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Observation on the regeneration of the plasmodium of the Myxomycete *Didymium xanthopus* (Ditm.) Fr.

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1. INTRODUCTION

A remarkable regeneration capacity is characteristic of myxomycetes in the plasmodial stage. It is connected most probably not only with the primitive character of this group of organisms but with the fact as well that the plasmodia unprotected by any membrane are often exposed, under natural conditions, to injuring agents.

In laboratory cultures this regeneration capacity greatly facilitates the multiplication of plasmodia by transfering their fragments on a fresh substrate. A detailed study of the regeneration processes following a mechanical injury of the plasmodium presents not only a scientific interest, but may be of some importance for laboratory practice. In spite of numerous studies concerned with the physiology of myxomycetes little is known however about their regeneration capacity. Besides occasional observations a study on regeneration was made by Skupieński (1953) who described the macroscopical changes occurring in injuried plasmodia of *Didymium squamulosum* Fries.

The study reported in this paper was undertaken with the aim of examining the microscopical changes following the injury of the plasmodia of *Didymium xanthopus* and those occurring during the course of regeneration. The study was completed by an investigation on the dependence of the regeneration ability on the age of the culture and the local differentiation of the plasmodium.

2. METHOD

The experimental material, the myxomycete *Didymium xanthopus*, was collected in the form of a sporangium found on a old rotten pine trunk in July 1958 in a small wood in the vicinity of Busko.

Plasmodia were obtained by sowing out the spores on a 2,5% carrot extract solidified with 1,5% agar; 3 weeks after the spores has been sown out lemon yellow coloured plasmodia appeared. They were subsequently cultured on oat agar prepared after Howard (1931).

Cultures were maintained in vegetative state by inoculating them successively (every 6—7 days) on the above mentioned nutrient medium in test tubes kept in a dark thermostate at 20°C.

The material for experiments was obtained by transferring fragments of the plasmodium from the stock culture (from oat agar) on a washed nonutritive agar on Petri plates. Observations were performed 1, 2, 3, 5, 10 days following inoculation directly on the plates. A Lumipan microscope equipped with a condensor for small magnifications and an objective (10 × or 5 ×) was used.

The plasmodium was cut by means of an arrangement shown in fig. 1. A narrow piece of a rasor blade was fastened in a ring attached to the objective; the blade was situated about 3 mm. below the lens of the

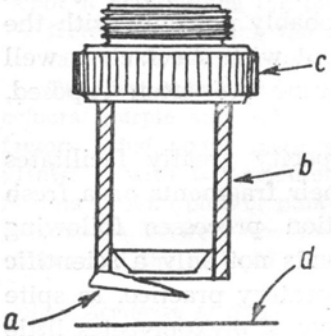


Fig. 1. Arrangement for cutting plasmodium threads: *a* — piece of rasor blade; *b* — fastening ring; *c* — microscopical objective 10 ×, *d* — plane of object

objective and was not visible in the microscopic field. A swift lowering of the microscopic tube permitted to make a straight cut through a plasmodial vein in a desired place. A rapid lifting of the microscopic tube made possible the observations of the cut fragment as early as 1 sec. after operation.

An apparatus described by Zurzycki (1958) was used for registering the speed of the streaming protoplasm.

Fragments of veins and of the frontal part of plasmodia provided the material for investigations on regeneration capacity of small isolated fragments. These fragments were previously immersed in water for 5—10 minutes in order to clean them from the agar substratum. The fragments were then transported on fresh agar in a Petri plate on which they were directly observed.

The migration and growth of the plasmodia developed from fragments of different age and size were observed in Rayan's tubes filled with agar medium (oat agar). The tubes, 1.4 cm. in diameter, were 30 cm. long. The plasmodial fragment was introduced into one end of the tube and the successive positions of frontal part of the plasmodium were read every day in 24 hours intervals. Each series is the mean of 20 determinations.

3. RESULTS

The course of regeneration

Even a slight contact of a plasmodial vein with a rasor blade, without cutting it, caused an immediate inhibition of the streaming of protoplasm on the length of 0.5—1.5 mm. extending to both sides from the place of contact (Plate I, fig. 5—8). A local thickening, of the vein in this place was visible for a few seconds following the mechanical stimulation (Plate I, fig. 6). The zone of inhibited motion decreases after 10—30 sec.; a local inhibition, however, in the place the stimulus was applied remains still for some more minutes. Finally the influx of protoplasm produces an increase of broadth and thickness of the vein in the place the operation was performed (Plate I, fig. 8). After 3—5 minutes the blocked vein becomes again accessible to protoplasmic streaming. Initially the channel of streaming protoplasm is narrow in the place of application of the stimulus and for this reason the motion rate of protoplasmic granules locally increases. The thickening of the cortical layer of protoplasm disappears after some more minutes and the vein regains its normal appearance (Plate I, fig. 8).

A complete cut made through a vein with a sharp blade leaving both fragments in the very place causes a cycle of phenomena presenting some differences according to the thickness of the cut vein, its physiological state and the age of plasmodium. The observed phenomena may be classified into 3 types:

Type I. Outflow of protoplasm is characteristic of this type. It occurs only after cutting thick veins (200—300 μ in diameter) of a normal plasmodium. The cut is followed by an immediate outflow of endoplasm. Probably the mechanical stimulus is capable of causing the gelatinisation of the whole protoplasm in a large vein in a short time and for this reason the inner pressure in the vein presses out the liquid endoplasm (comp. photos 9—12, plate I). After removing the blade the two fragments of the cut vein join again and the effused protoplasm causes the formation of large globular blisters of about 1 mm. in diameter. About 1 sec. after the cutting operation the shape of the effused protoplasm is final and is the result of the solidification of its peripheric part (Plate I, fig. 10). Protoplasmic streaming in the vein is inhibited within 1—1.5 mm. distance extending to both sides of the injured place. Within 2—5 sec. following operation a contraction of the effused protoplasm takes place and lasts for about 20 minutes (Plate I, fig. 11). Subsequently the effusion maintains its shape and dimensions unchanged for a longer time. The motion of protoplasm in the more removed parts of the plasmodium causes an increase of the rate of protoplasmic streaming in side veins. It may also

produce their thickening. Sometimes a secondary break of the ectoplasmatic layer is observed as a consequence of the pressure exerted by the streaming protoplasm. Sometimes a secondary break of the first protoplasmic outflow and an increase of its size following injury were also observed. Finally in few cases a new effusion is formed in the distance of about 1 mm. from the injured place within 30—60 sec. (fig. 2). In the course of time the zone of inhibited motion shrinks and after some minutes

