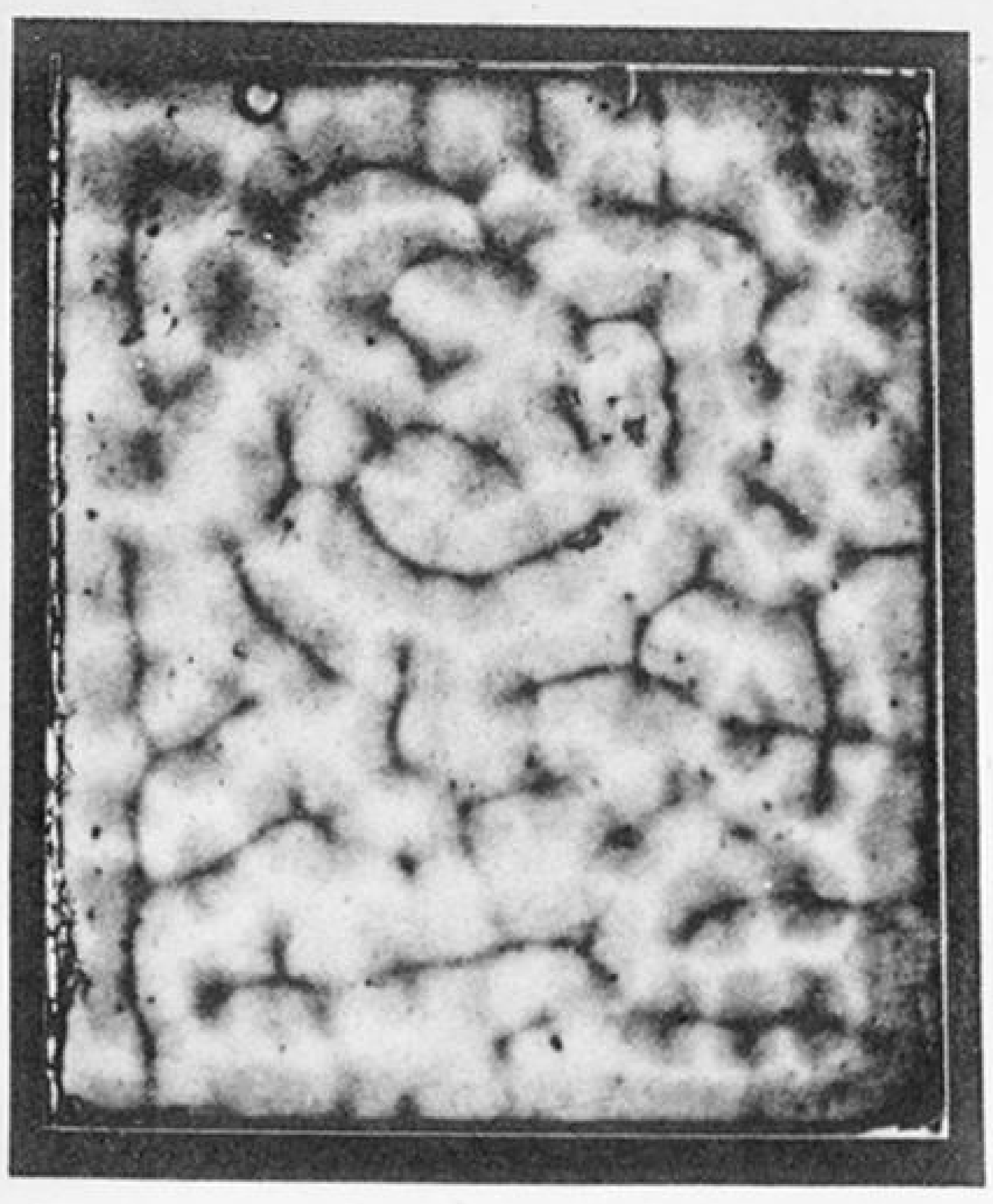
Fig. 3.—Three stages photographed from the side.

Figs. 4–6.—Stages in the movements of Euglena in an upright cell with the upper surface of the water in contact with air, photographed at intervals of five minutes.

Fig. 7.—Hermetically sealed cell, showing Euglena at the base. The vertical streaks are the downward-moving streams of Euglena. The upper part of the liquid contains very few of the organisms.

Fig. 8.—Hermetically sealed cell, showing Euglenæ aggregating from a homogeneous surface film, brought to the upper surface of the liquid in the cell by allowing light to fall perpendicularly upon it. As soon as the Euglenæ had accumulated at the surface in a homogeneous layer, the cell was placed in the dark, and immediately the aggregation began as shown in the figure.