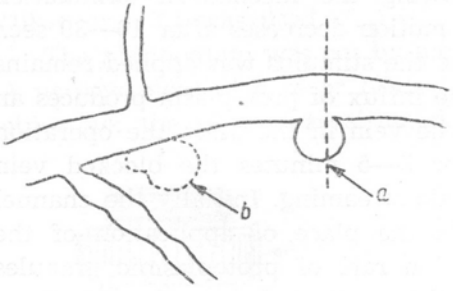


Fig. 2. Secondary effusion of protoplasm: *a* — plane of cutting with primary effusion; *b* — secondary effusion appeared 30 sec. after operation

the communication in the vein is restored. In this period a gradual decrease of the size of the effusion and its resorption are observed (Plate I, fig. 12). After 0.5—1.0 hour the shape of the effusion in the injured place does not much differ from normal protrusions occurring in other places of the vein.

Type II is characterised by a gelatisation and contraction of the fragments of the cut vein followed by regeneration processes resulting in a fusion between the two fragments. Processes of this type occur most frequently either in veins of average size or in small ones in young plasmodia whereas in old plasmodia they are observed in thick veins and as a rule are characteristic of cuts made in the frontal part of the plasmodium.

The course of processes occurring after cutting a vein can be seen from fig. 13—19 (Plate II); a sudden inhibition of protoplasmic streaming takes place immediately after injury. It extends to the length of 2 mm. to both sides from the place of operation. If there is a side vein in the cut fragment the inhibition of motion spreads as far as the side vein, but it also may extend over it. A contraction of both the cut fragments and the formation of a small fissure between them are visible already 1 sec. after injury. This contraction increases within the following 5—15 sec. and results in an enlargement of the fissure the size of which may attain even 150 μ (as a rule 50—100 μ) (Plate II, fig. 15). Within the following 20—30 sec. the zone of inhibited motion begins to shrink. Unless the protoplasm streaming from more remote parts of the plasmodium finds an outlet in side veins, a considerable mass of protoplasm is accumulated

in the injured place. This accumulation leads to a thickening of the corresponding vein (or veins) and results in a rounding up of their outlines. The accumulated protoplasm is not removed even in the periods of reverse streaming, in contrast to type III.

The protoplasmic streaming starting in the zone of inhibited motion after about 10—20 sec. (sometimes even earlier) is not a mass transport of protoplasm normally observed in an injured vein. Initially it is of local character, restricted to short distances and occurs irregularly at a low rate and is, as a rule, interrupted by longer periods of stagnation (Fig. 3).

It is only in the later periods that the streaming becomes more coordinate, extends over longer distances and becomes rhytmical. Its rate, however, is still much lower than the rate of streaming shown by a normal vein. Due to this streaming, however, larger quantities of

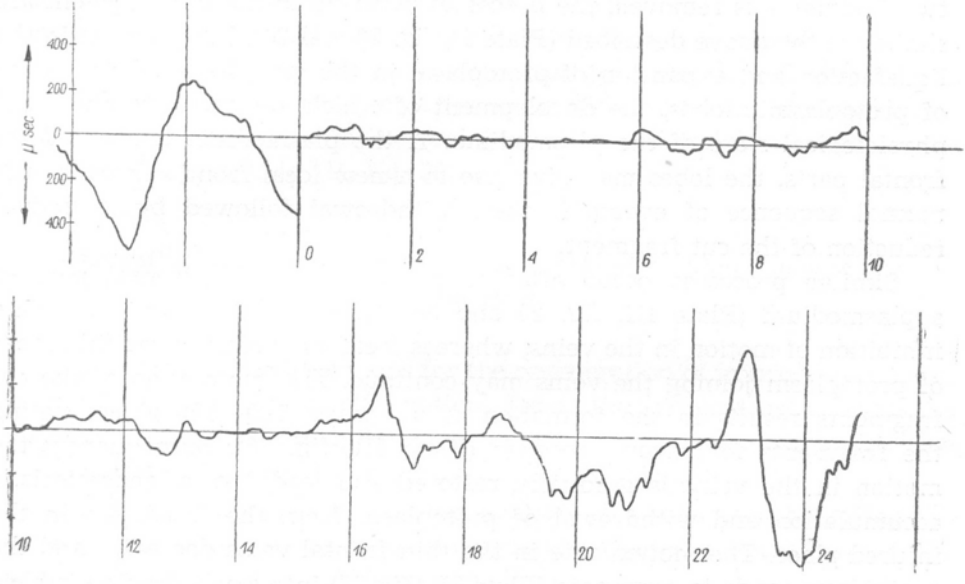


Fig. 3. Speed of protoplasmic streaming in the operated thread: X-axis — time in minutes (0 — time of operation), Y-axis-speed of protoplasmic streaming in μ/sec. The record was taken at a point distant 300 μ from the plane of cutting

protoplasm are gradually transported to the injured place; the result is a formation of lobes of protoplasm. The two parts of the cut vein approach each other and eventually they join after 5—15 minutes, depending on the intensity of the protoplasmic streaming (Plate II, fig. 17). In the place of junction the cortical layer of the vein is relatively thick and remains thick for a long time. Finally a channel is formed by means of which endoplasm may flow again in the veins.

Initially some disturbances in streaming may occur if the rhythms in the both fused fragments are not concordant as to their phases. Within few minutes the protoplasmic streaming regains its regular character and the motion rate which was low before the restoration of communication in the veins increases in later period considerably (Fig. 3).

The place where the injury was performed is visible on the vein for a long time in the form of an accumulation of protoplasm (Plate II, fig. 18) or an inflection in the vein caused by an unsymmetrical fusion of the two fragments. Also the optical properties (light refraction) are modified in the place of junction. In plasmodia showing strong vitality (young plasmodia on a humid substratum) the accumulation of plasma in the place of injury initiates the formation of a new vein with side veins extending along the line of cutting on the agar substratum.

If the vein is cut without injuring the agar substratum and one of the two fragments is removed, the processes occuring in the left fragment are similar to the above described (Plate II, fig. 20 and 24). They consist in the liquefaction and expansion of protoplasm in the cut place and formation of protoplasmic lobes, the development of which depends on the local physiological state of the plasmodium. If the plasmodium tends to form frontal parts, the lobes may give rise to a new local front; otherwise the normal sequence of events is their withdrowal followed by a gradual reduction of the cut fragment.