Fig. 9.—Two stages showing the beginning of the aggregation in a horizontal tube with very few Euglenæ, photographed from the side.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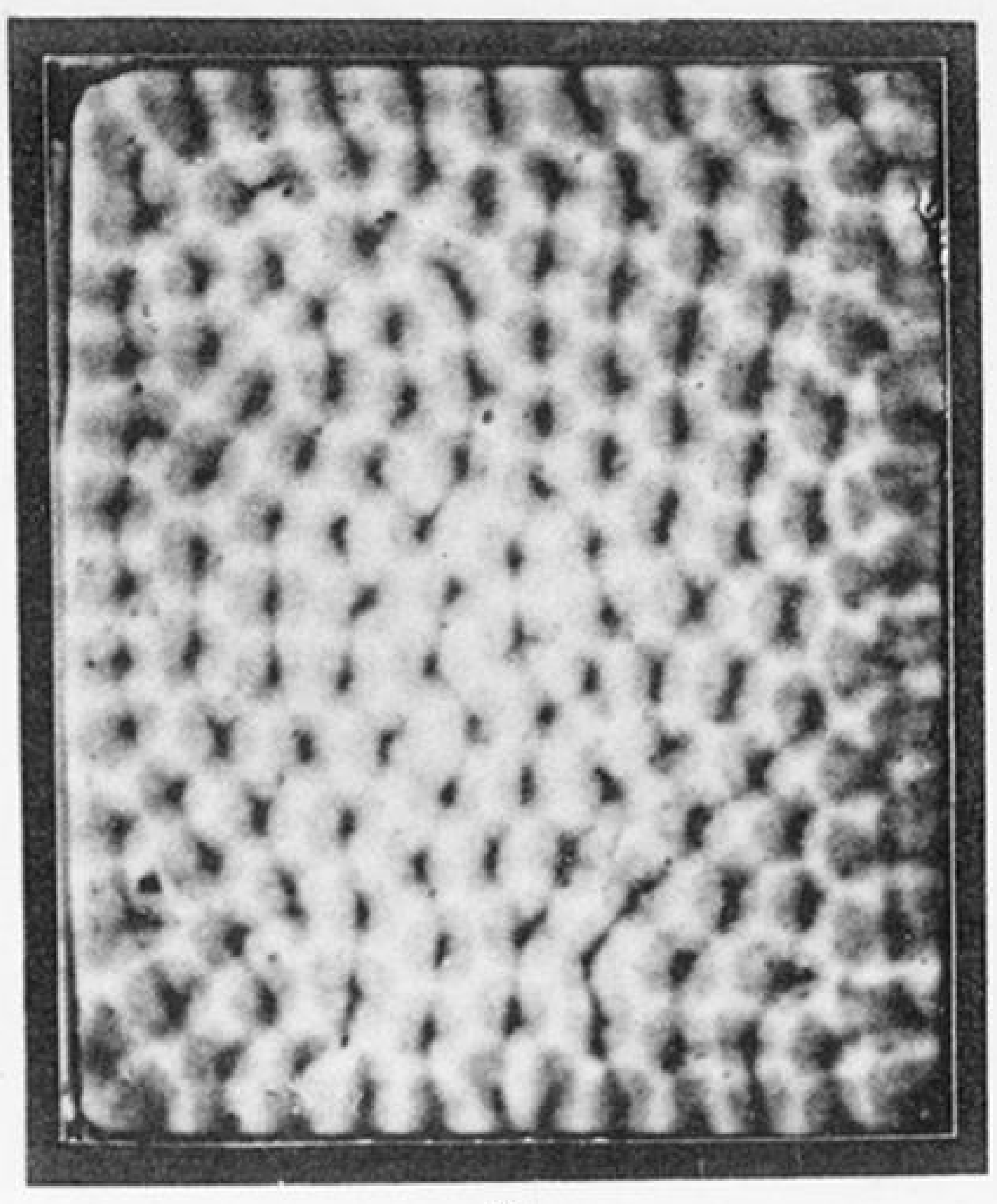
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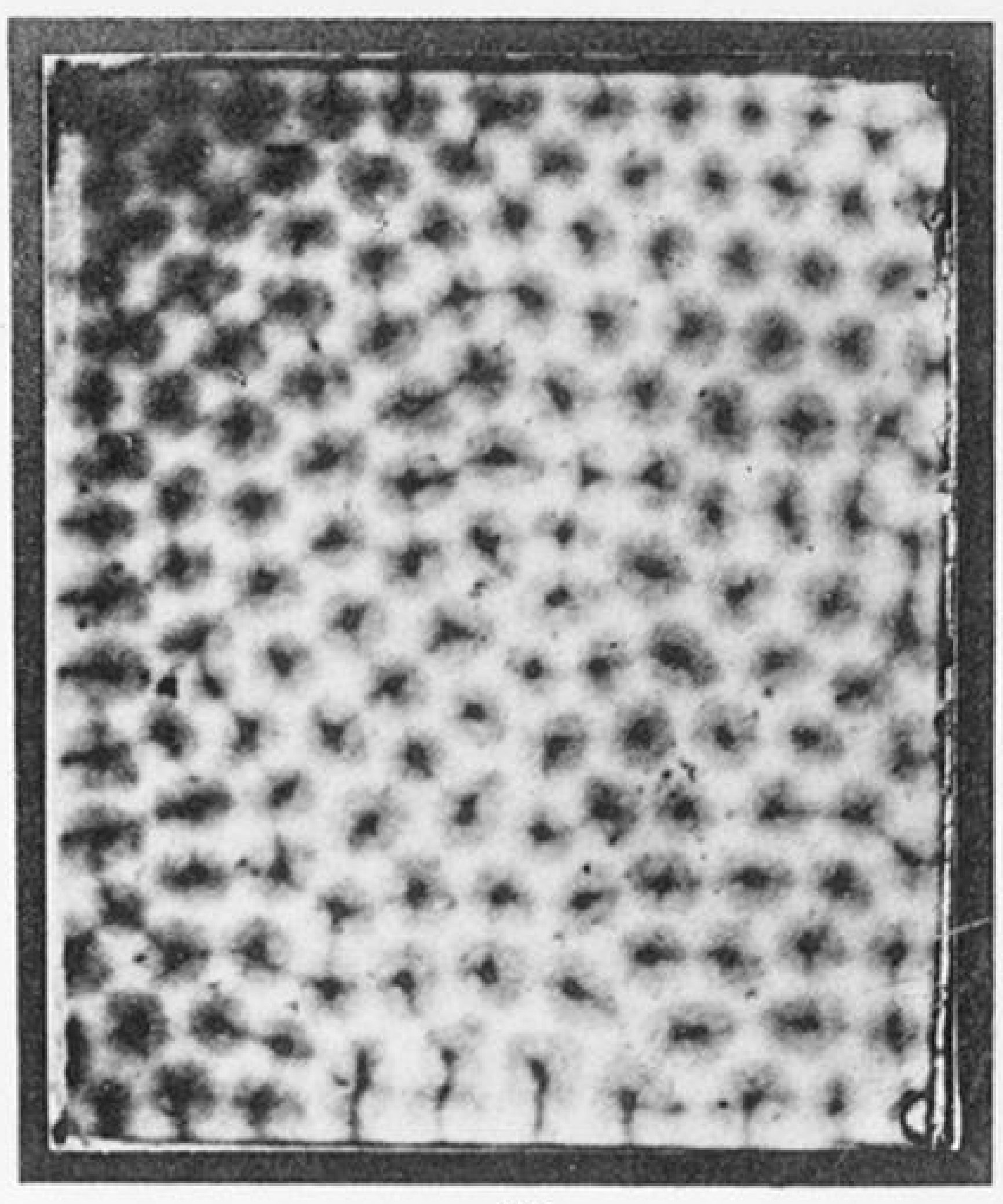
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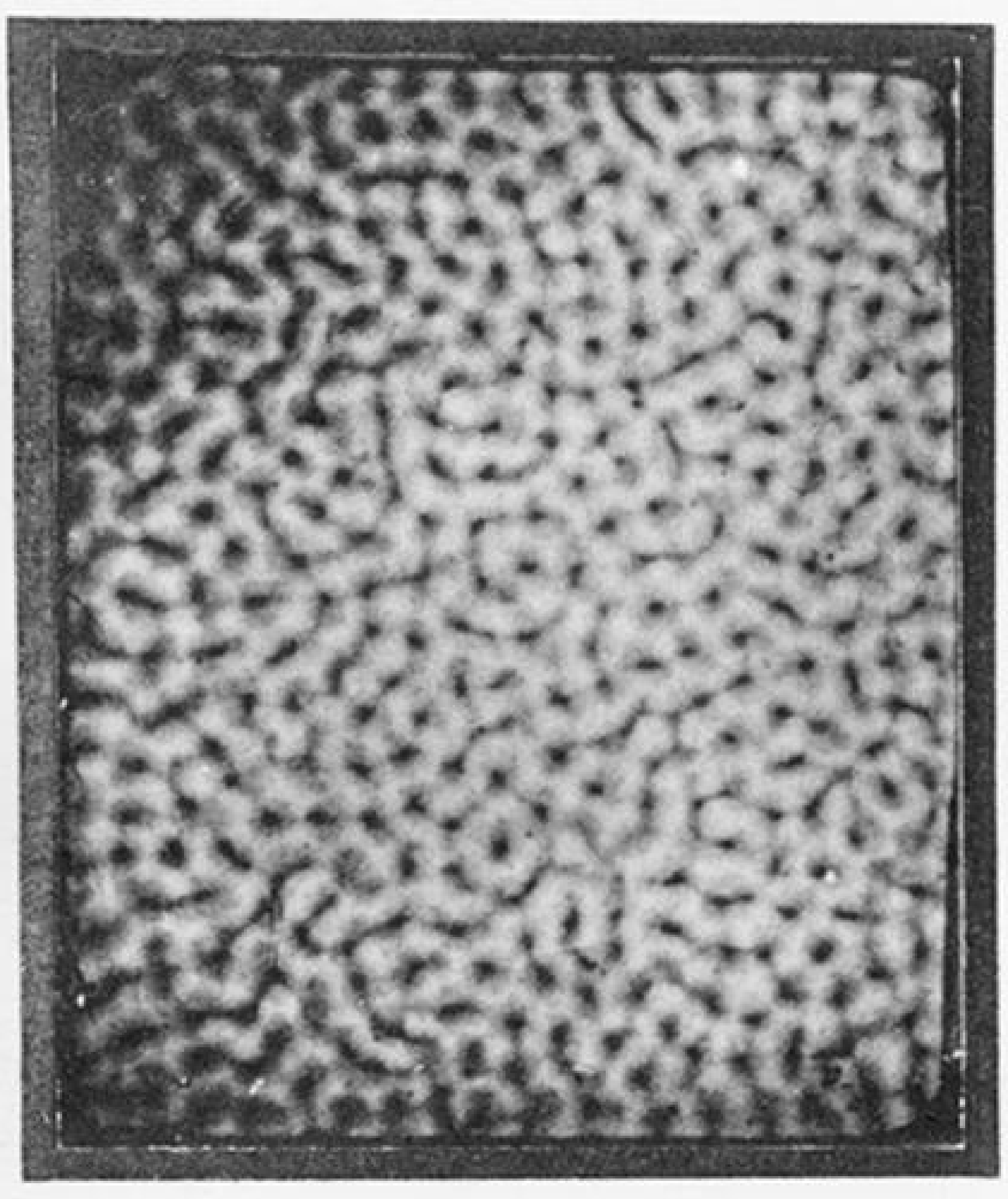
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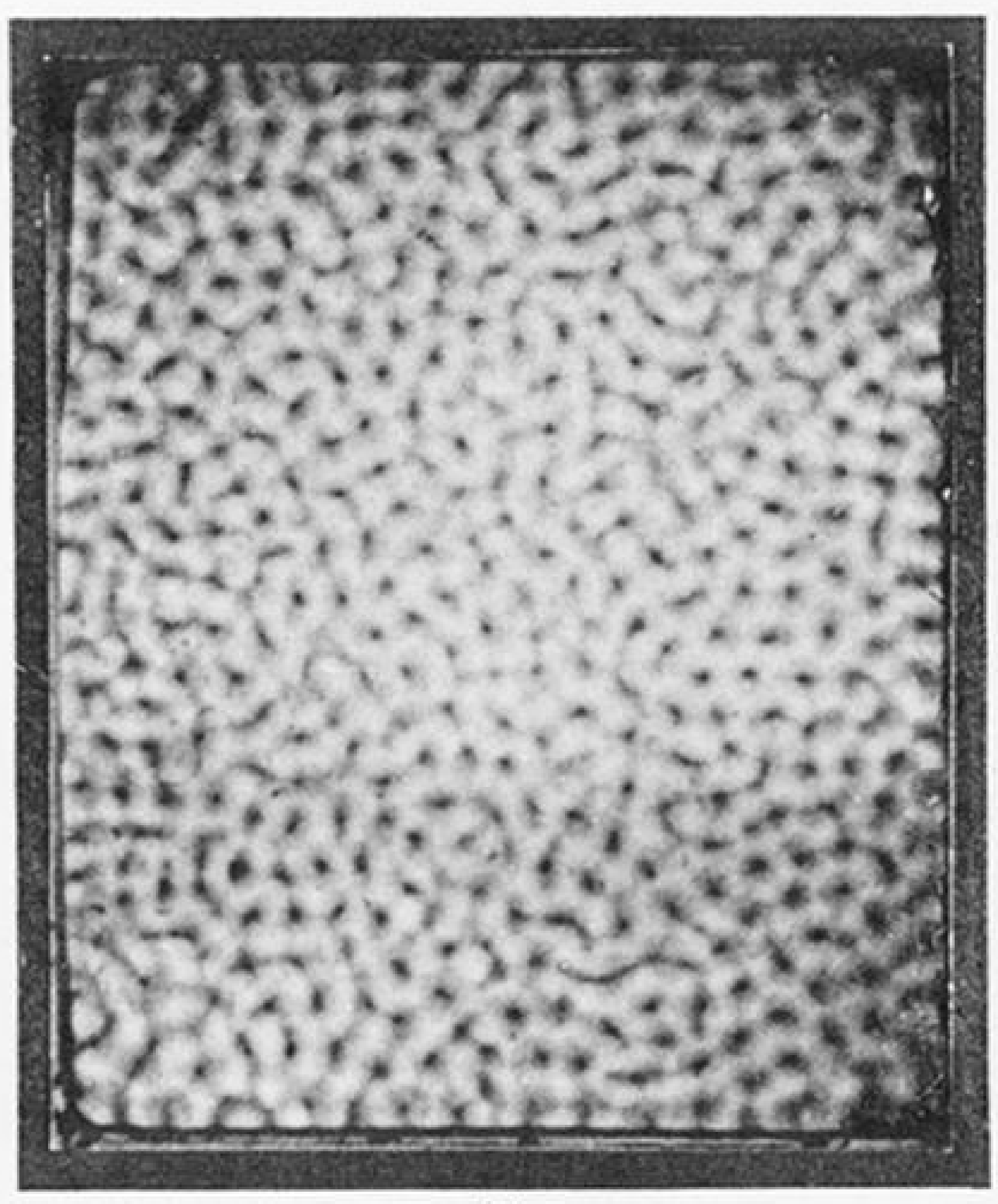
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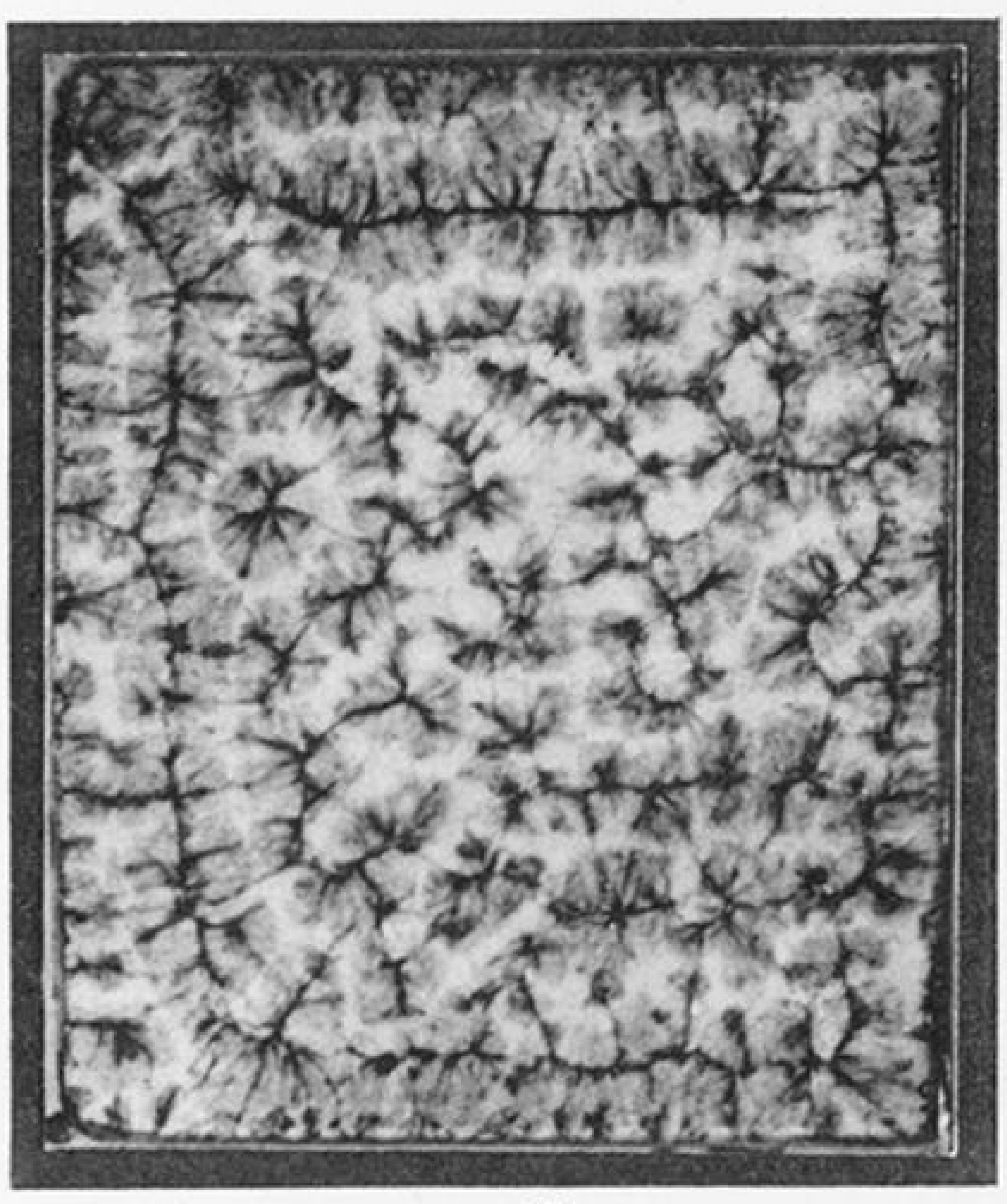
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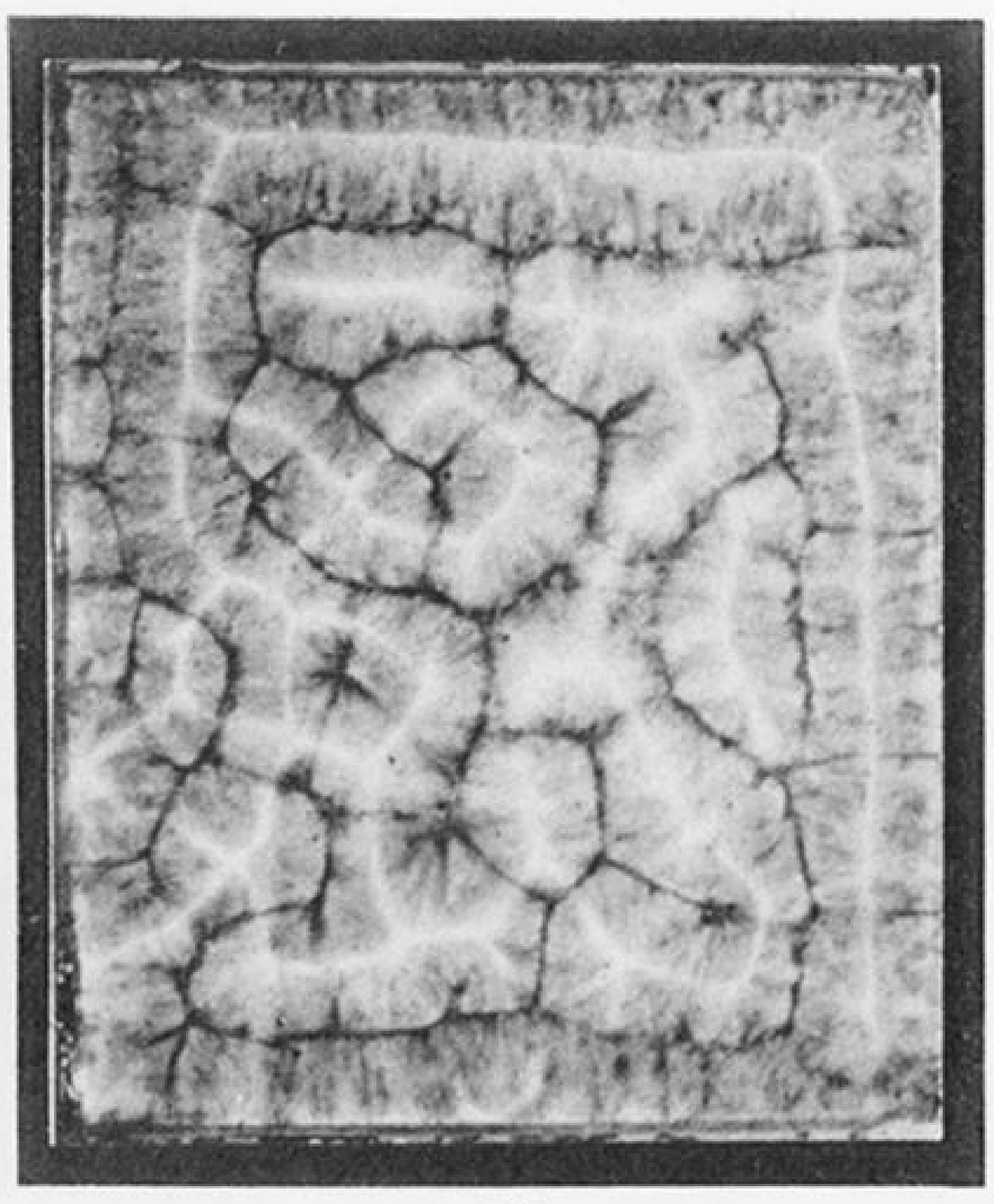
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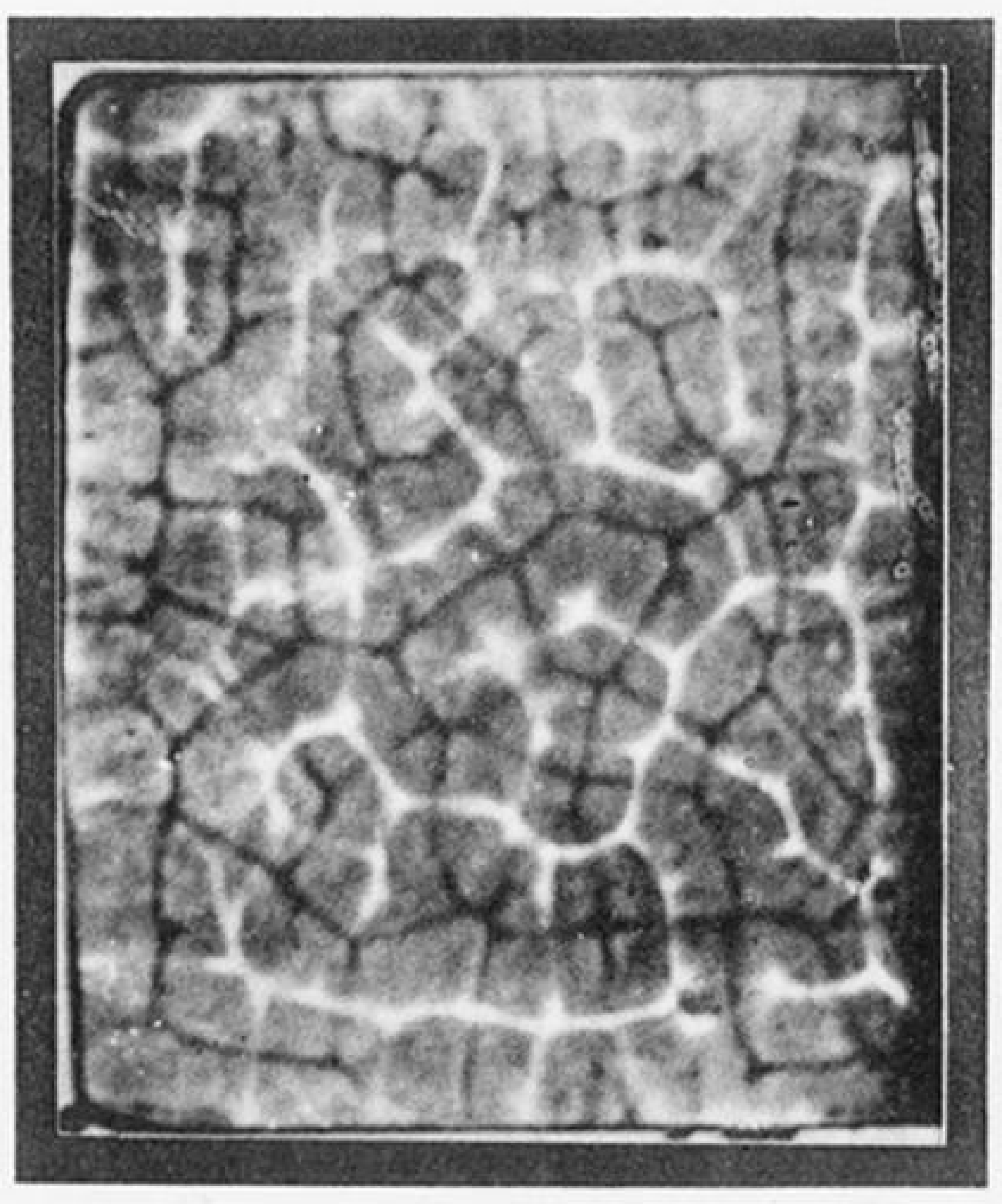
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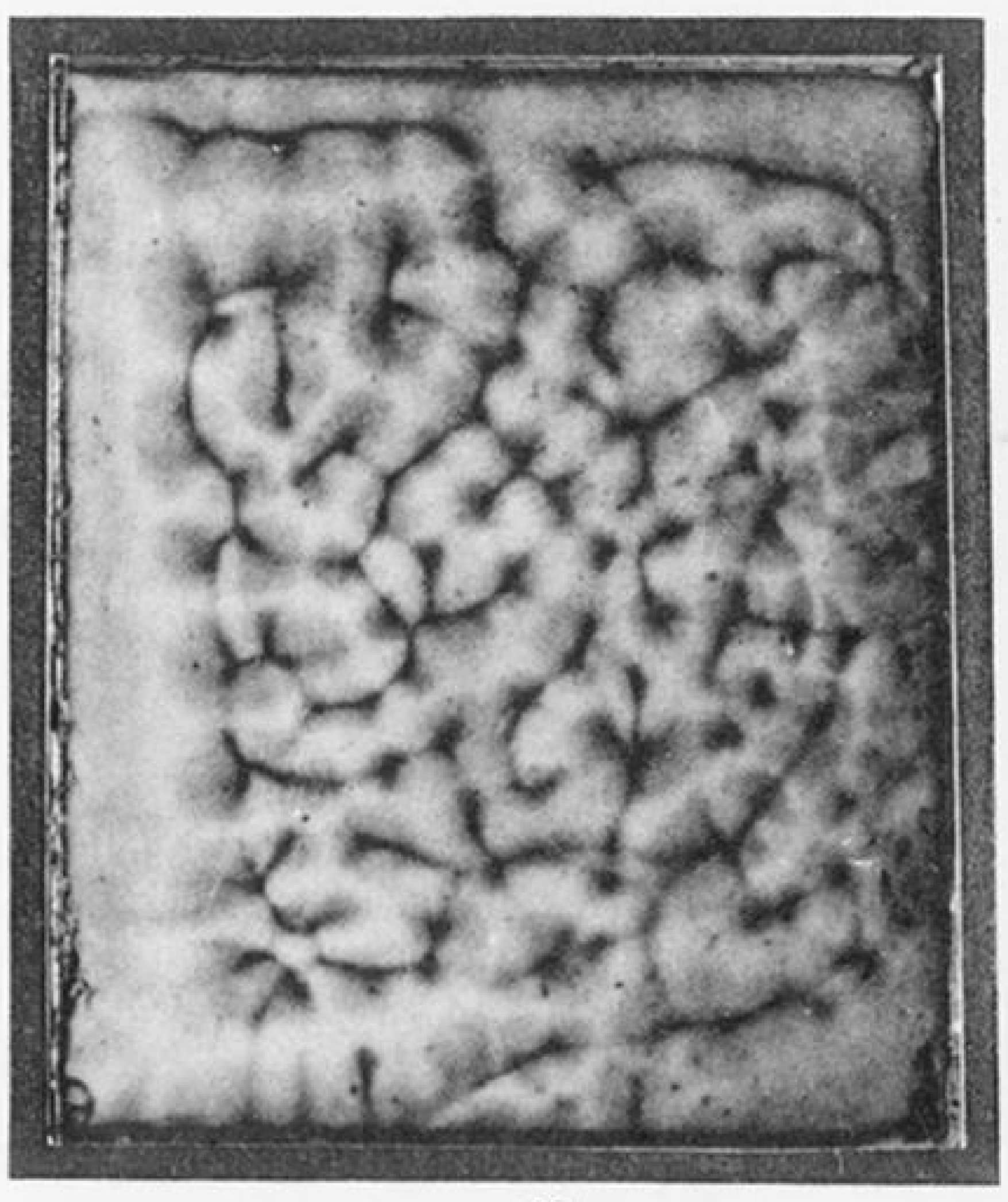
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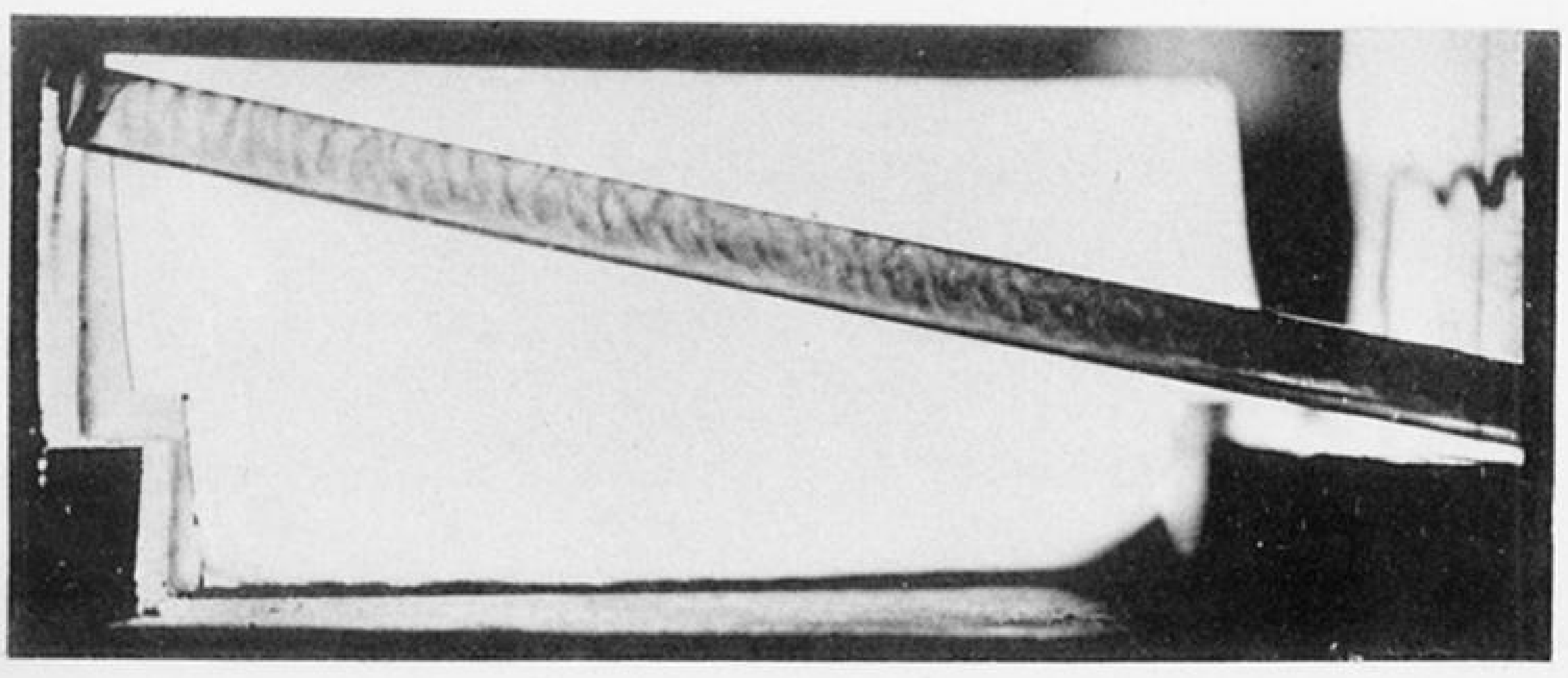
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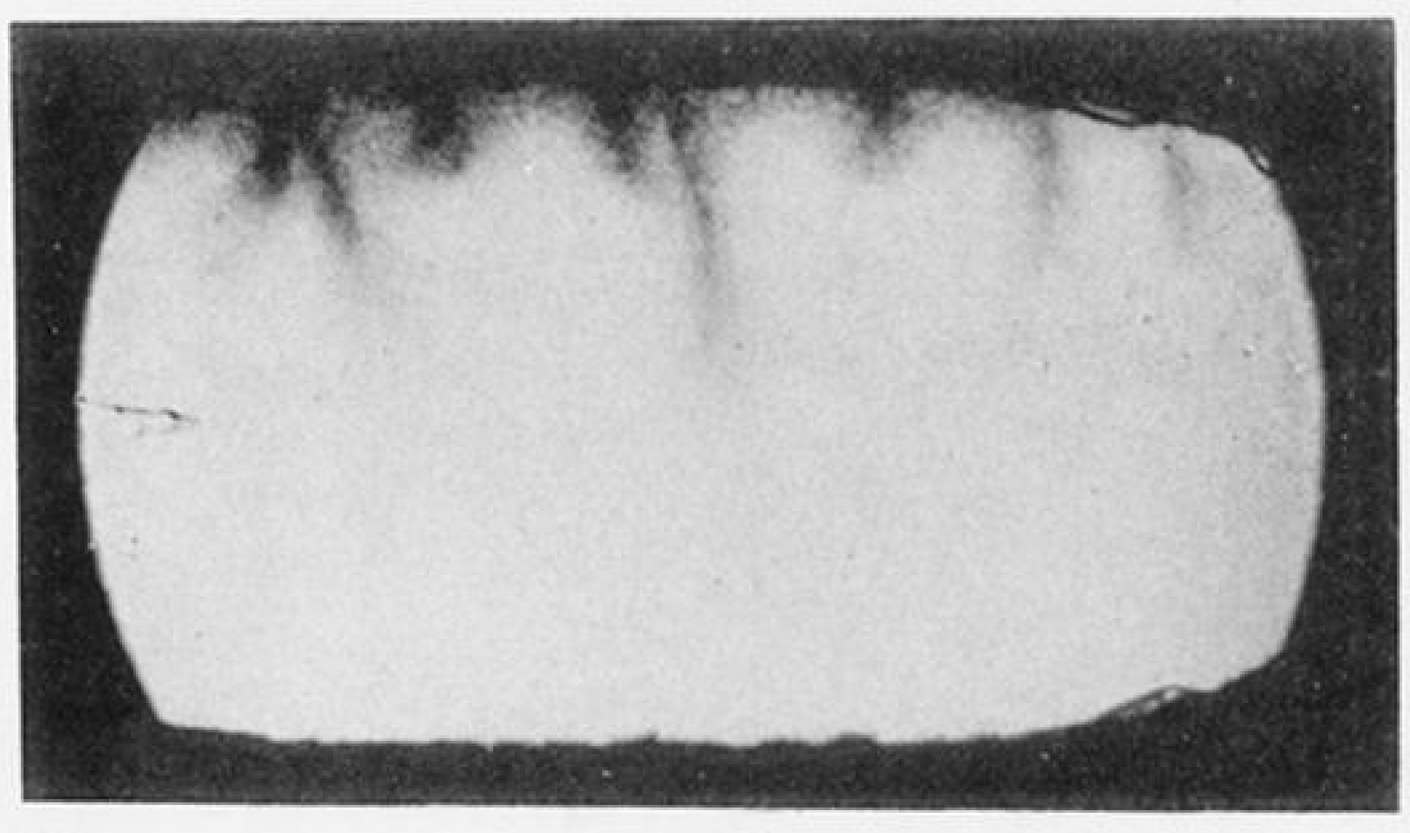
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PLATE 33.