Similar processes occur after injury of a healthy frontal part of a plasmodium (Plate III, fig. 25 and 30). The cut causes an immediate inhibition of motion in the veins, whereas local streaming in the thin lobes of protoplasm joining the veins may continue. The contraction of the cut fragments results in the formation of a fissure (100—150 μ) separating the fragments for several seconds (Plate III, fig. 26). Subsequently the motion in the veins is gradually restored and leads to a characteristic accumulation and withdrowal of protoplasm from the fragments in the injured place. The motion rate in the thin frontal veins decreases and the protoplasm tends to aggregate (Plate III, fig 27) into small droplets which, however, spread again after some minutes and form a delicate network of small veins. The wound edges of the two vein parts gradually cicatrize, their outlines become irregular and after a time they fuse (Plate III, fig. 28). Fusion takes place first between the principal veins and subsequently along the whole length of the cut. Protoplasm is accumulated along the line of the cut and this accumulation leads to the formation of a new vein during the course of further expansion. From this accumulated protoplasm starts the spreading of thin lobes of protoplasm.

Type III. This type of regeneration is not a regeneration in the exact meaning of this term — because it leads finally to a disparition of the injured vein (Plate III, fig. 31 and 36). It is observed only in young

plasmodia in small anastomosing veins, whereas in older or less healthy ones it may also appear in thicker veins, sometimes also in the frontal part. The cut is immediately followed by an inhibition of motion in the vicinity of the operated place and a contraction of the cut fragments. In contrast to the previously described type where the contraction is transient and attains its highest degree after 10—15 sec., in this type the contraction is permanent and increases with time (Plate III, fig. 32 and 33). The protoplasmic streaming which gradually appears in the zone where the motion was inhibited causes a withdrawal of protoplasm from the injured place. Only exceptionally a greater accumulation in the cut fragment may temporary appear. The plasma does not accumulate in the wounded place and at the moment of maximal withdrawal of endoplasm from this place a further contraction of the injured part of the cut vein is observed. It is manifested by the shortening of the vein fragments. Their contraction proceeds at an irregular rhythm by fits and starts. The contraction period lasts 1—3 sec. and is followed by a period of stagnation. This may be caused by the resistance resulting from the adhesion of the plasmodium to the substratum. After some minutes a complete liquidation of the vein is observed and the protoplasm is completely withdrawn from the injured vein (Plate III, fig. 36).

The influence of potassium oxalate solution on the regenerative processes

The necessity of calcium ions for the regeneration of injured protoplasm is widely known. Their elimination from the environment causes an inhibition of surface precipitation reaction which is a necessary condition of cicatrization of the wound (H e i l b r u n n 1956).

Using 0.1 M solution of calcium oxalate as a substance bounding the calcium ions observations were made on the role of calcium ions in the process of regeneration of the plasmodium.

The flooding of the plasmodium cultured on agar with the above mentioned solution causes its decay in a short time. The first and immediate effect is an inhibition of protoplasmic streaming in the veins. In some cases it occurs in a fraction of a second, in other cases (in thicker veins as a rule) it takes some seconds to occur. After 10—20 sec. the protoplasmic streaming is restored (in the same direction as before the inhibition); but soon it becomes irregular. A simultaneous contraction of the gel layer in the veins is observed. It results in an increase of pressure of endoplasm that causes in turn splitting of this layer and formation of outflows of protoplasm. Globular outflows 1 mm. in diameter are visible even with a nacked eye. The first ones appear about 60 sec. following

application of the potassium oxalate solution. In contrast to the previously described effusions resulting from mechanical injuries of veins, outflows of protoplasm in this case do not show any distinct connection with protoplasmic streaming. Surface vacuolization is observed in numerous parts of the plasmodium, especially in the central ones. Hyaline vesicles appear on the surface of smaller and bigger veins as well as on the surface of the outflows. After 2—3 minutes a typical granulation of protoplasm takes place. The yellow pigments washed out from the protoplasm form stains on the agar. After 4—6 minutes ragged fragments of veins and colourless granules of protoplasm are the only remnants of the intact plasmodium.

Since the immersion of the whole plasmodium in a Ca ion bounding environment causes its complete degeneration in a very short time, an attempt was made to examine the influence on regeneration of locally administered potassium oxalate. The operation consisting in cutting the plasmodium vein (as formerly described) with the modification, however, that the blade was moistened with 0.1 M potassium oxalate solution. The course of regeneration in this case differs somewhat from that previously described (Plate IV, fig. 37—44). An inhibition of protoplasmic streaming on a certain length and a contraction of the cut vein fragments are observed; the very place of operation, however, is undistinctly outlined, shapeless (Plate IV, fig. 37 and 38) and after 1—2 minutes a distinct layer separating the healthy protoplasm from the injured is formed (Plate IV, fig. 39 and 40). A similar wound barrier is described by Seifriz (1955).

In places where the vein was blocked by a wound barrier accumulation of protoplasm is observed. From these places on the expansion of the plasmodium begins (Plate IV, fig. 42). The granular remnants of protoplasm dispersed among the centres of expansion are not involved in the process of regeneration.

Regeneration of the plasmodium from small fragments

Small fragments of veins from the frontal part of the plasmodium were amputed by means of a rasor blade in order to examine the regeneration capacity of a new plasmodium from isolated fragments. These fragments were transported on pure 1.5% agar.

In all cases, the regeneration process is as a rule similar to the above described (comp. photos, plate V). The vein or lobe fragment cut off from the frontal part of the plasmodium is in the gel state and in this form it is transferred to a new substratum. After 15 minutes a certain rounding of its outlines and pulling in of plasmatic threads is observed. This process is doubtlessly connected with a partial liquefaction and contraction of

protoplasm. After 15—60 minutes thin frontal lobes begin to expand (Plate IV, fig. 41; Plate V, fig. 51). With very small fragments they spread equally in all directions; the fragments form slowly a new network of veins which initiates a new round or elliptic plasmodium (Plate V, fig. 49 and 52). It is only when the fragment of the frontal part is considerably greater that the newly formed plasmodium is fanshaped and has a clearly distinct frontal part. When a fragment of the frontal part or of a small or averagely thick vein regenerates, the accumulated protoplasm disappears earlier than when a fragment of a thicker vein starts the regeneration process. After 1—3 hours the transferred fragment is no more visible but is transformed into a new plasmodium with distinct frontal and distal parts which begins to migrate on the substratum (Plate V, fig. 53 and 54).

When compared with regeneration of cut veins the regeneration of small fragments is characterized by a complete lack of protoplasmic streaming in the amputed fragment. The change from gel into sol state is accompanied by a slow spreading of protoplasm over the substratum and by formation of thin lobes and veins around the transferred fragment. The size of the isolated fragment is of no importance for the speed of formation of a new plasmodium. Three regenerating fragments, one about 0.2 mg. and two much smaller, under 0.01 mg., are shown in photos (Plate V, fig. 45 and 49). The fresh weights of these fragments were estimated from their dimensions, the weight of fragments taken from similar veins having been determined previously by means of a semi-microanalytic balance. The three fragments begin to form protoplasmic lobes albost simultaneously. The only difference is that in greater fragments the primary accumulation of protoplasm is visible for a longer time, whereas, in very small ones it disappears completely in about 30 minutes.

When comparing the regeneration of fragments taken from the frontal part with that of fragments taken from distal veins of the same plasmodium no essential differences in the course of regeneration are observed (Plate V). Some differences, however, especially in the initial period could be observed depending on the age of the plasmodium the fragments were taken from.

In regenerating fragments taken from old plasmodia protoplasmic lobes appear a little earlier than in fragments from young plasmodia.

The above described course of regeneration is observed only if the plasmodial fragments are transferred on fresh agar in the way mentioned in section 2, i.e. with no trace of the old substratum adhering to the fragments. Fragments of old cultures, on the other hand, transferred with a layer of agar on which they developped (as it is generally practised) show a poor migration ability on fresh agar and often perish.

Influence of age and size of the implant on the growth and migration of the plasmodium

In connexion with the fact that the regeneration capacity depends on the size and the age of the fragments, there arose the question of what size and age implants should be used to obtain cultures of uniform growth.

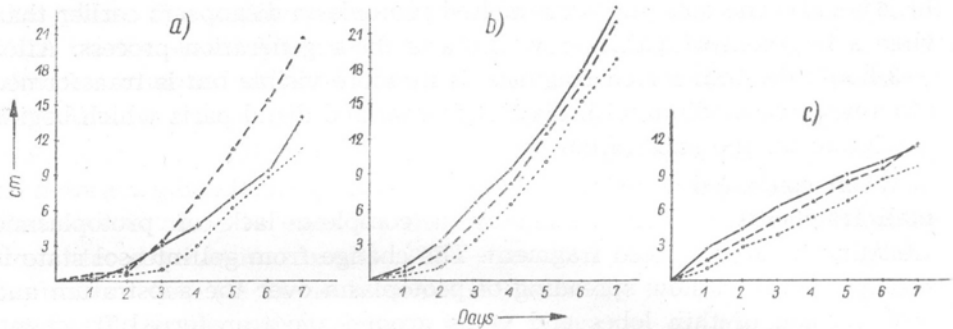


Fig. 4. Growth and migration of plasmodium in Rayan's tubes. Fragments of the frontal part used for inoculation were taken from 4 days (*a*), 8 days (*b*), 14 days (*c*) old cultures. The sizes of fragments were about 10 mm² ....., 40 mm² ————, 150 mm² ————, X-axis — time in days, Y-axis — distance in cm

The method of culturing plasmodial fragments on oat agar in Rayan's tubes was applied for investigations on this problem (R a y a n, B e a d l e and T a t u m 1943). The cultures were maintained in the dark at 20°. In these conditions the plasmodia may be cultured for 20 days.

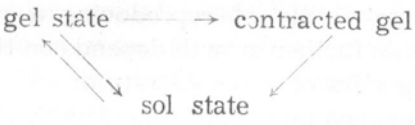
Fragments taken from the frontal parts of plasmodia of about 10, 40, 150 mm² area, together with a thin layer of culture agar were transferred to one end of the Rayan's tubes. The positions of the moving fronts of the plasmodia were read daily. Results are given in fig. 4, *a*—*c*. Compared with fungi the migration of plasmodia shows less regularity. This can be explained by the fact that the successive positions of the plasmodia are the result of their migration and growth (sometimes it happens that the plasmodium temporarily migrates in oposite direction). It results from the graphs that the most regular growth and migration are observed with 8 days old plasmodia and are only to a small extent dependent on the size of the fragments used for transplantation. With older material the migration of the plasmodium is much slower; with younger material — a delayed stabilisation of the migration rate and an obvious influence of the size of the implant are discernible. It must be stressed that plasmodia grown on oat agar in small Petri dishes provided the stock material for these observations. This is a detail that is important from the methodical

view point, because 8 days old cultures are at the climax of their development. In these conditions the size of the inoculum is without influence on rate of growth and migration of the plasmodium provided the growth rate has attained its constant value (after about 2 days).

DISCUSSION

In all cases, a sudden cut of the plasmodium caused an immediate inhibition of protoplasmic streaming along a certain length of the injured vein and its gelatisation. This phenomenon is a known reaction to a shock stimulus and was many times described (Winer and Moore 1941, Seifriz and Erstein 1941 and others).

In all cases the cut is followed by a more or less intensive contraction of the two fragments. Before their subsequent expansion a part of protoplasm at least becomes liquid, this being shown by sporadically occuring protoplasmic streaming. The observed phenomena may be compared with the scheme proposed by Landau (1959) to explain the changes of sol into gel state occuring in amoebas. According to this scheme the expansion of the contracted gel may only occur by passing through the sol state.



The temporary and local protoplasmic streaming discernible in the zone of inhibited motion presents some interest from the point of view of mechanics of motion. These streamings are not a mass transport of plasm but rather local streams which obviously are not synchronised either between themselves nor with the mean stream. It is only after a considerably length of time that their range enlarges and a coordination with the main stream takes place. This phenomenon presents some similitude with the beginning of the rotation movement in *Elodea cells.* It is always predeced by local streams of sliding type whose intensities increase till they change into a coordinate rotation movement.

According to Linsbauer (1929) a mechanical shock exerts a similar effect on the protoplasmic streaming in *Chara* cells.