Figs. 10–14.—Aggregation of living Euglenæ in a flat hermetically sealed cell, $\frac{5}{16}$-inch deep, placed horizontally in the dark.

Fig. 10.—Two and a half minutes after being placed in the dark. Network grouping.

Fig. 11.—Four minutes after being placed in the dark. Separate groups beginning to form.

Fig. 12.—Six minutes after being placed in the dark. Separate groups.

Fig. 13.—Seven minutes after being placed in the dark. The groups are beginning to break up into smaller ones.

Fig. 14.—Ten minutes after being placed in the dark. Small groups. The Euglenæ remained in this condition for several days.

Fig. 15.—Dead Euglenæ aggregating from a somewhat loose surface film.

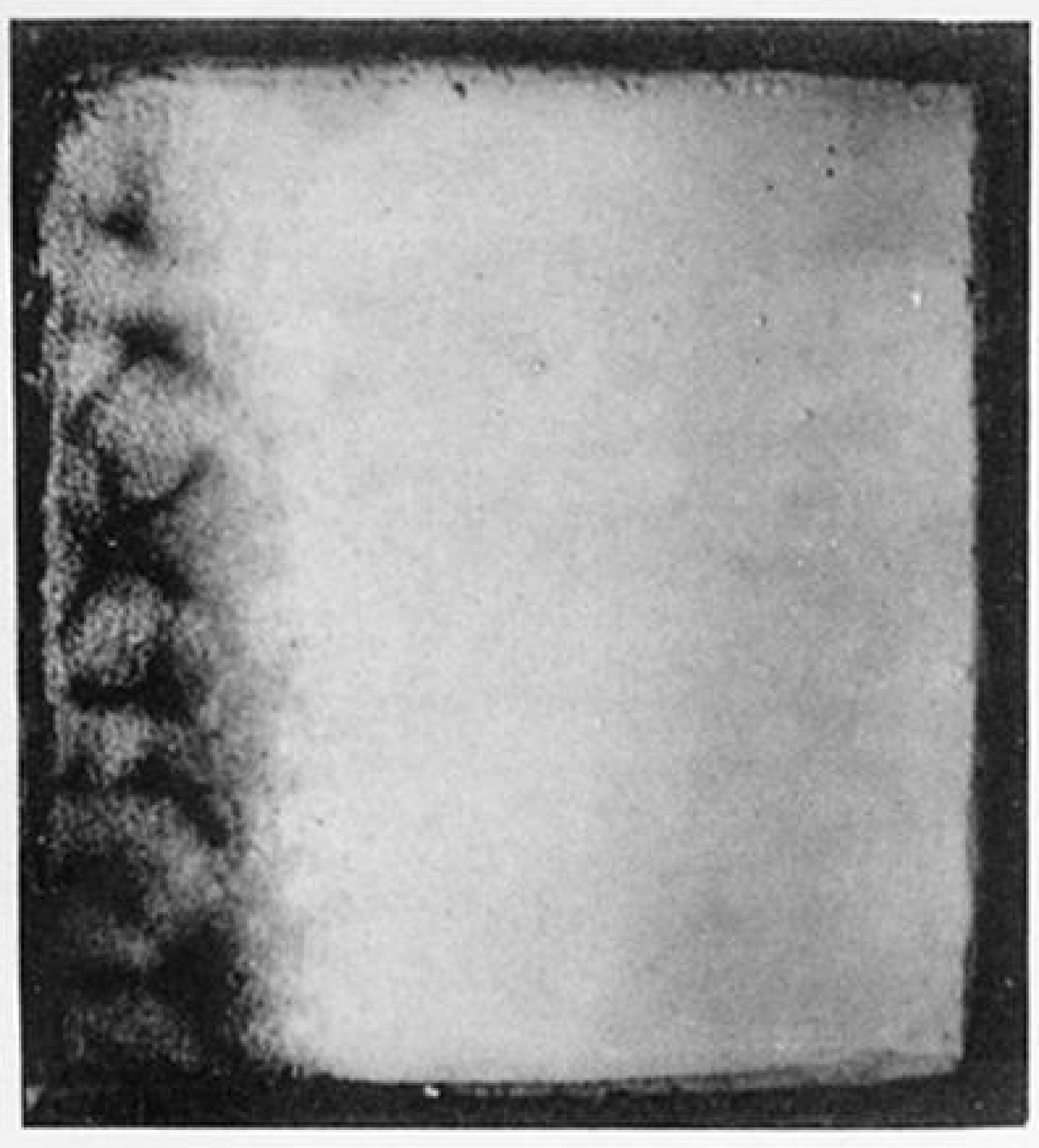
Fig. 16.—Dead Euglenæ aggregating from a firmer and more coherent film.

Fig. 17.—Manganese dioxide falling from a surface film formed by the settling of the precipitate to form a layer on the bottom of the vessel, which was then turned gently upside down.

Fig. 18.—Living Euglenæ falling from a surface film, obtained by attracting the Euglenæ to the bottom of the vessel, by means of an appropriate arrangement of the light, to form a homogeneous layer, which was then turned upside down.

Fig. 19.—Euglenæ in a slanting tube. The tube was first placed horizontally in a dark chamber. As soon as the grouping had begun the tube was tilted, and it was then seen that the streams of Euglenæ became bent into a vertical direction, thus showing that the streams are under the influence of gravity.