In myxomycetes a local injury of ectoplasm causes an inhibition or a disturbance of motion in the place of injury, blocking of the vein (on a certain length), accumulation of protoplasm, local changes of the direction of streaming etc. As a rule no effusion of protoplasm from the veins is observed, this process being inhibited by an immediate gelatisation. The cut of thick veins only, with a large endoplasmic channel is accompanied by an effusion of protoplasm which, however, rapidly (about 1 sec.) forms a gel membrane. This membrane prevents, a further effusion

of protoplasm. In contrast to *Nitella* (Strugger 1949, Kamiya 1959) this phenomenon is observed even after an immersion of the plasmodium in water.

If a plasmodium is placed in a medium capable of bounding calcium ions (0,1 M solution of potassium oxalate) the appearing symptoms are characteristic of a lack of surface precipitation, described by Heilbrunn (1956) and Strugger (1949). The veins undergo a spontaneous bursting accompanied by frequent effusions of protoplasm. However, a great amount of Ca ions present in the endoplasm interferes with the effusion and contributes to the formation of a new gel membrane on the surface of the effusing endoplasm. A far going destruction and a final decay of the plasmodium are the result of the action exerted by potassium oxalate.

When the injuring factor is applied locally, it affects only a small fragment which is separated from the rest of the vein by a wound barrier. In the noninjured parts the regenerative processes proceed in a normal way.

Our study has shown that fragments of plasmodia possess a remarkable regeneration ability. Even a very small fragment of about 0.01 mg. fresh weight has not lost its ability of reproducing a new plasmodium on a suitable substratum. Its further growth depends on the culture conditions and the chemical properties of the substratum.

The observed phenomena are in agreement with the results of investigations published by Skupieński (1953). Although a different species *(Didymium squamulosum)* was used in his investigations, the rates of the regenerative processes in both cases are nearly the same.

SUMMARY AND CONCLUSIONS

1. A detailed description is given of the processes following an injury made on a vein or the frontal part of plasmodia of the myxomycete *Didymium xanthopus*. The first effect is an inhibition of protoplasmic streaming in the plasmodial vein on a certain distance from the injured place. The effects of cutting a vein of the frontal part of the plasmodium into two fragments are their contraction and moving away from each other.

Regeneration processes may be classified into 3 types:

a) The first is characterised by an effusion of protoplasm which joins again the cut fragments. The effusion is followed by a restoriation of protoplasmic streaming and a resorption of effused protoplasm.

b) In veins of average size or in frontal parts there is no plasmatic effusion after a cut. The two fragments contract and move off. After some time the streaming of protoplasm is restored. Plasma expansion in the

injured place leads to a fusion of the cut fragments and reestablishment of the former state.

c) In small anastomosing veins the cut fragments contract and move off. The restored movement of protoplasm and a further contraction lead to the resorption of the two fragments by the larger veins.

2. Disturbances of protoplasmic streaming observed after a performed cut of the vein and in the course of regeneration present some similarity to analogous processes occuring in higher plants.

3. A great regeneration ability of even very small fragments isolated from the plasmodium was established. This ability seems to be independent, in a wide range, on the size of fragments and on the place these fragments were taken from. To a certain extent it depends on the age of the plasmodium.

4. Treatment of the plasmodium with 0,1 M solution of potassium oxalate bounding Ca ions induces symptoms indicating the absence of a surface precipitation reaction in the protoplasm. The symptoms are similar to those observed in protozoa and in plants spiecies with cell membranes. After 5—10 minutes the plasmodium becomes completely destructed and perishes.

5. The most regular growth and migration rate are shown by plasmodia whose inocula were taken from cultures in the period of optimal growth. For the examined species this condition is fulfilled by 8 days old cultures grown on oat agar.

I am indepted to Prof. Dr. F. Górski and Doc. Dr. J. Zurzycki for their kind advices during the course of the work.

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Explanation of the plates

Plate I

Figs. 5—8. — Changes following touching a midle size thread of plasmodium without cutting it. *5* — before operation, *6* — 2 sec. after operation. The arrow indicates the place of touching; *7* — after 35 sec.; *8* — after 5 minutes

Figs. 9—12. — Effusion of protoplasm after cutting a thick vein: *9* — before operation, *10* — 1 sec. after operation; *11* — after 5 sec.; *12* — after 25 min.

Plate II

Figs. 13—19. — Regeneration of the injured thread: *13* — before operation; *14* — 1 sec. after operation; *15* — after 1.5 min.; *16* — after 12 min., *17* — after 17.5 min.; *18* — after 25 min.; *19* — after 50 min.

Figs. 20—24. — Regeneration processes following the removing of a fragment of a cut thread: *20* — before operation; *21* — after 2.5 min.; *22* — after 35 min.; *23* — after 60 min.; *24* — after 75 min. Magnification as in Plate I

Plate III

Figs. 25—30. — Regeneration processes after cutting the frontal part of a plasmodium: *25* — before operation; *26* — 10 sec. after operation; *27* — after 5 min.; *28* — after 14 min.; *29* — after 22 min.; *30* — after 75 min.

Figs. 31—36. — Reduction of an injured thread.: *31* — before operation; *32* — 40 sec. after operation; *33* — after 4 min.; *34* — after 9 min.; *35* — after 15 min.; *36* — after 17 min. Magnification as in Plate I

Plate IV

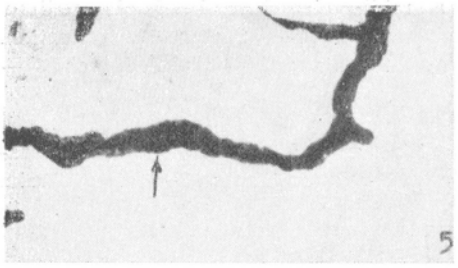
Figs. 37—44. — Course of regeneration after cutting the thread with a rasor blade moistened with 0,1 m potassium oxalate solution: *37* — 1 sec. after operation; *38* — after 20 sec.; *39* — after 3 min.; *40* — after 12 min.; *41* — after 15 min.; *42* — after *30* min.; *43* — after 1 hour; *44* — after 2 hours. Magnification as in Plate I

Plate V

Figs. 45—49. — Regeneration of plasmodium from small isolated fragments of a thread: *45* — immediately after transfering to a new substratum; *46* — after 30 min.; *47* — after 1 hour; *48* — after 1,5 hour; *49* — after 2.5 hour