Fig. 20.—Shallow upright cell in which Euglenæ are just beginning to fall from an upper layer. Magnified about 4 diameters.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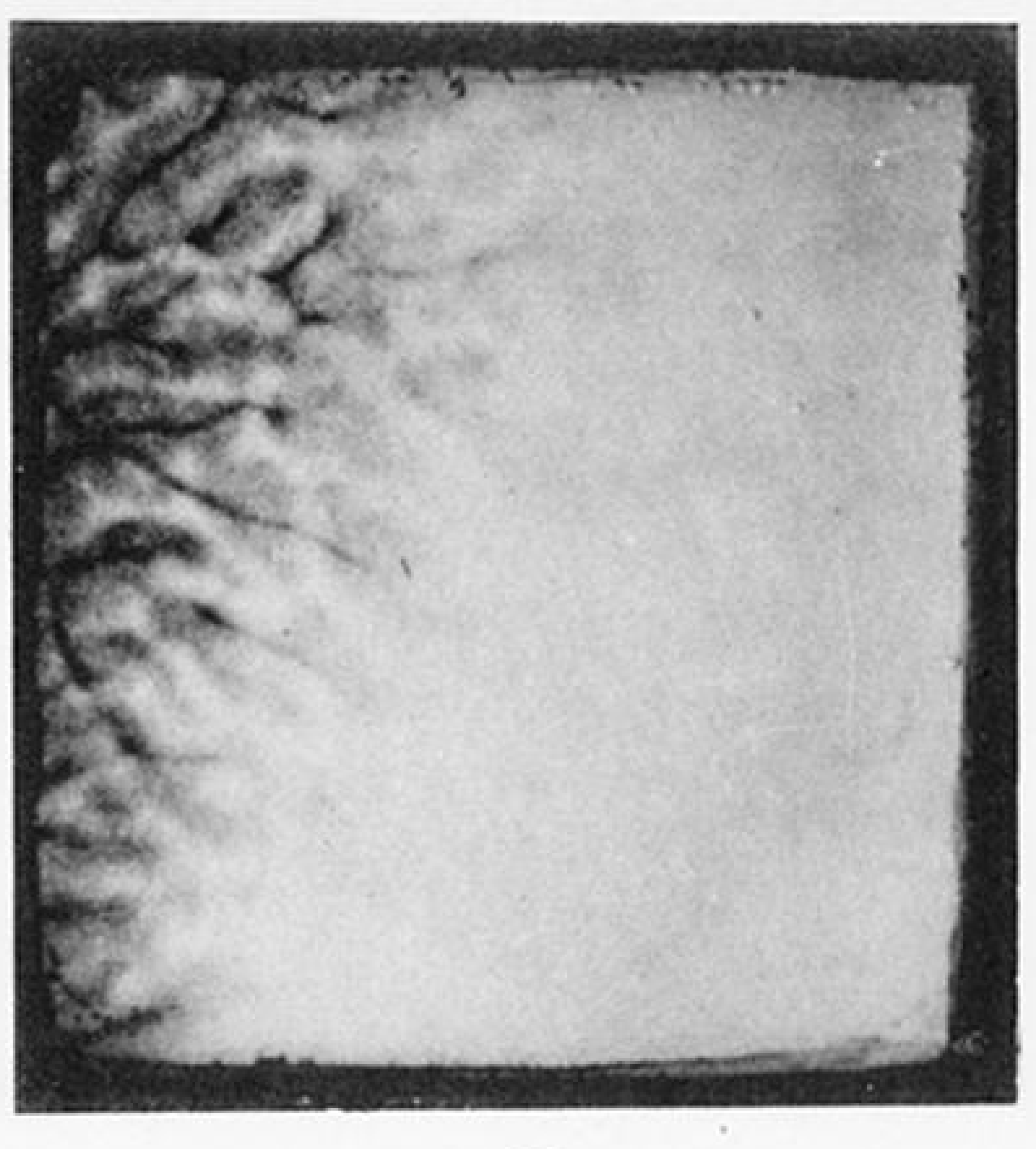
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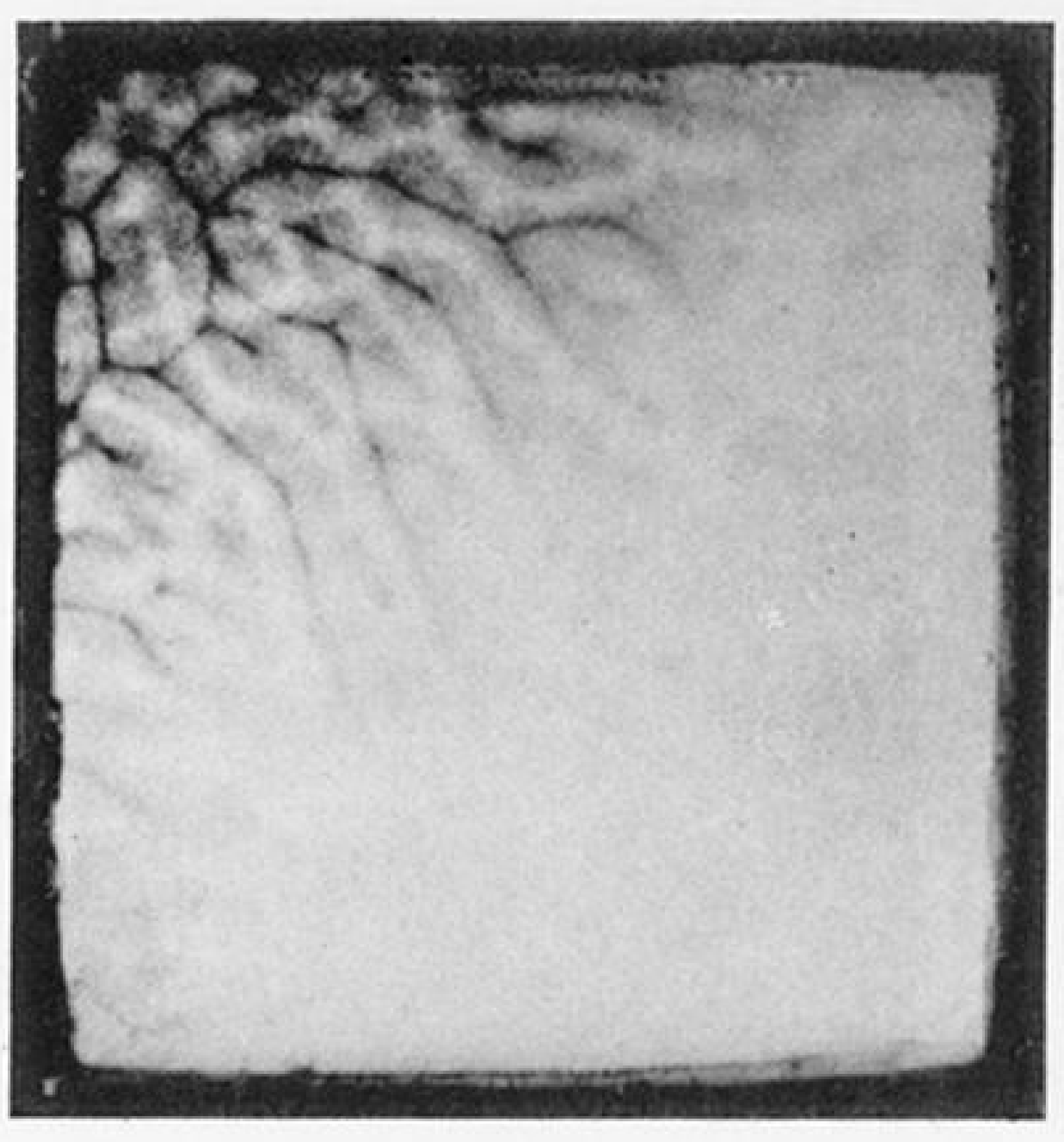
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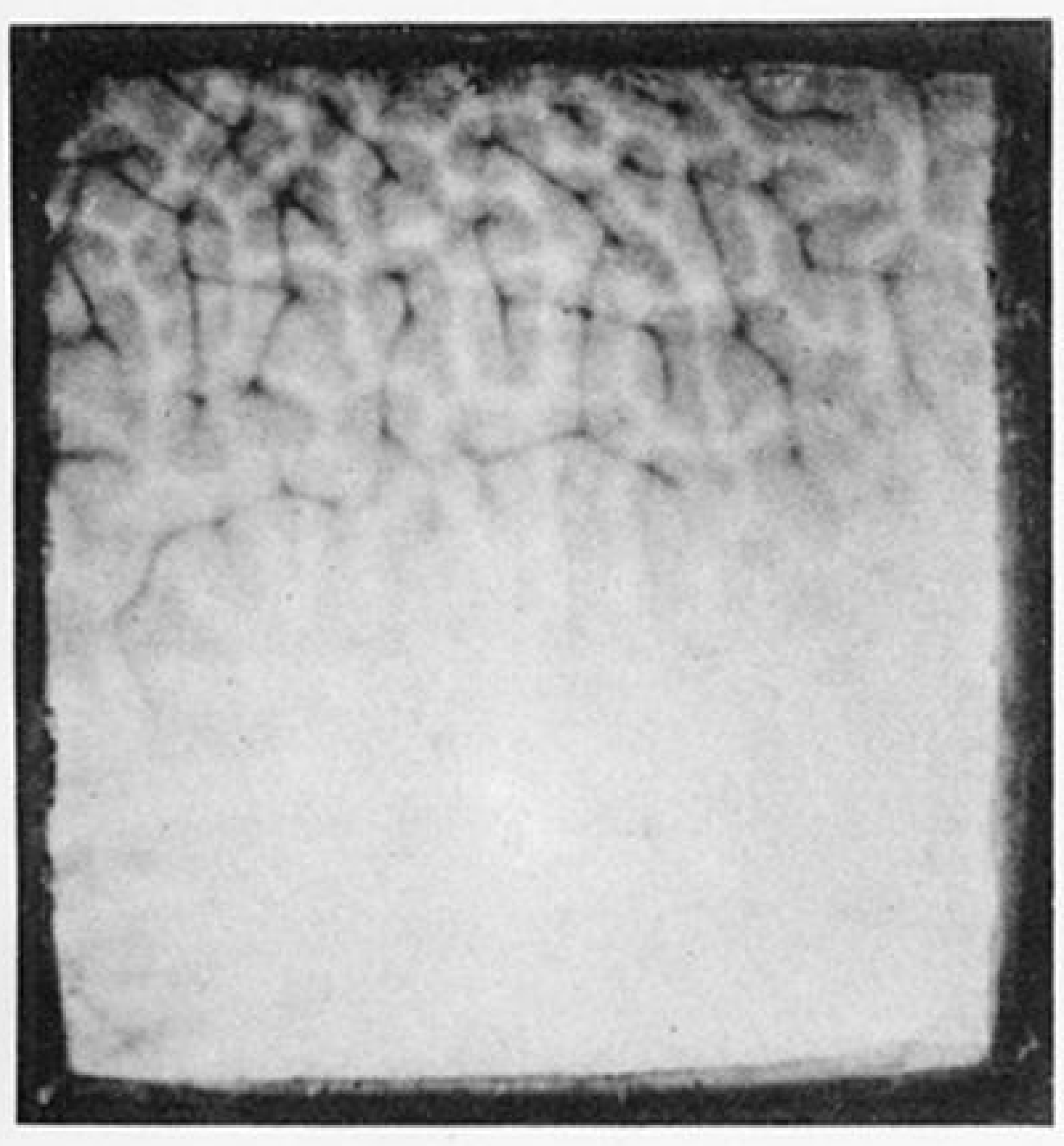
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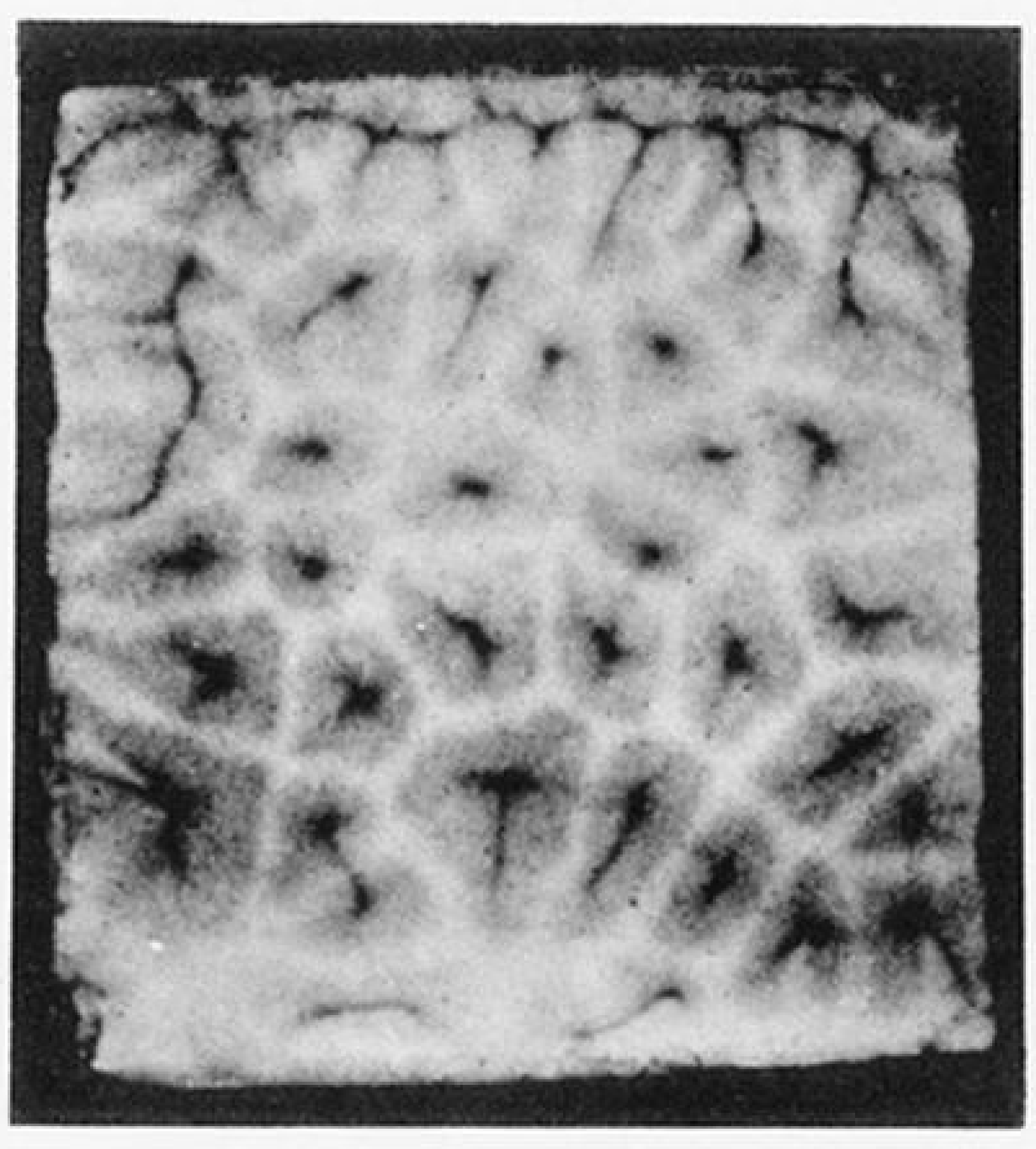
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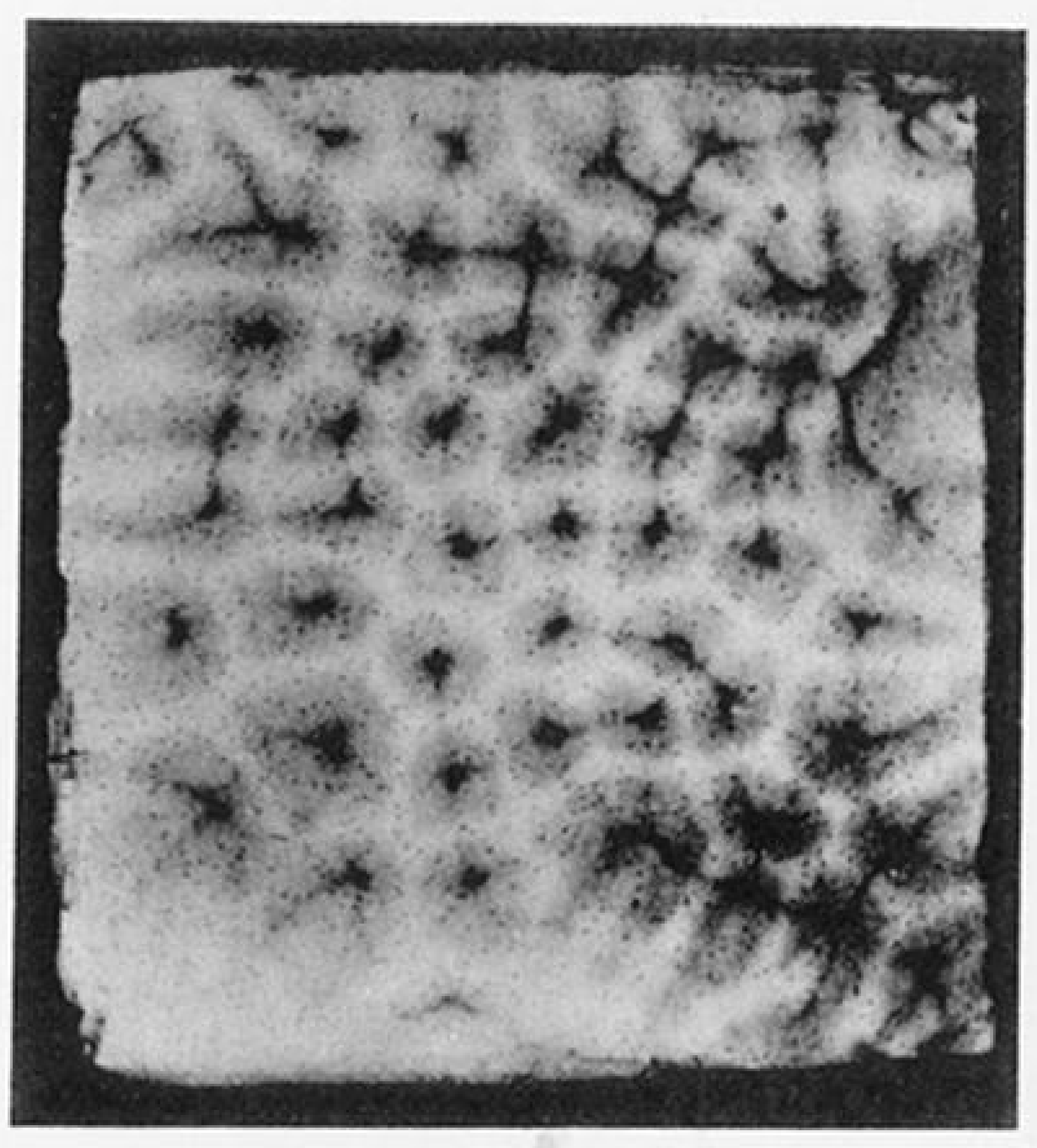
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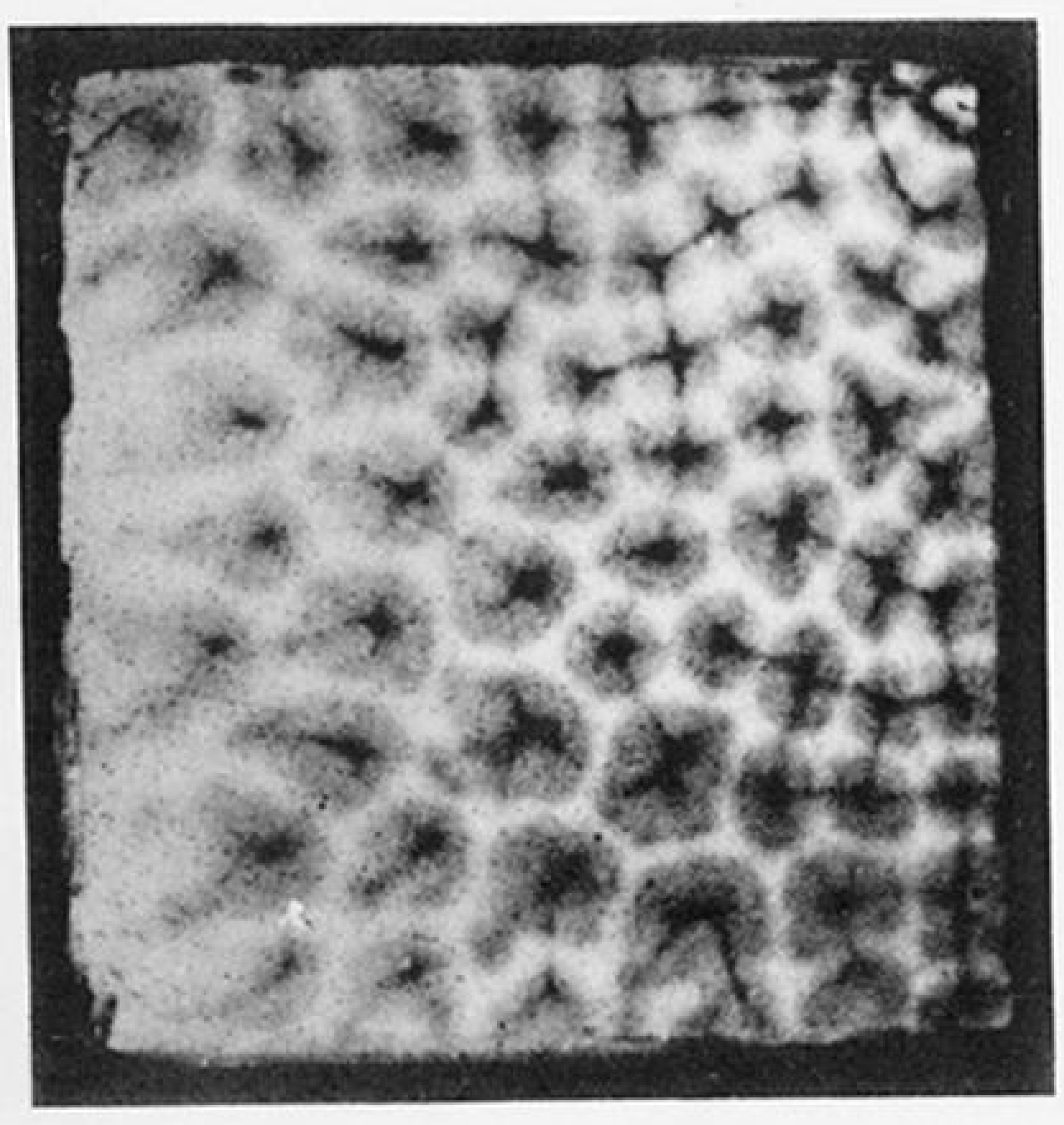
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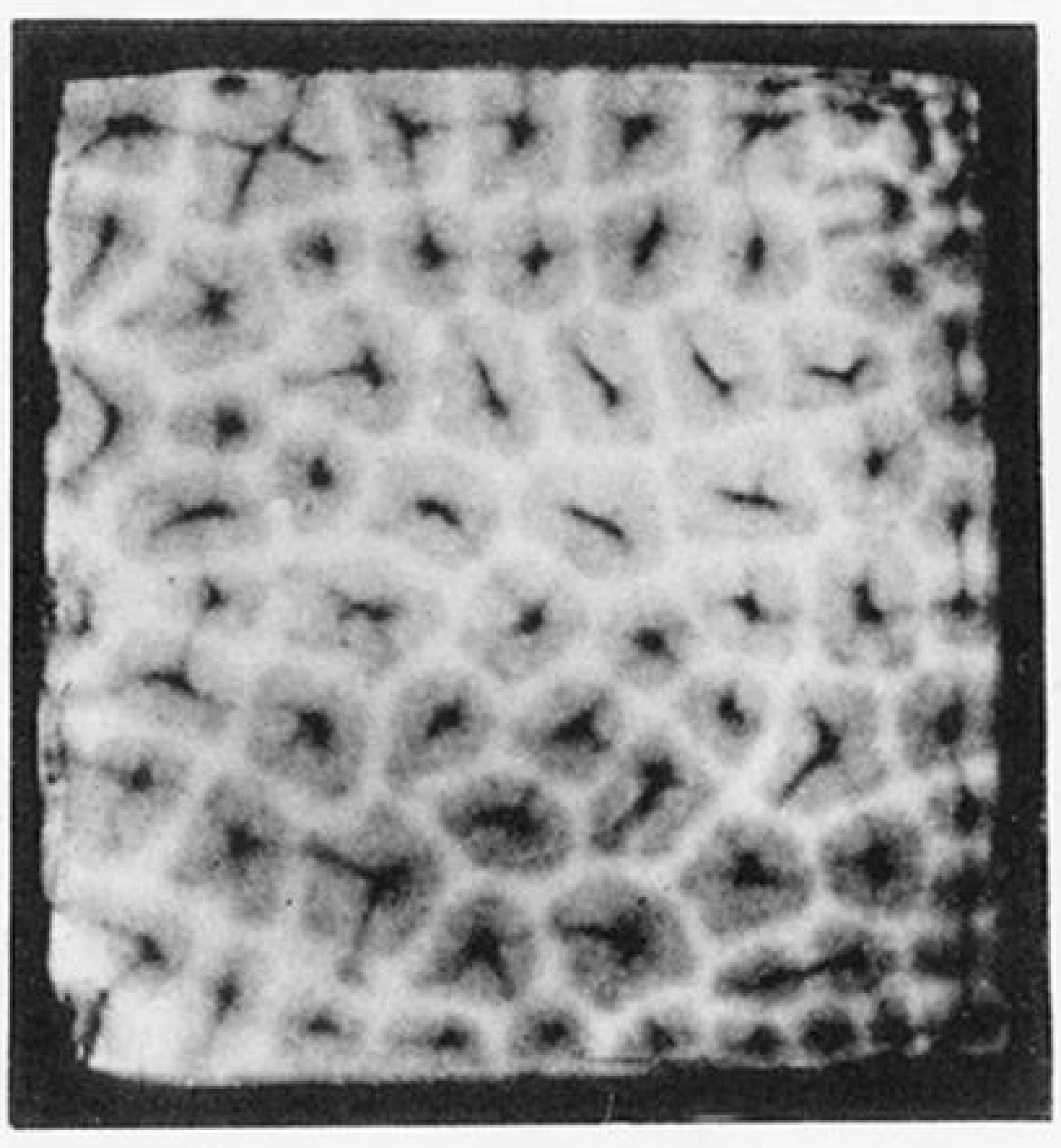
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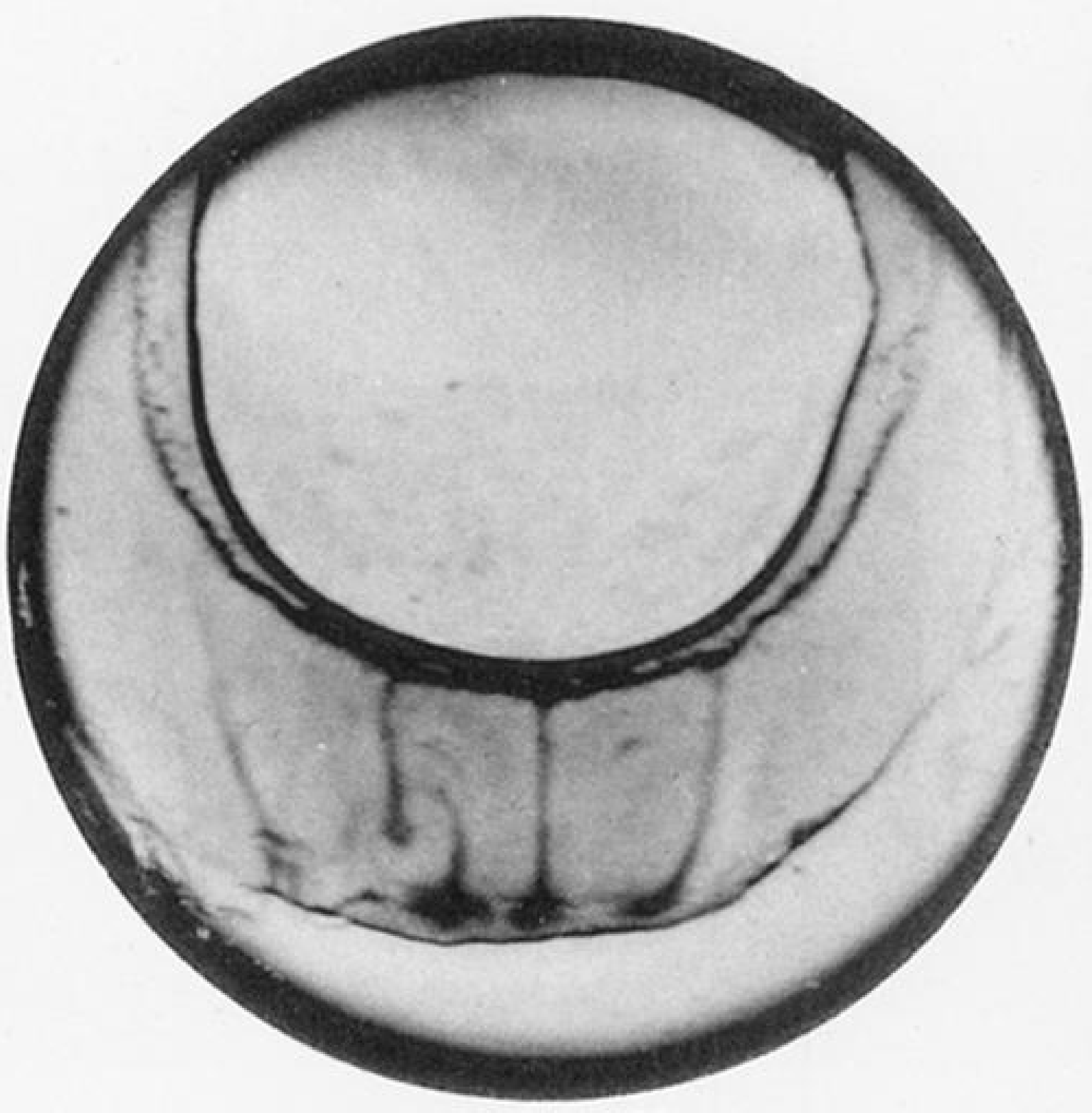
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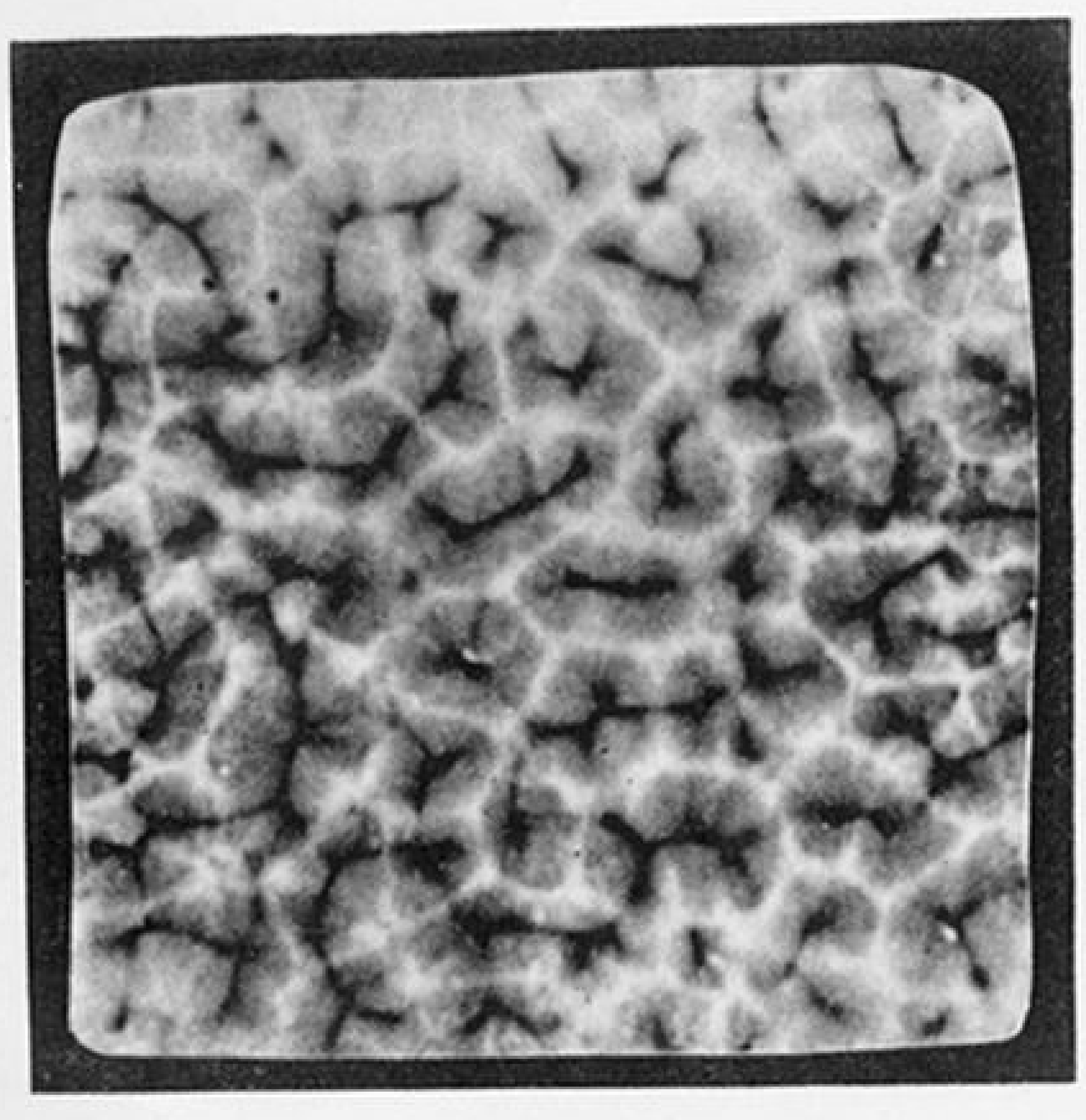
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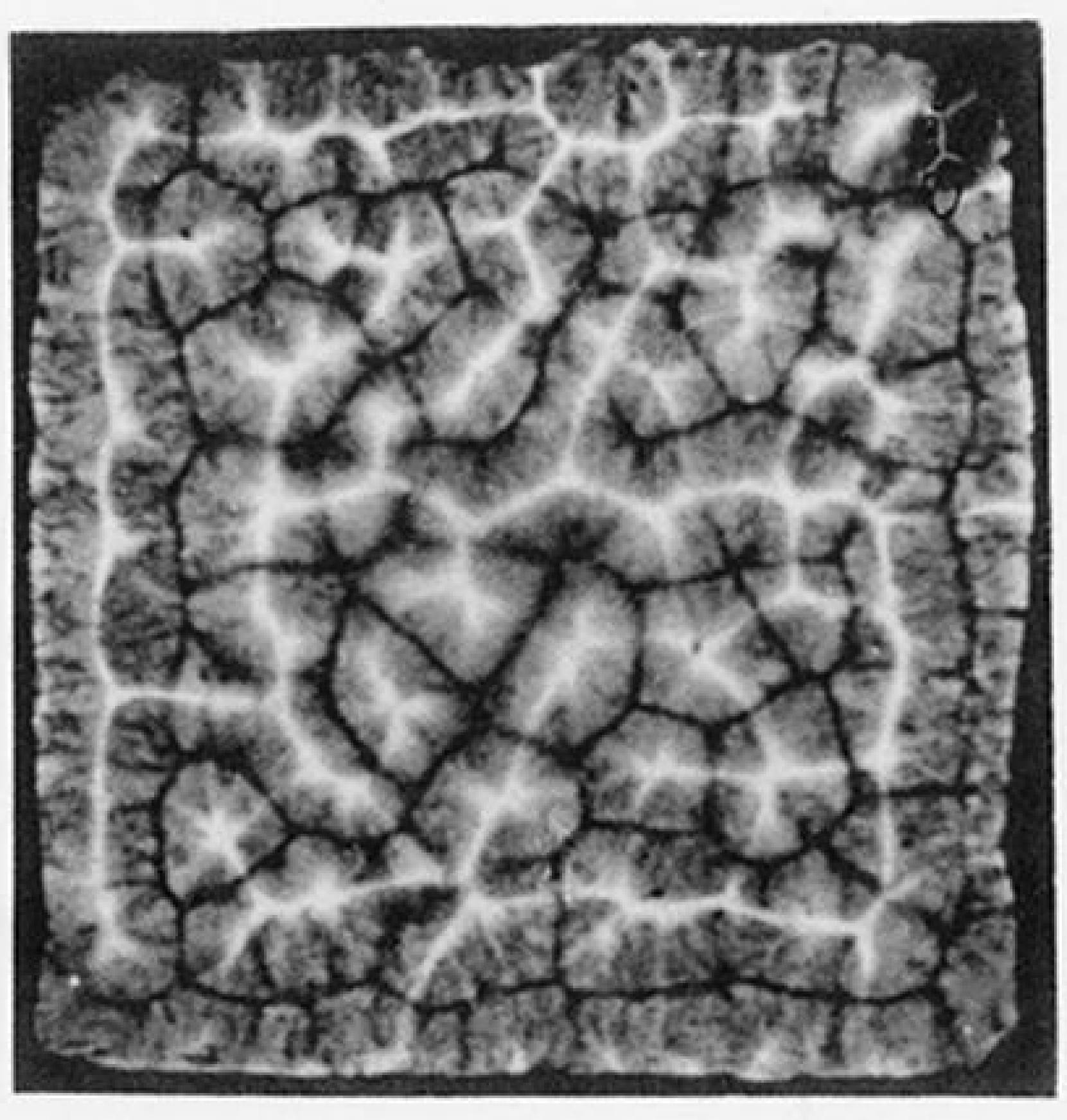
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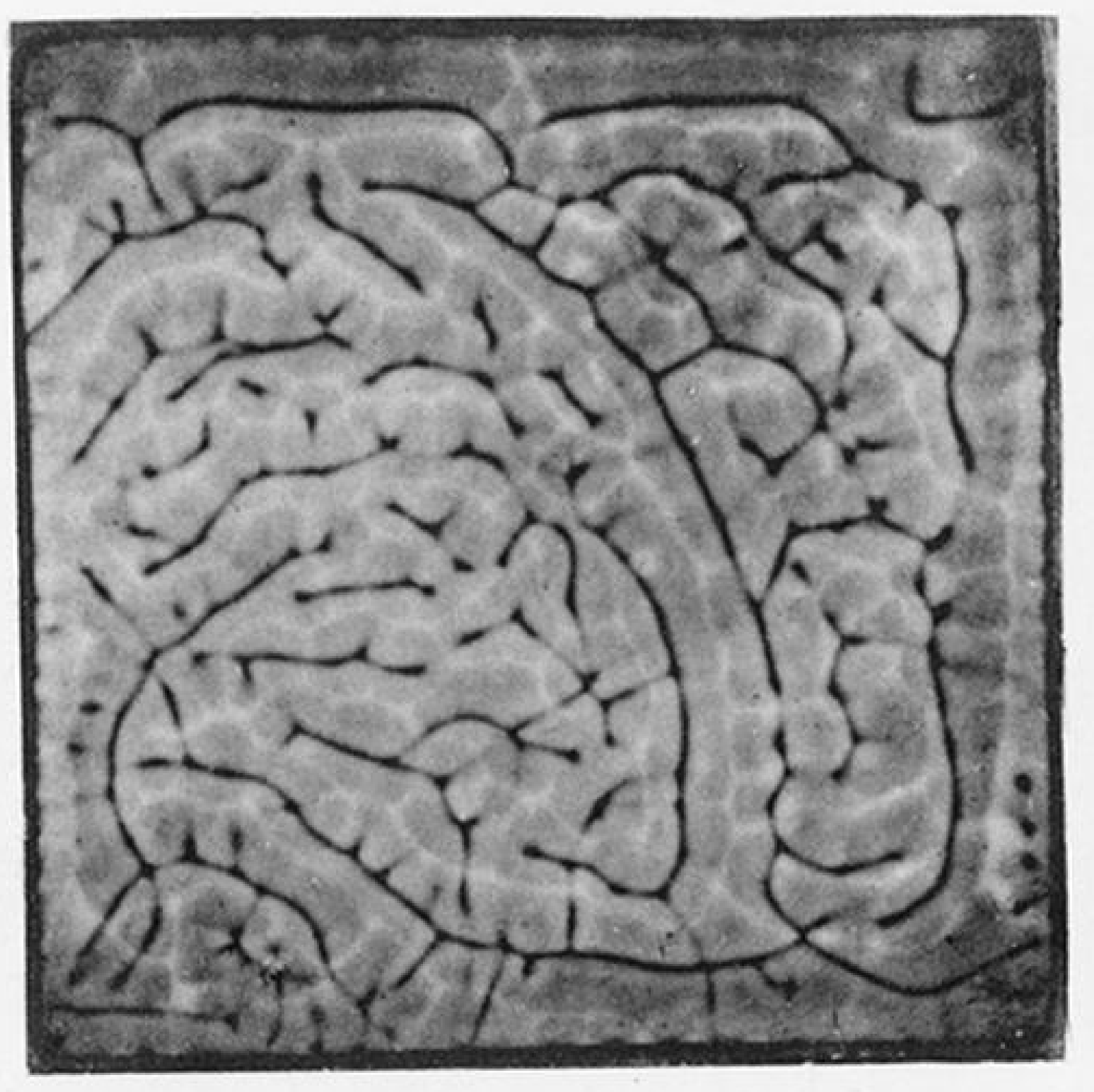
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PLATE 34.

Fig. 21.—Cell in which Euglenæ were brought to one side by allowing light from one side to fall upon them. They formed there a dense mass. Two minutes after being placed in the dark they began to aggregate, as shown in the figure, photographed from above. The following figures (22–24) show successive stages.

Fig. 22.—At the end of four minutes.

Fig. 23.—At the end of six minutes.

Fig. 24.—At the end of eight minutes. The Euglenæ, at the end of 20 minutes, had spread themselves all over the cell in separate groups, as shown in fig. 28, and ultimately into smaller groups as in fig. 14 (Plate 33).

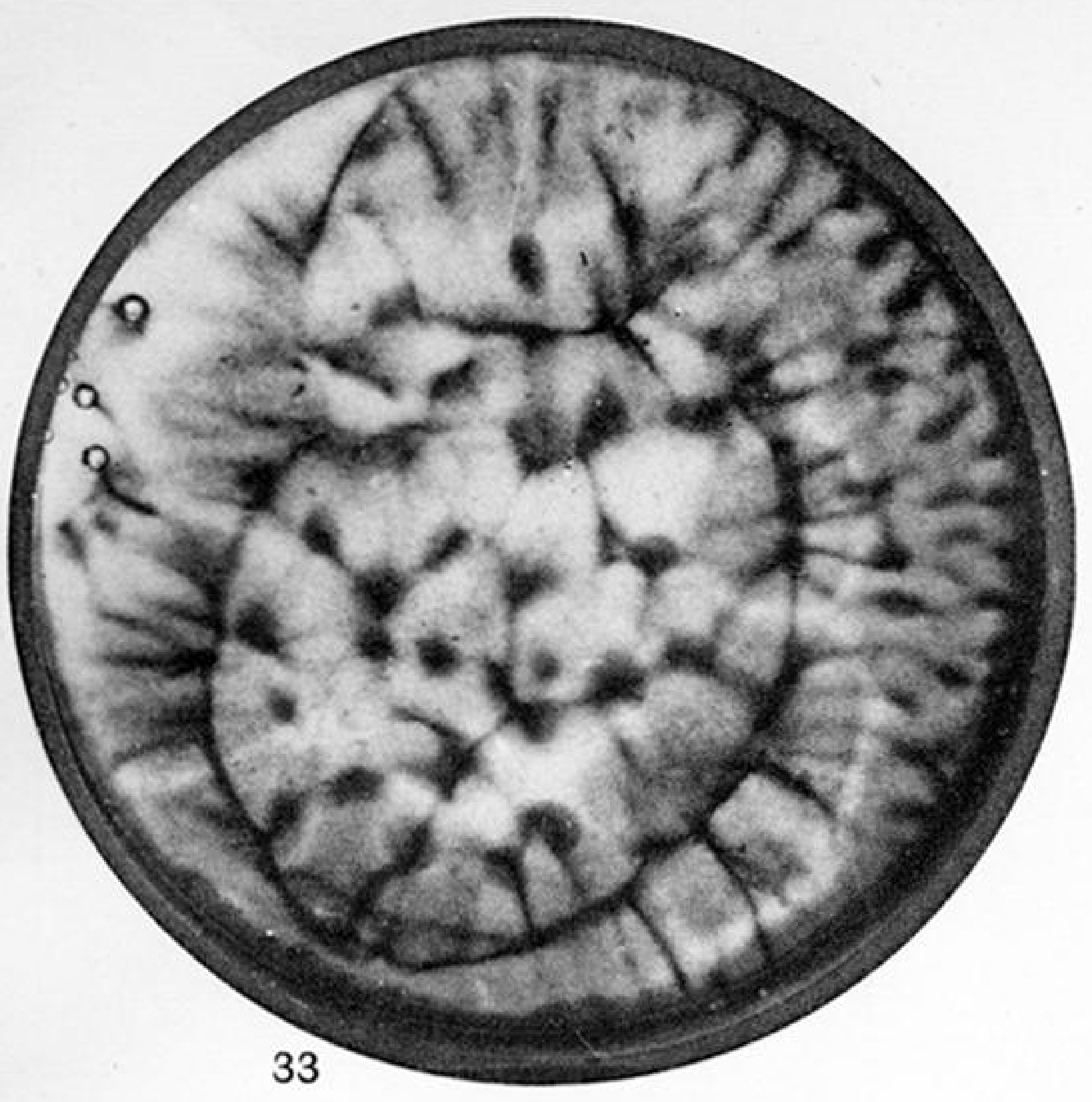
Figs. 25–28.—These figures show the aggregation of Euglenæ from a diffused state in a shallow cell, when fewer are present than in Plate 33, fig. 10. The separate groups are formed almost at once, and are much larger, breaking up later into smaller ones, as shown in the figures.

Fig. 29.—Osmic dioxide precipitate, in dilute glycerine, to retard the downward movement. A netlike arrangement of the precipitate is first of all formed, which soon breaks up into more or less separate groups as shown in the figure. *Cf.* figs. 26–28.

Fig. 30.—Osmic dioxide in dilute glycerine. Grouping formed from a film not quite so coherent as in fig. 29, that is, it had not been allowed to stand quite so long before it was turned upside down, and was photographed at a slightly earlier stage than fig. 29.

Fig. 31.—Spirilla in a very shallow cell, in which, by capillary attraction, the surface of the water had become much curved. The bacteria formed a dense layer just beneath the surface of the water, at the level of the optimum oxygen layer, from which downward moving streams were set up. Magnified about three or four times.