Figs. 50—54. — Regeneration of plasmodium from a fragment of the frontal part of a plasmodium: *50* — immediately after transfering to a new substratum; *51* — after 1 hour; *52* — after 2 hours; *53* — after 2.5 hour; *54* — after 3.5 hour

Plate I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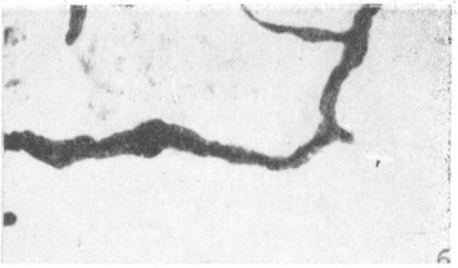
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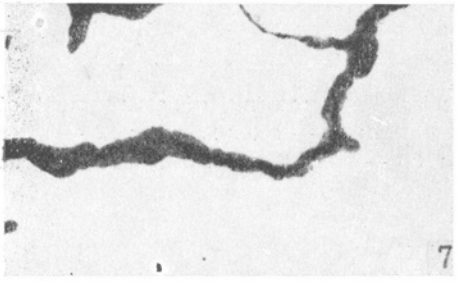
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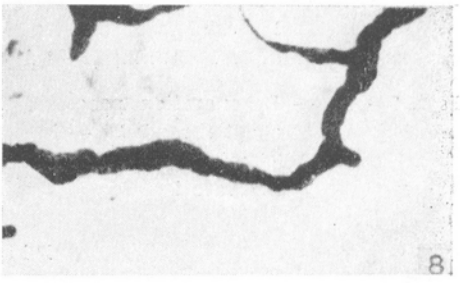
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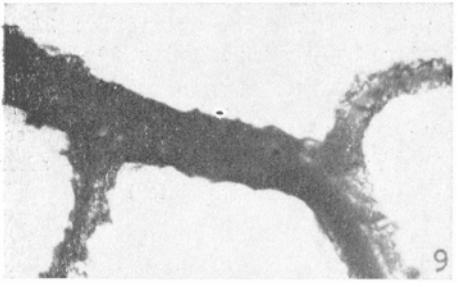
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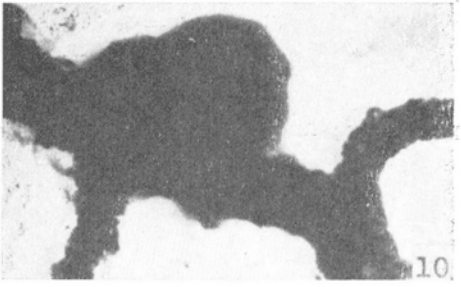
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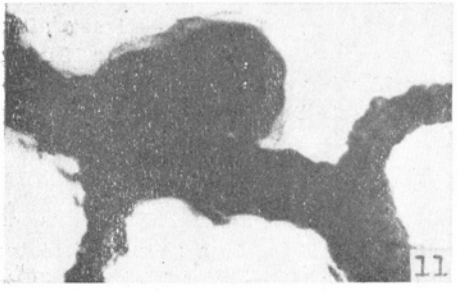
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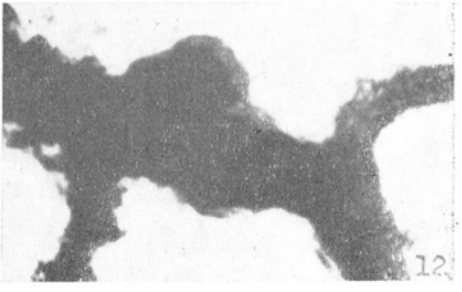
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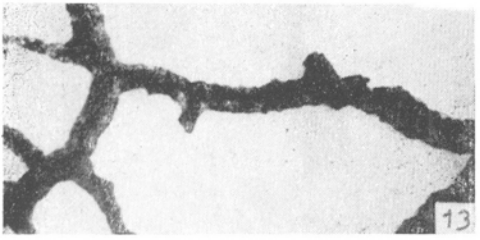
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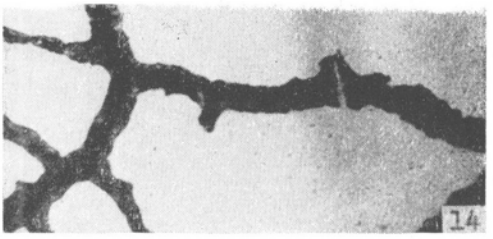
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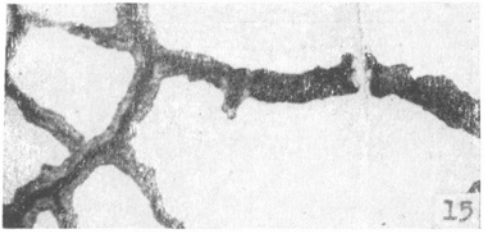
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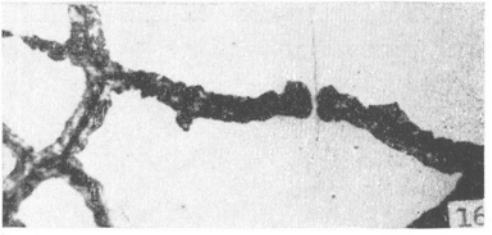
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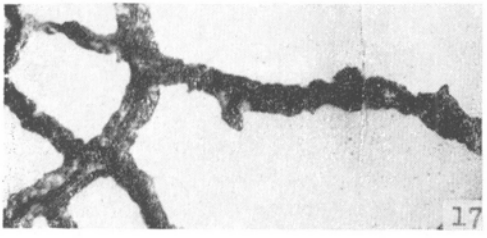