Fig. 32.—Figure produced by the oxidation in air of a solution of Watalu developer. Later stage than fig. 37.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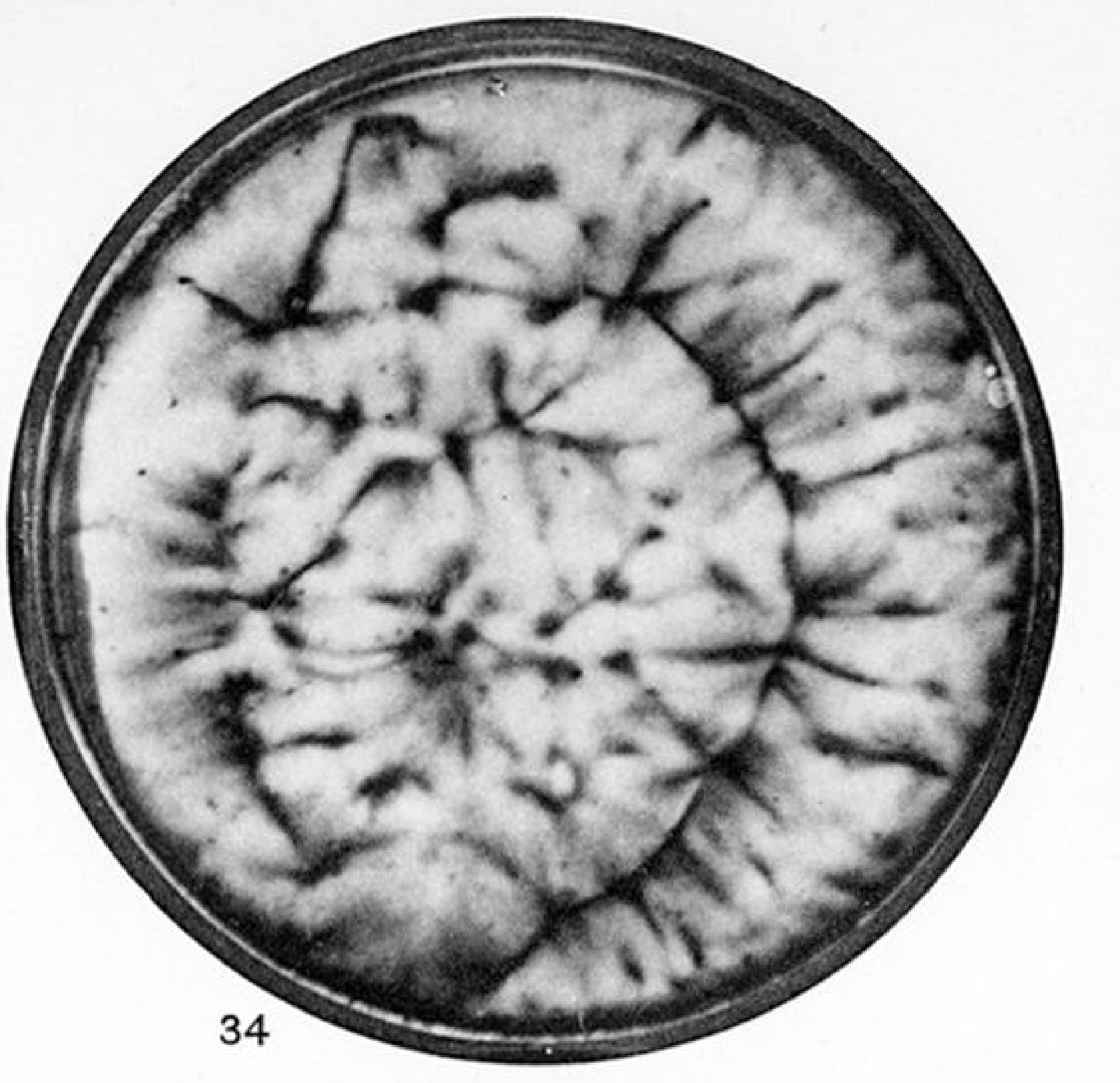
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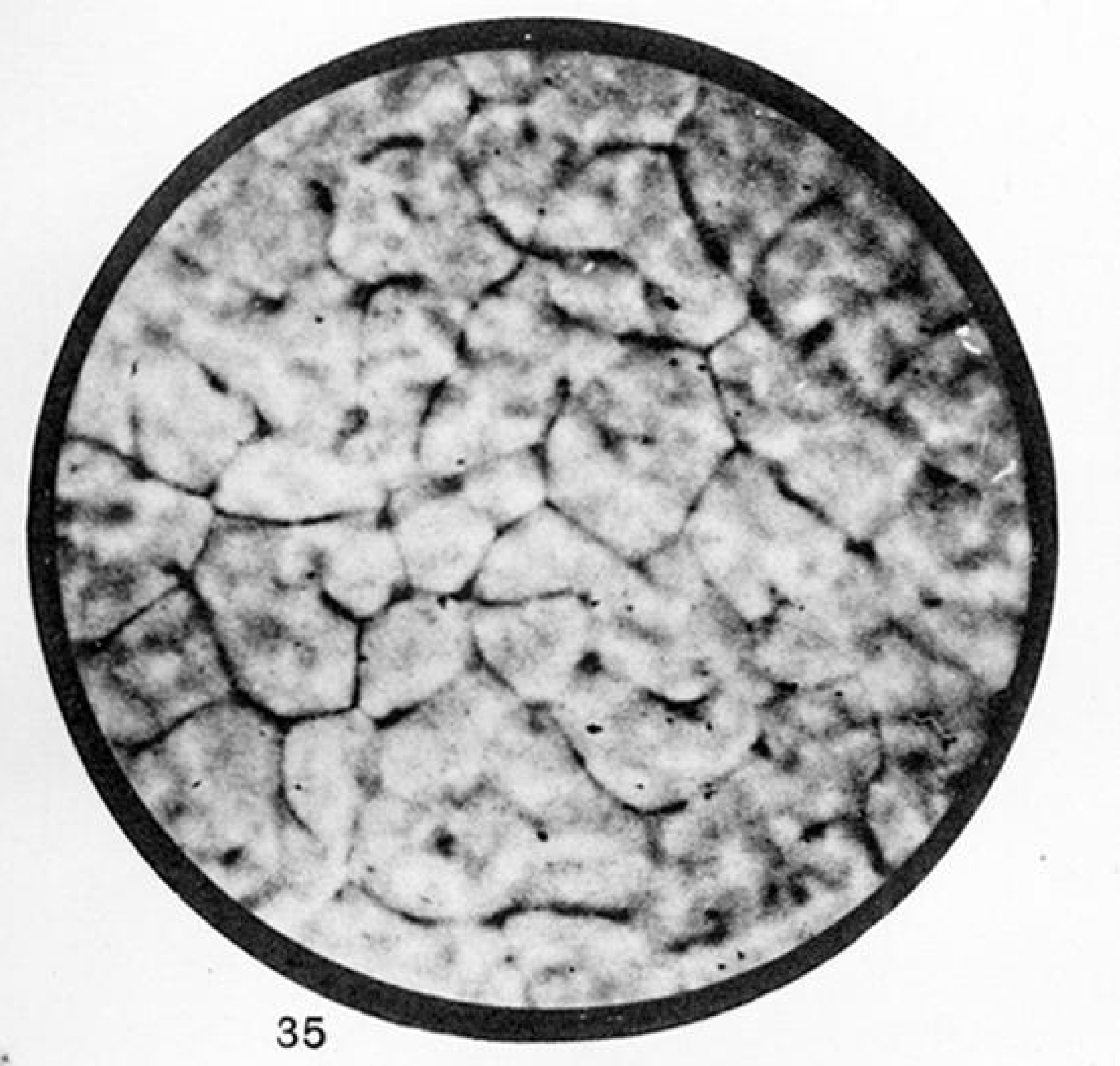
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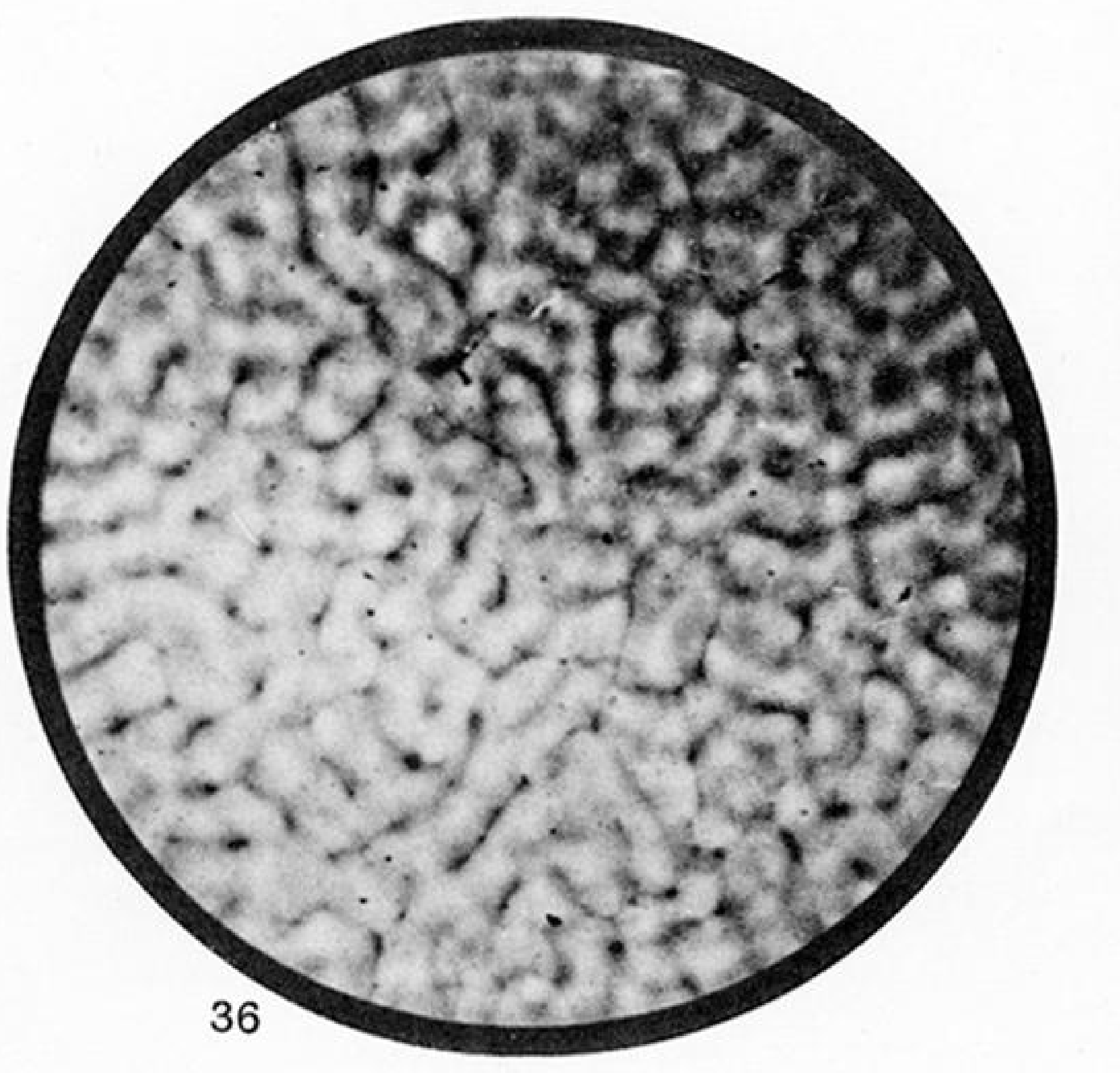
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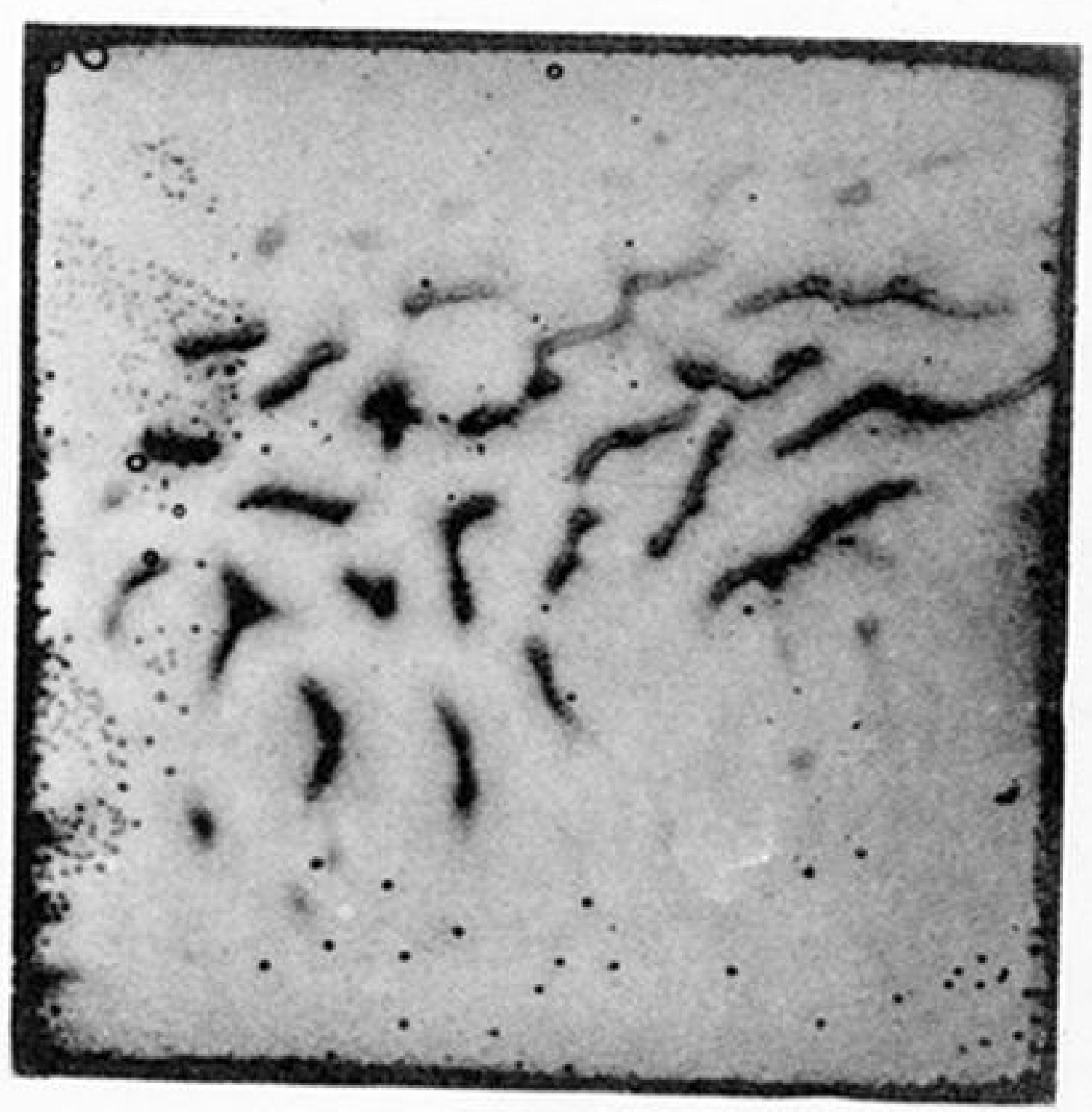
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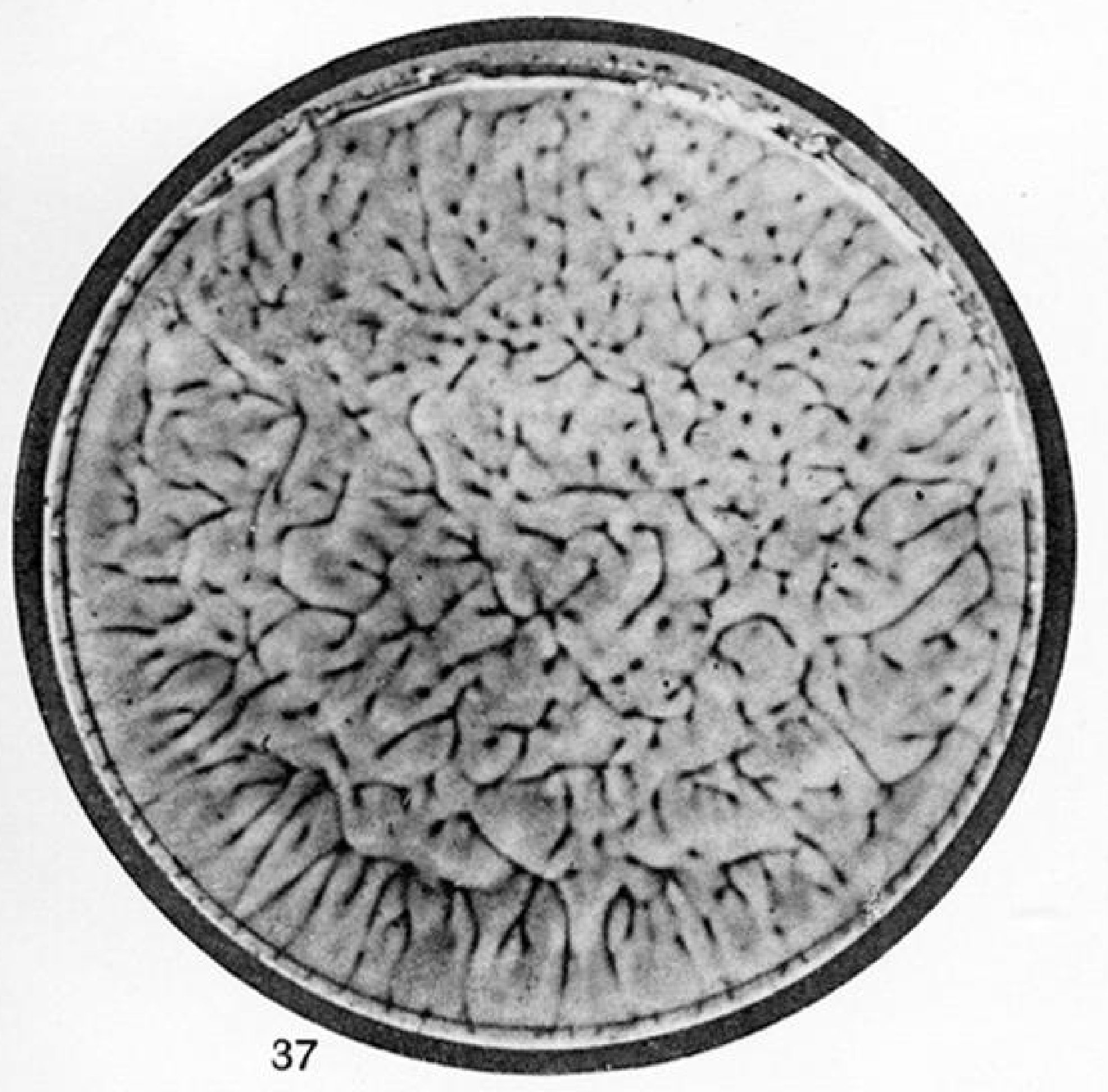
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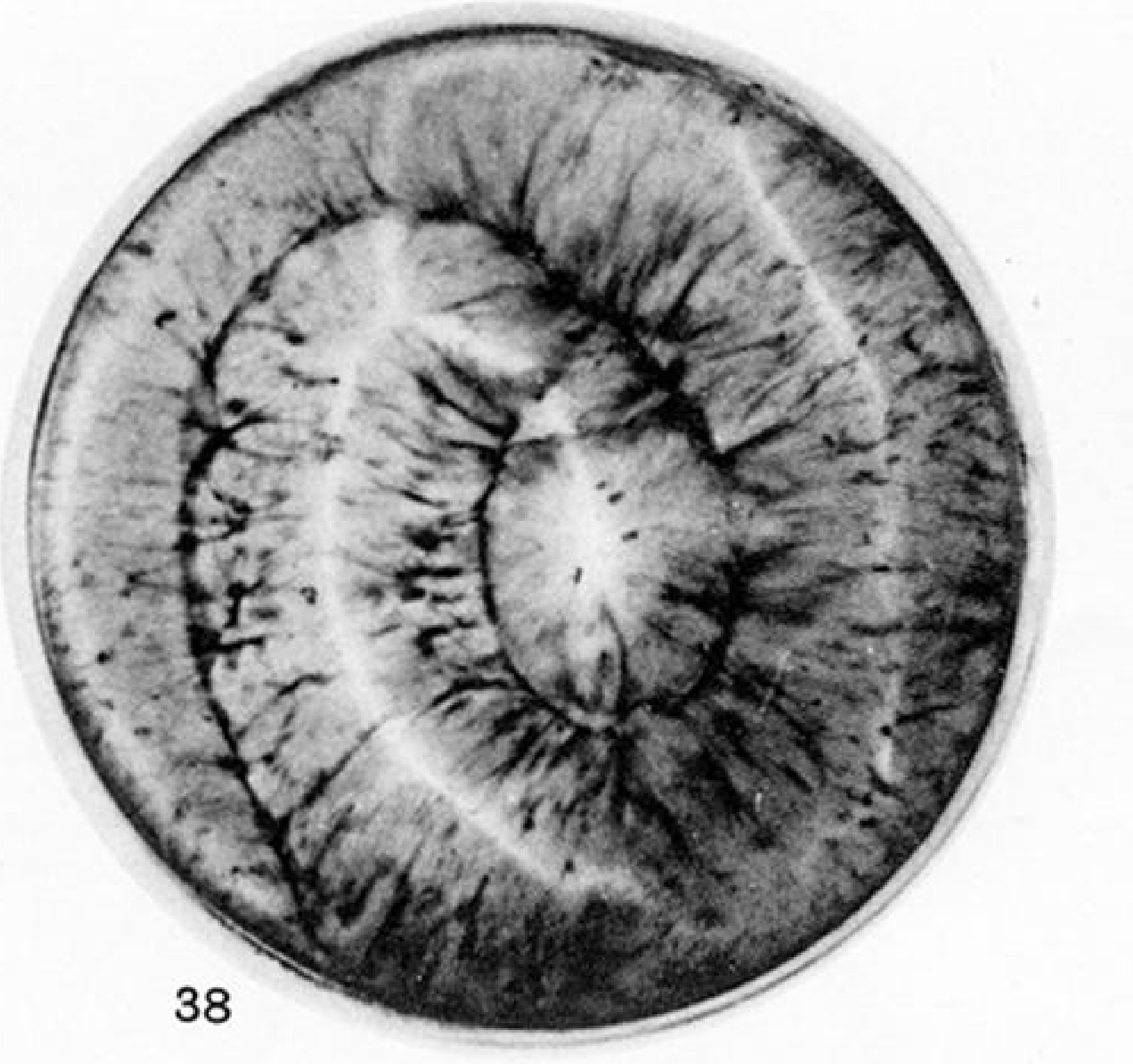
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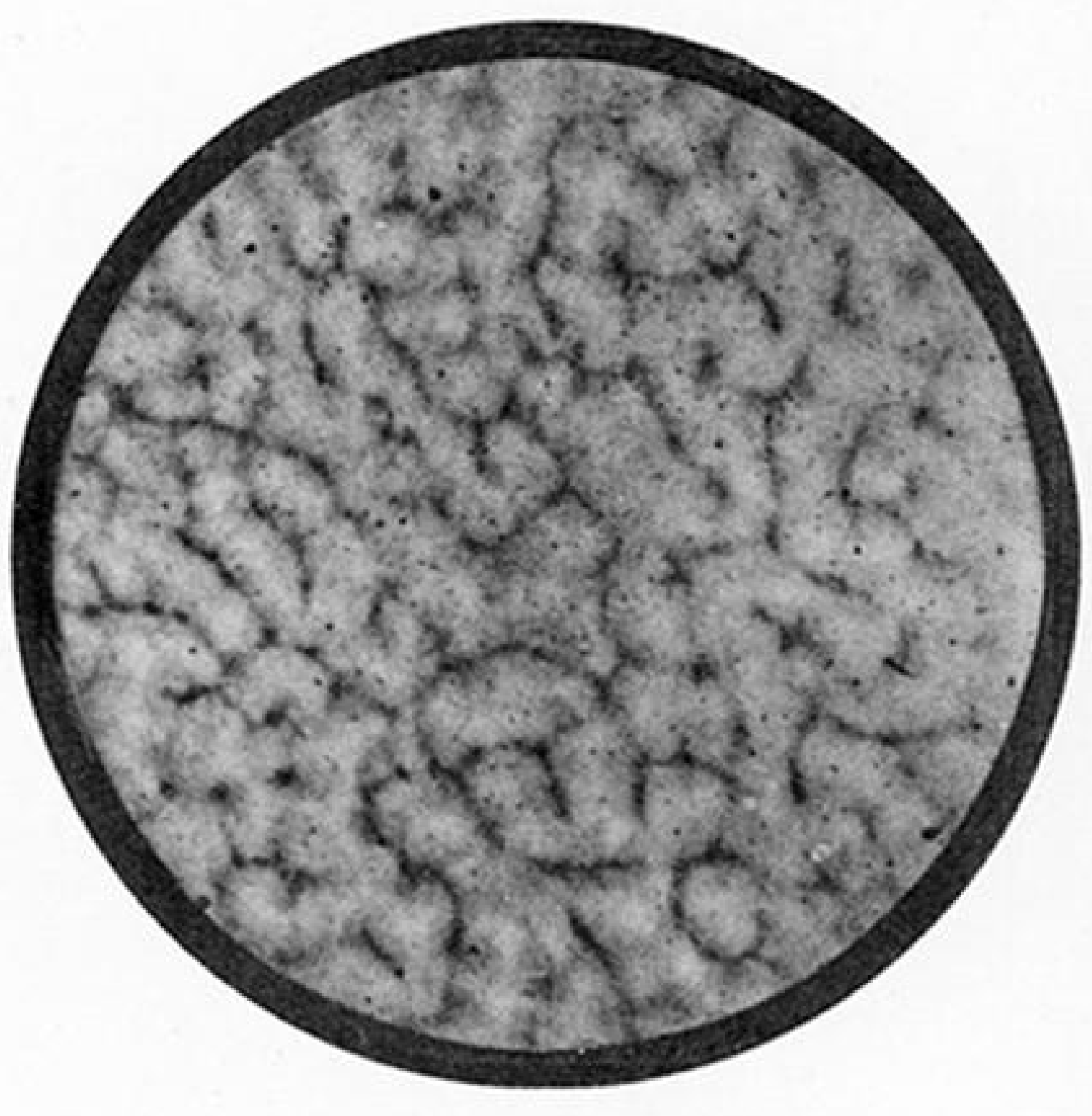
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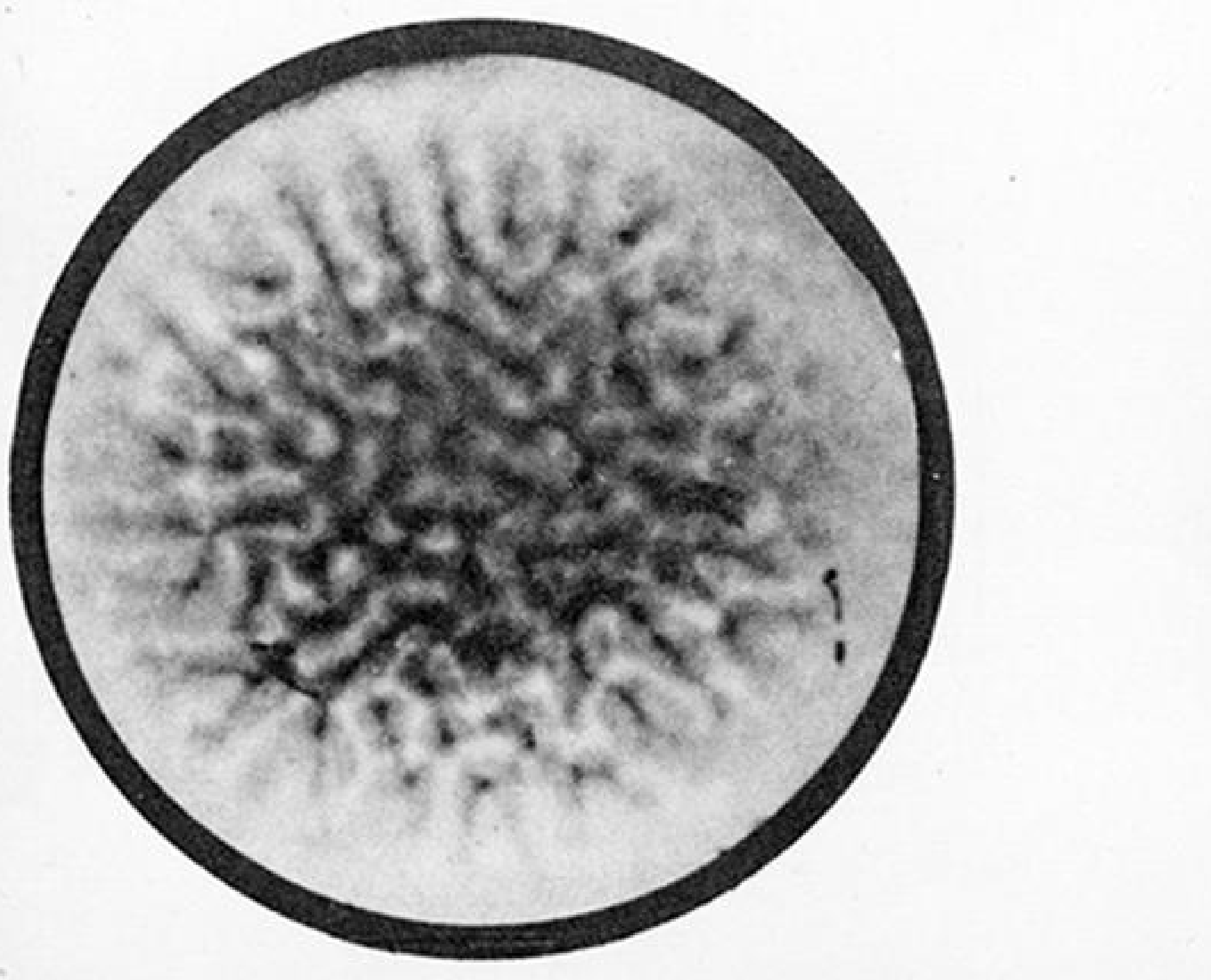
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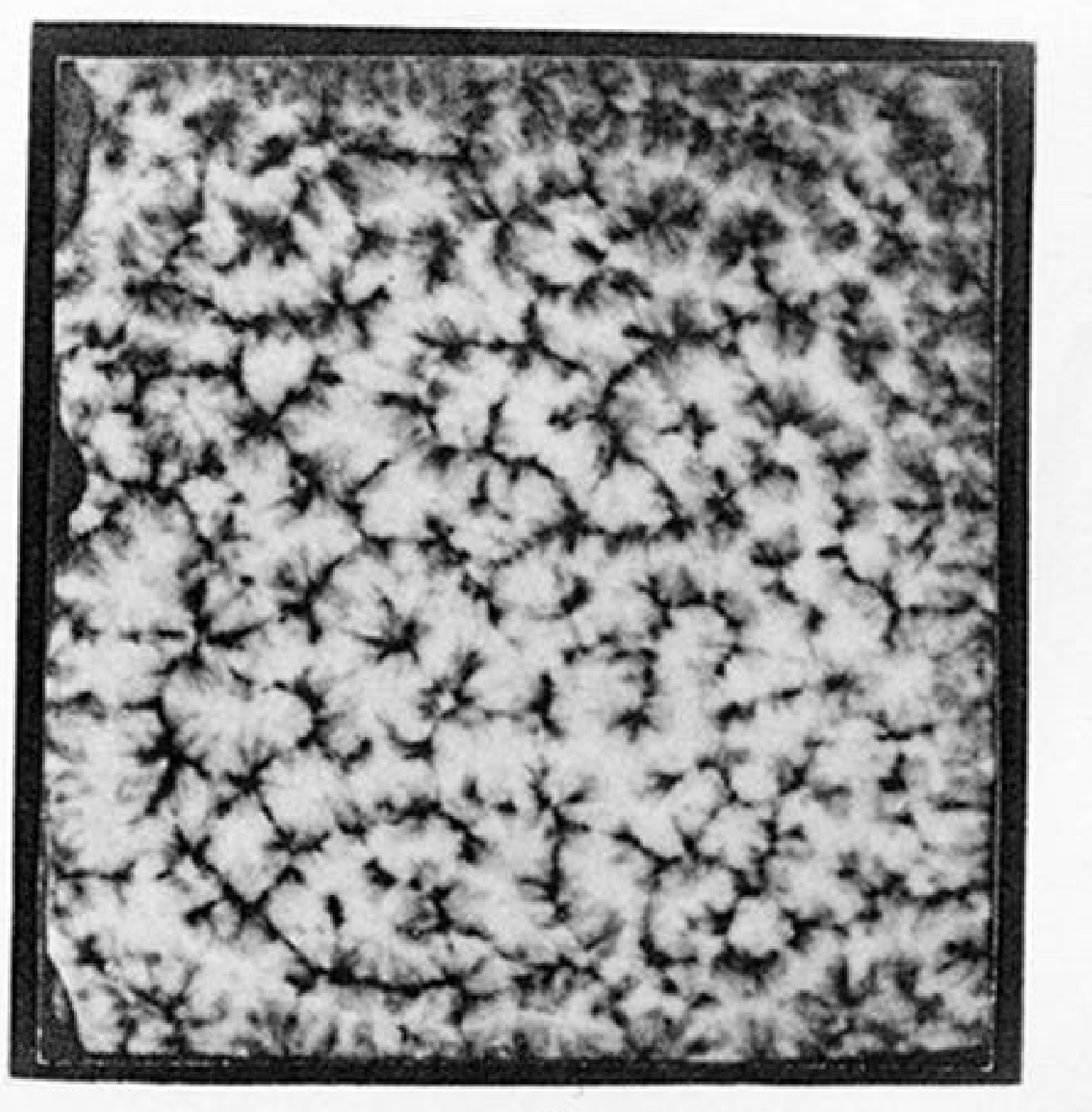
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Plate 35.

Fig. 33.—Living Euglenæ falling from a film which had been obtained by allowing light to impinge upon the lower surface of a cell $\frac{1}{2}$ inch deep, which was then turned upside down.

Fig. 34.—The same Euglenæ killed in osmic acid solution and allowed to settle to the lower side of the cell to form a homogeneous film which was then turned upside down. The Euglenæ are just beginning to fall again and an aggregation is visible.

Fig. 35.—Living Euglenæ aggregating from the diffuse state in a cell $\frac{1}{2}$ inch deep. A large number of Euglenæ were present.

Fig. 36.—A later stage than fig. 35. The meshes of the network have become smaller, and there is some indication of a separation into groups, but the cell is too deep and the number of Euglenæ too large to allow a definite separation of the groups.

Fig. 37.—Figure produced by the oxidation in air of a solution of Watalu developer in a shallow cell.

Fig. 38.—Precipitate of manganese dioxide falling from a film in a cell $\frac{1}{2}$ inch deep. This cell is much deeper than those used in most of the experiments previously figured, and the movement of the water as the cell is turned upside down is sufficient to give a definite direction of movement to the surface film of precipitate. The main lines of the grouping are definitely due to this. Possibly the circular shape of the cell has something to do with it also. A similar arrangement is observed in figs. 33, 34, and 40.

Fig. 39.—Aggregation of Euglenæ in a shallow cell with sloping sides. The grouping is denser towards the middle and radiates more or less equally from the centre.

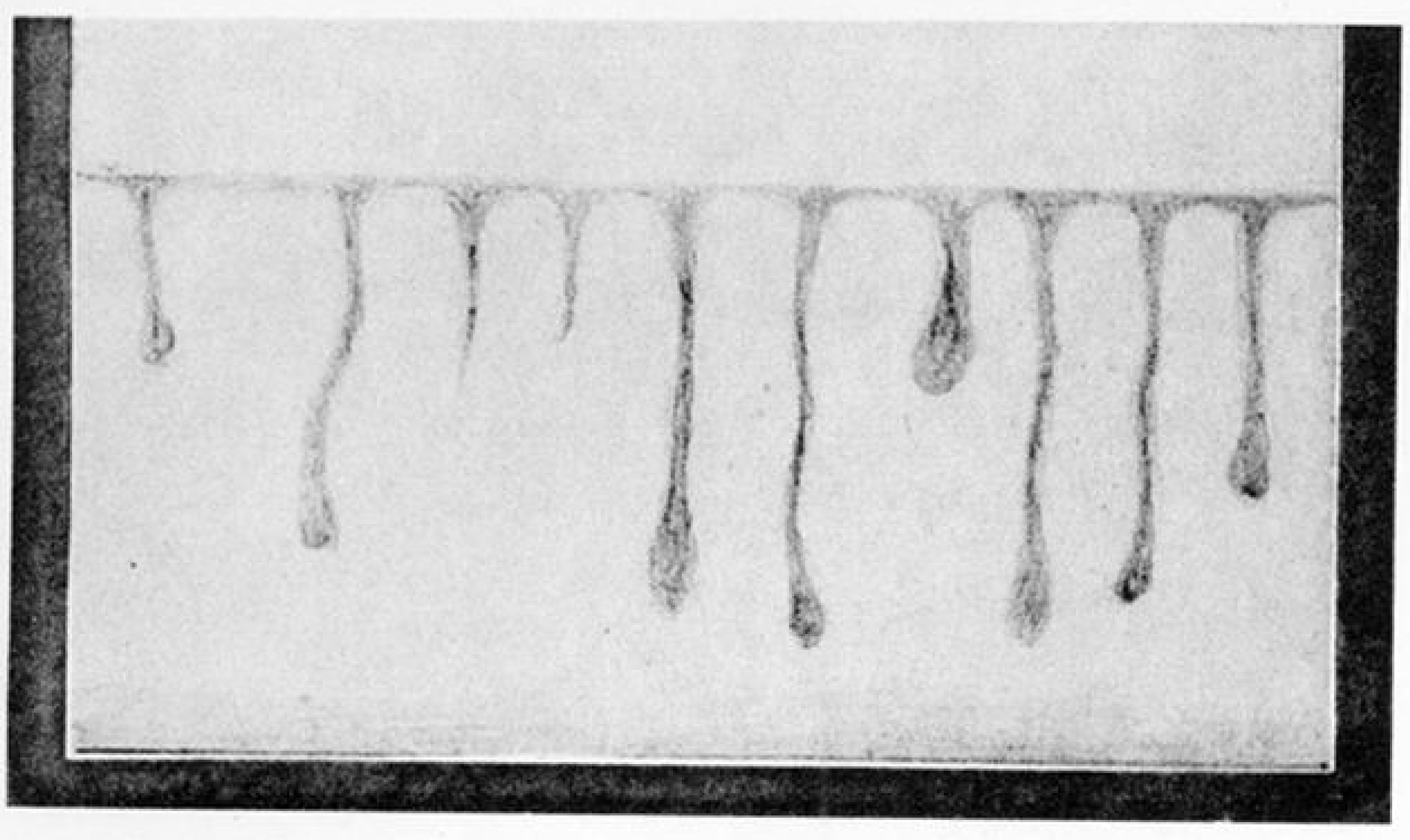
Fig. 40.—Aggregation of a precipitate of osmic dioxide (OsO_2) from a surface film in a shallow, hermetically sealed cell. The cell is smaller than in the other figures, but the same general result is obtained.

Fig. 41.—Euglenæ moving across from one side of the cell to the opposite side under the influence of light. The upper side of the cell in the figure was the light side. The Euglenæ move across in a series of ripple-like aggregations.

Fig. 42.—Cell as in fig. 41, but with a dark screen placed over it for two seconds. The Euglenæ in each group at once begin to sink and become drawn together into denser masses, which is clearly shown in the figure. The same effect would have been produced by a red glass.

Fig. 43.—Euglenæ aggregating into a network in diffuse light from above. The same effect would have been produced under green glass. Under red glass the Euglenæ aggregate as they do in the dark.