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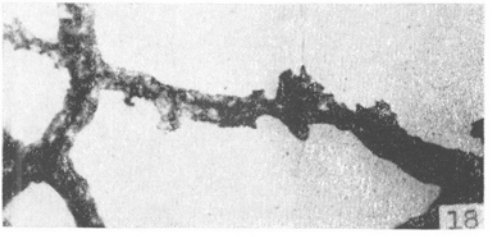
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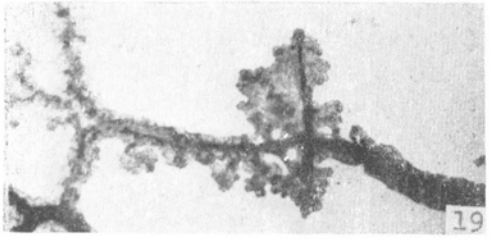
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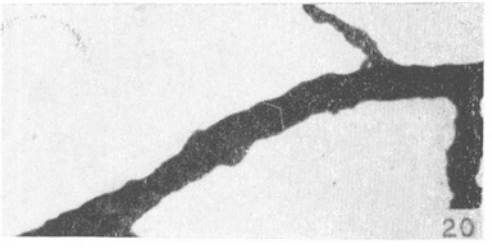
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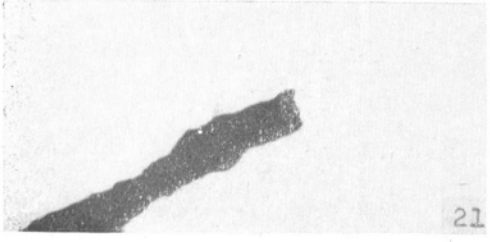
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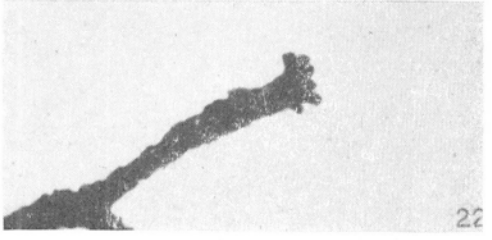
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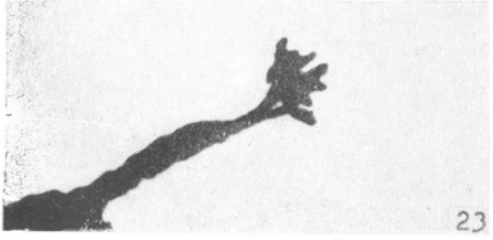
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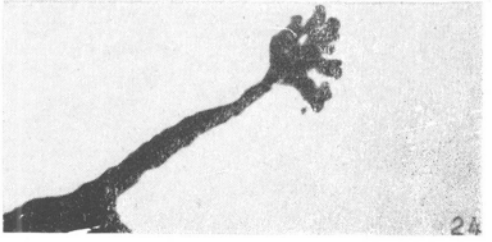
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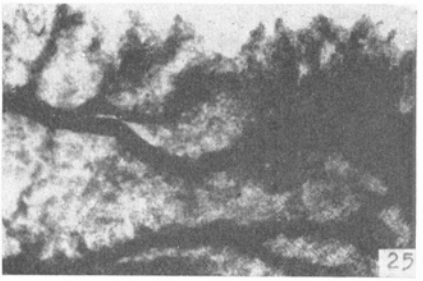
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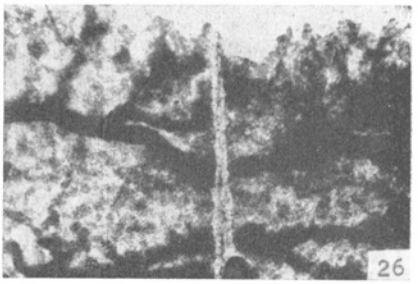
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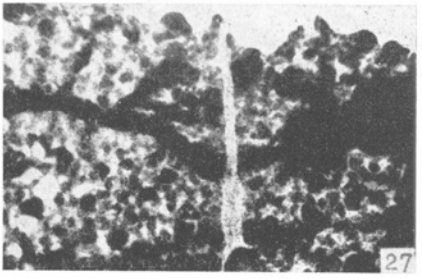
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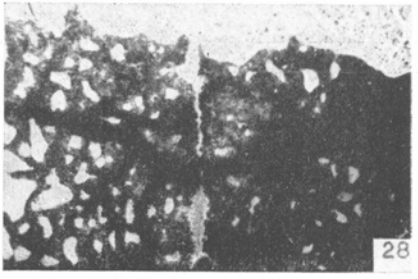
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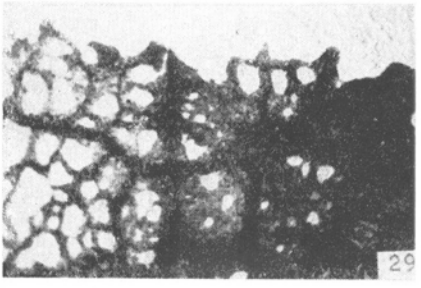
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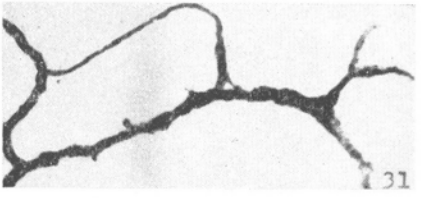
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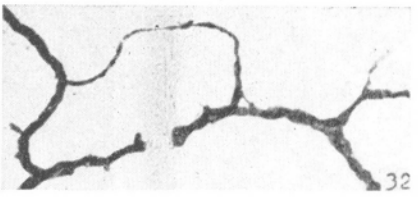
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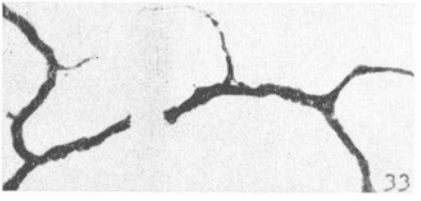