Fig. 44.—Chlamydomonas killed in osmic acid solution, just beginning to fall from a film in a shallow cell turned upside down.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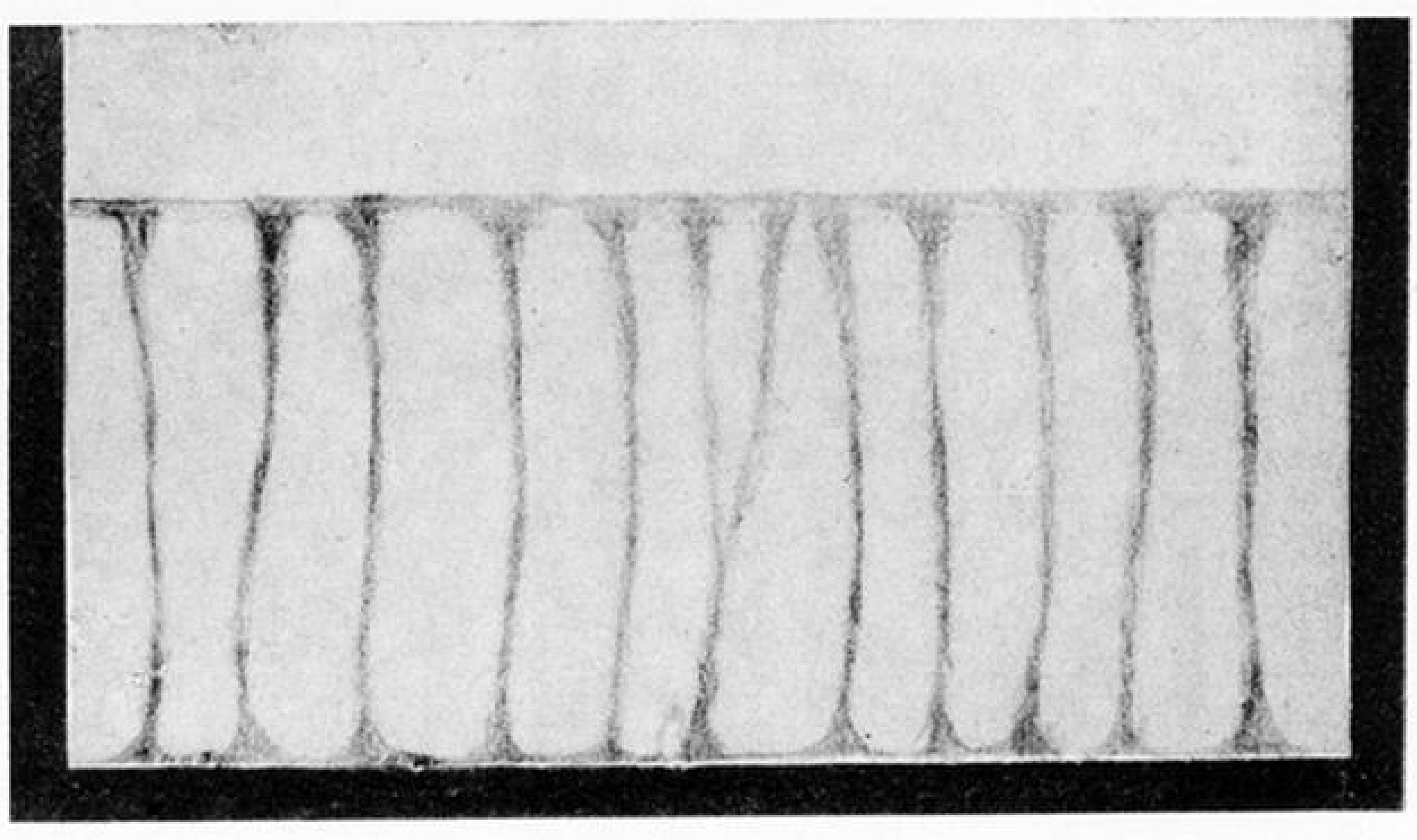
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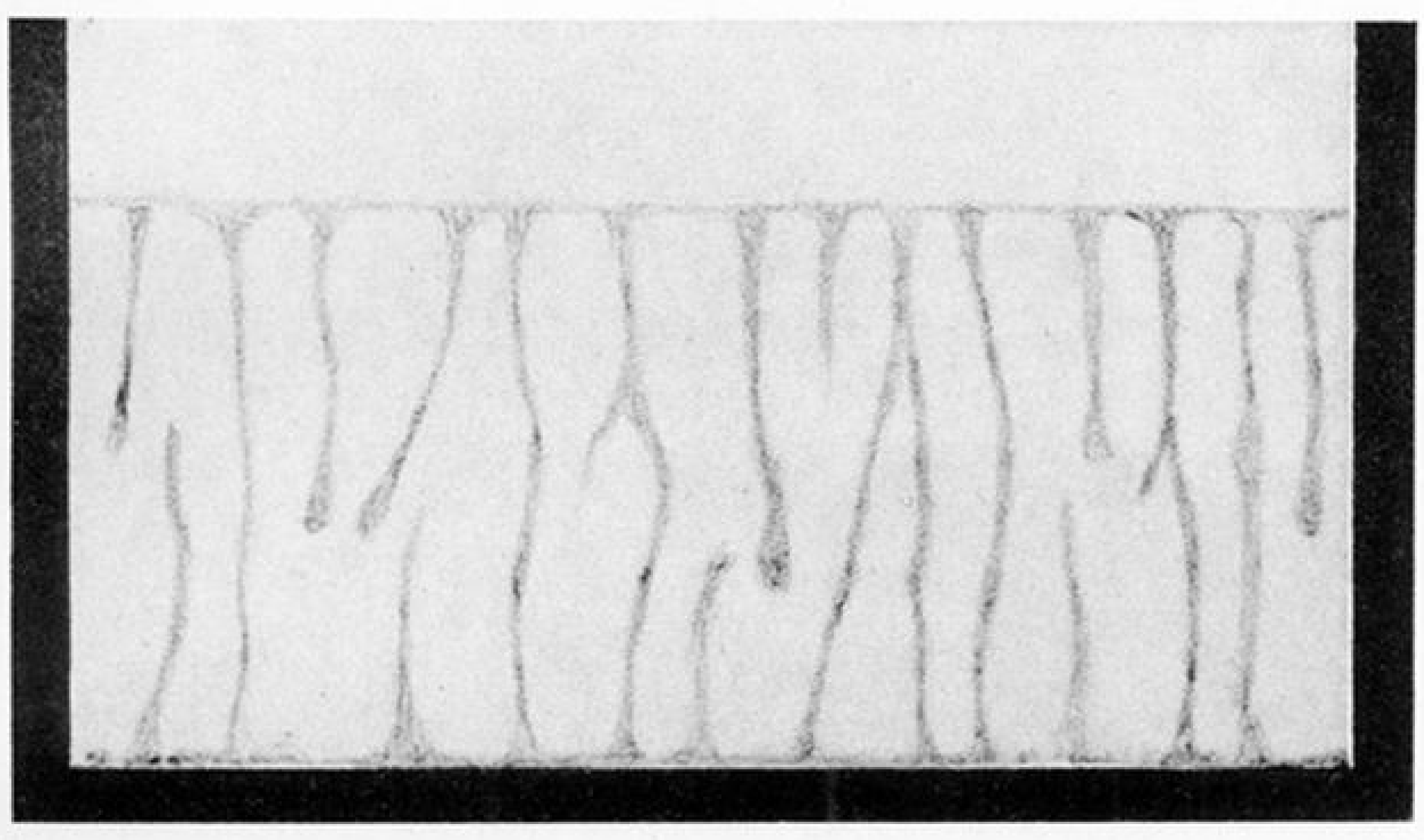
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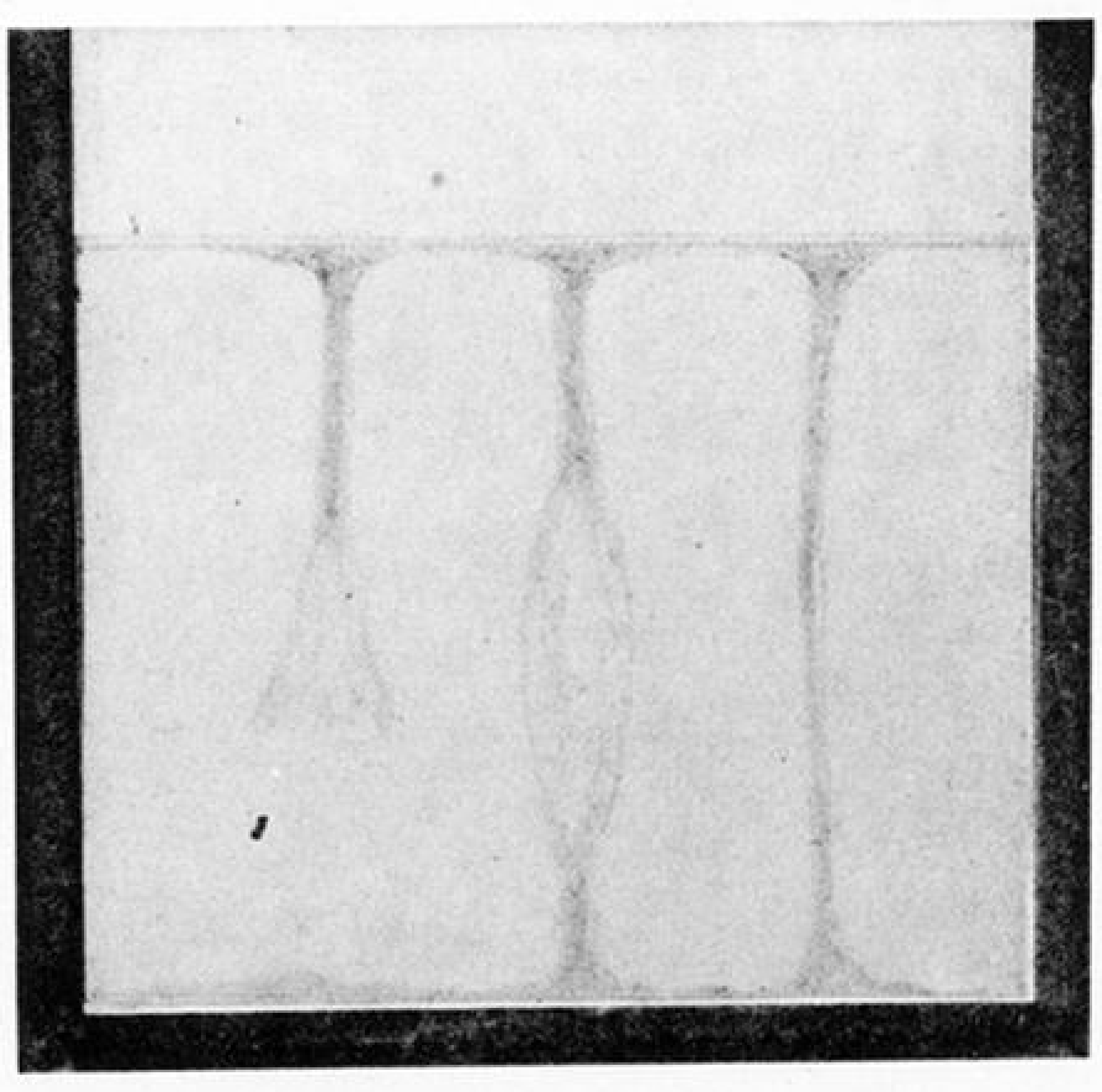
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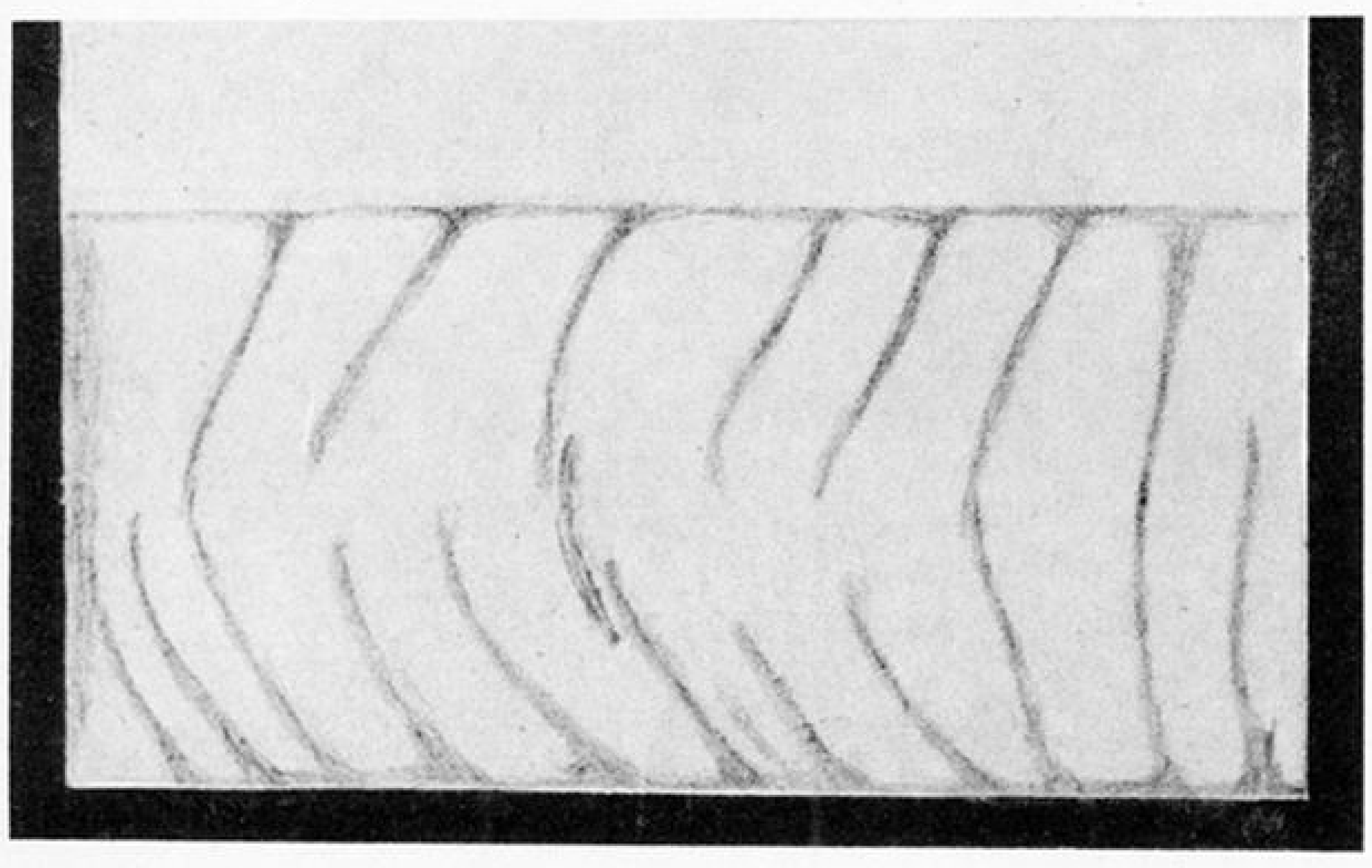
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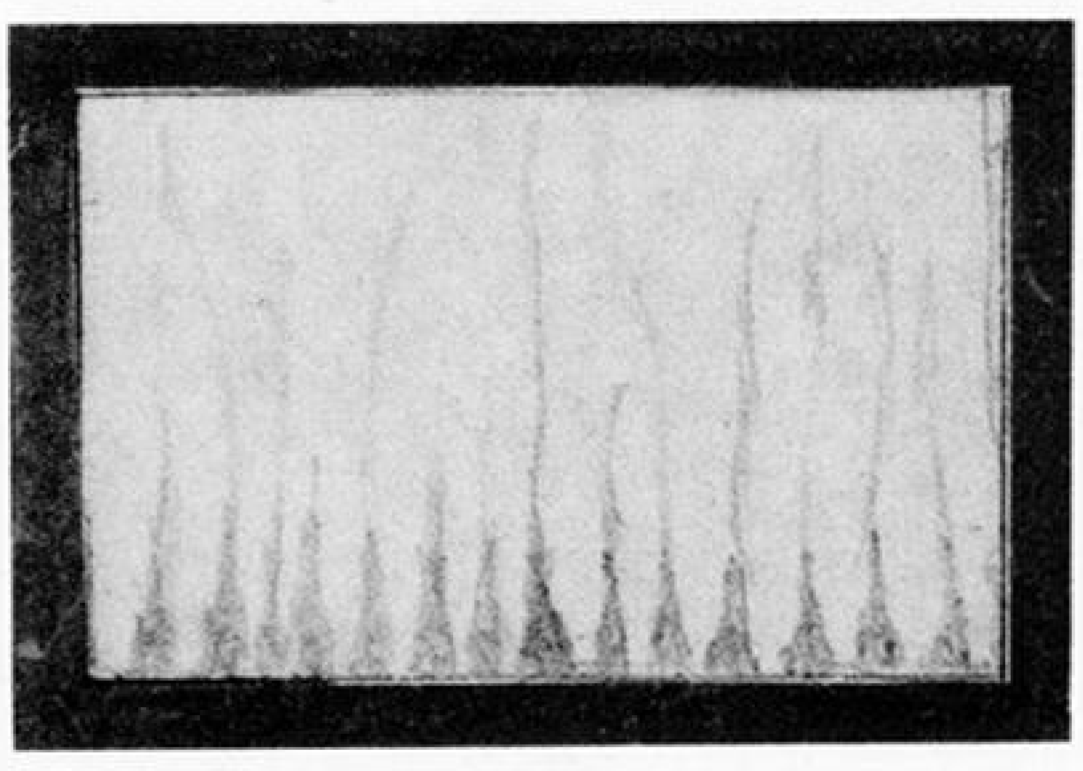
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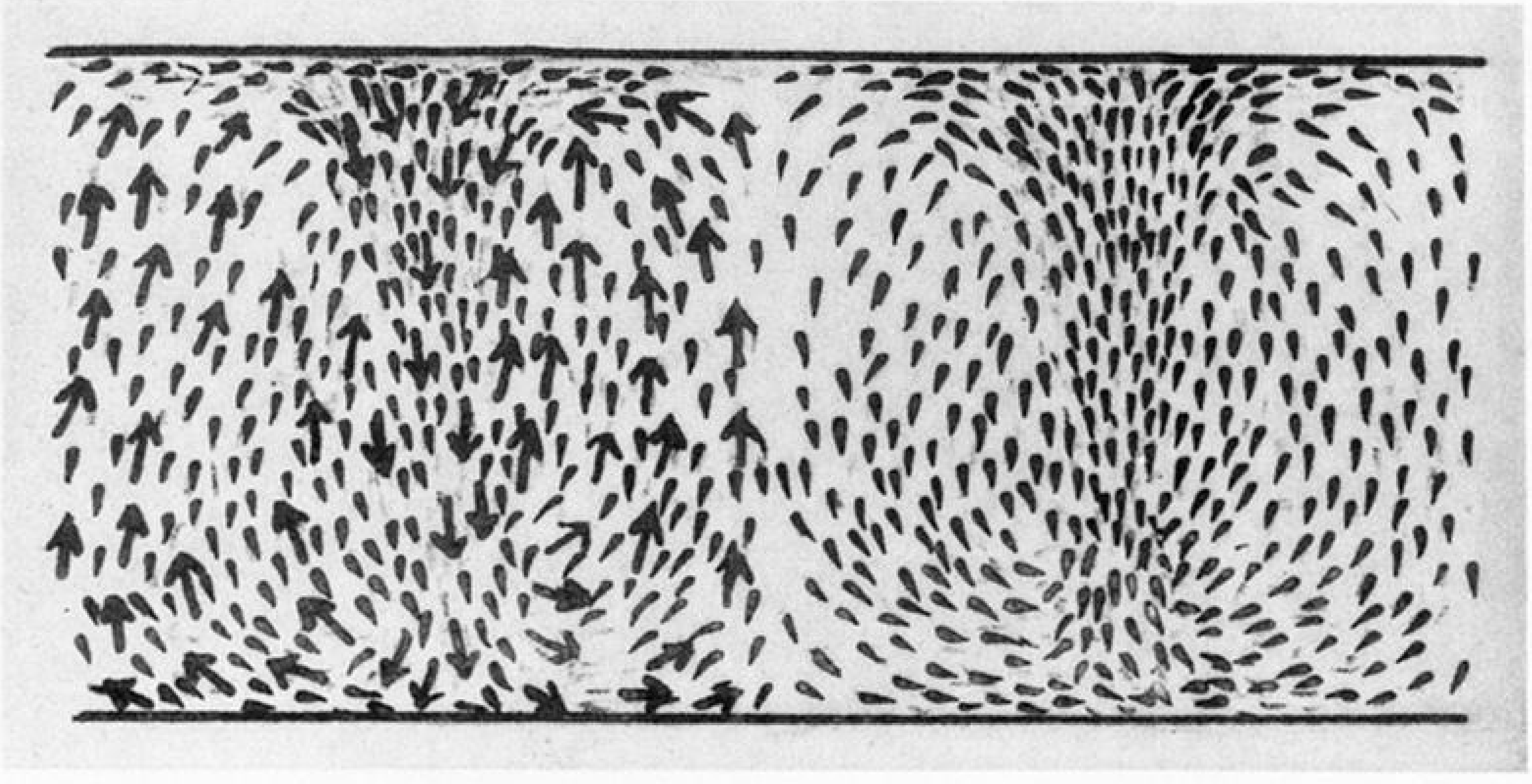
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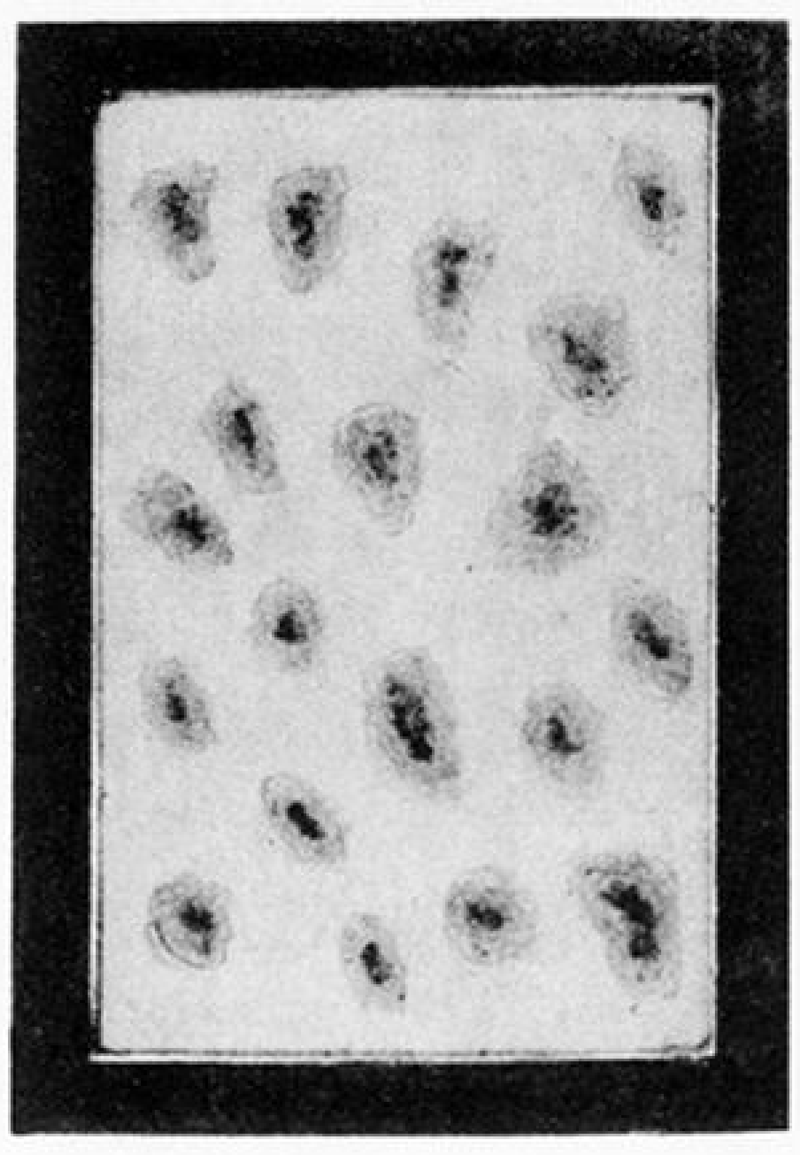
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PLATE 36.

Figs. 45–50.—Aggregation figures of *Glenodinium cinctum.*

Fig. 45.—Downward streaming just beginning in an upright cell with the upper surface of the water exposed to the air. Diffuse light.

Fig. 46.—A slightly later stage than fig. 45. The streams have in most cases reached the bottom of the cell.

Fig. 47.—A still later stage showing the streaming downward in lines. As soon as they reach the bottom, the organisms swim irregularly upwards again to reach the oxygen layer.

Fig. 48.—The effect of a stronger light falling on the cell from one side. The streams are bent towards the light, but for a time gravity still acts; the angles formed represent the resultant of the two forces; the aggregation gradually disappears as the organisms move towards the light.

Fig. 49.—This figure shows the effect of a strong beam of light concentrated by a condenser upon one of the moving streams. The organisms move away from the immediate vicinity of the light, which thus has the effect of causing an expansion of the stream (see the middle one of the three streams). If the light continues to act, the upper portion of the stream persists for some time, but the lower part disappears; the organisms are repelled too far by the light for them to become aggregated again below it (see the left hand figure). On the right hand of the figure is seen a stream on which the light is not falling.

Fig. 50.—*Glenodinium cinctum* in a hermetically sealed, upright cell. A very regular streaming up and down takes place at the bottom of the cell; a few of the organisms rise nearly to the upper surface, and descend in very delicate lines. The majority move up and down within a distance of about a quarter of an inch.

Fig. 51.—The grouping of *Glenodinium cinctum* in a shallow cell placed flat. The groups are well spaced.

Fig. 52.—Diagram showing the movements of *E. viridis* in two of the separate groups seen in fig. 1. There is a constant cyclic movement downwards and upwards in the general direction indicated by the arrows.