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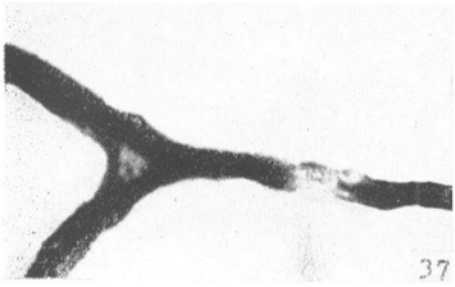
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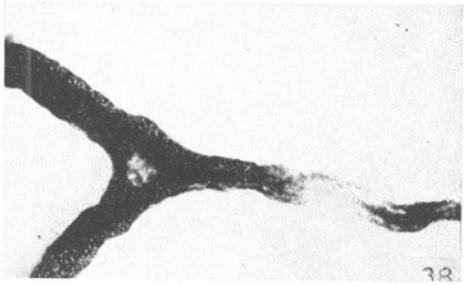
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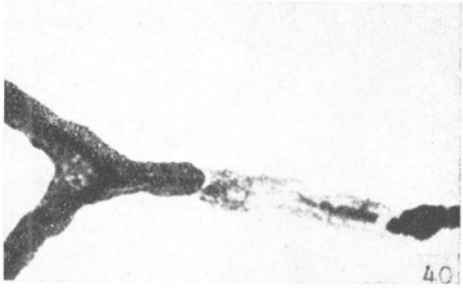
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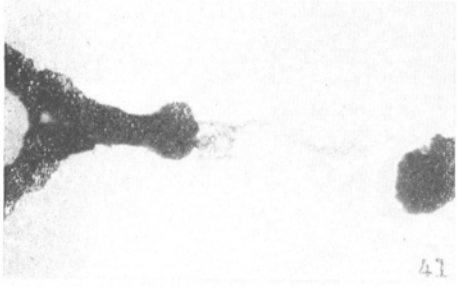
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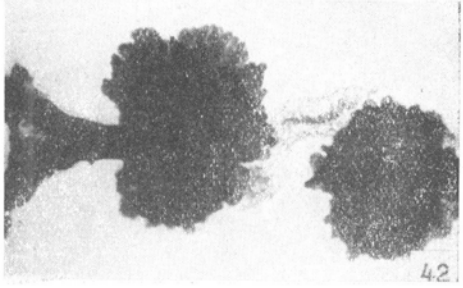
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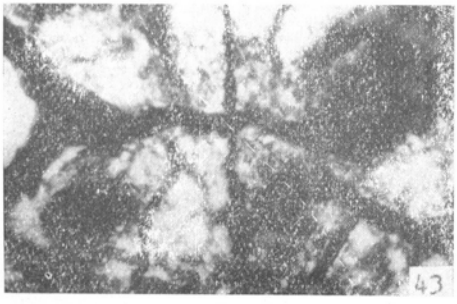
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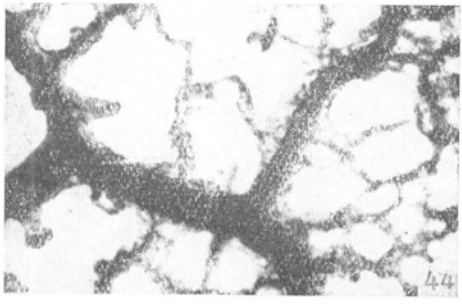
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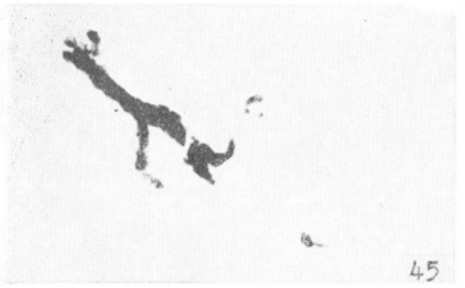
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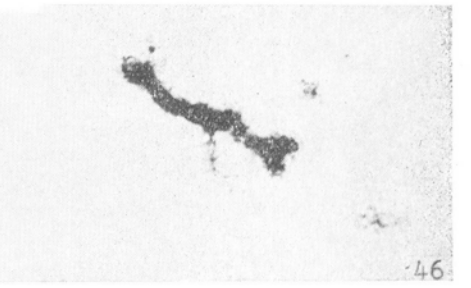
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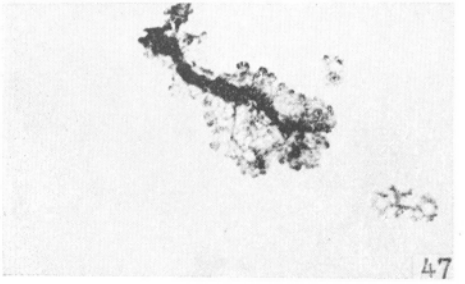
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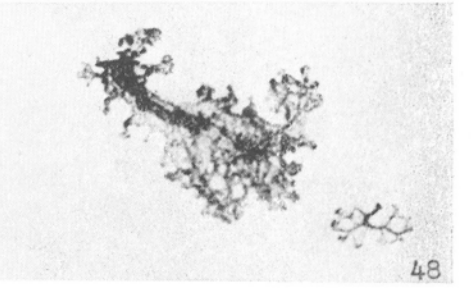
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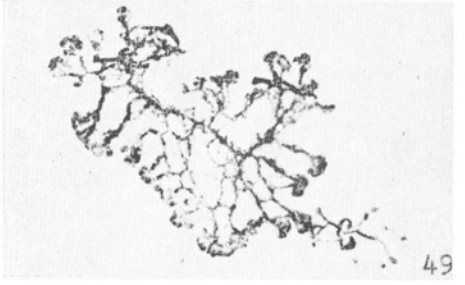
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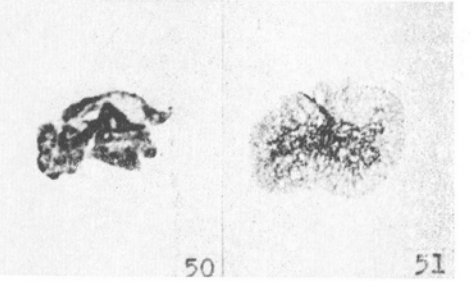
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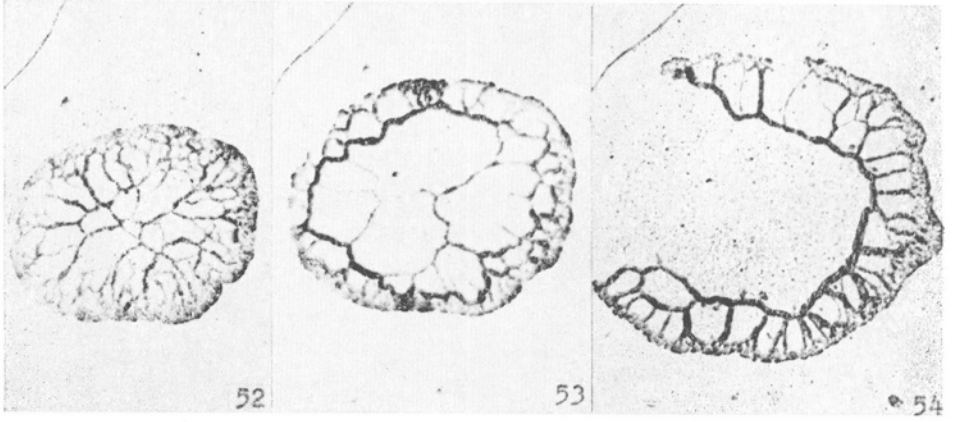
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