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Moving Colonies in Anaerobic Microbe Isolated from Vaginal Discharge*).

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During our studies on anaerobic organisms of the female genital tract, we were able to isolate from one case of leukorrhea, a microbe, the growth of which is accompanied by interesting phenomena. The description of these phenomena will be the subject of this communication.

This organism, which probably belongs to the genus Catenabacterium (Prévot, 1940), appears in young cultures as nonbranching filaments 30 to 60 μ long and short rods 2 to 3.5 μ long, arranged in very long chain formation. Segmentation in rods can be seen only in stained preparations since in unstained preparations a compact cell wall is seen to envelope whole filaments. The organism is not very strongly gram-positive in young cultures, becoming gram-negative as the cultures grow older, and is a strict anaerobe, very sensitive to oxygen. No flagella could be demonstrated with the use of several staining methods; motility of filaments in young but fully developed cultures could not be observed.

Chains may occasionally be tortuous, mostly by twisting of 2 filaments one along the other.

A striking feature is the presence of large thickenings in some rods or filaments. The thickened rod appears pear or club shaped and usually is located at one end of the chain or filament.

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At the beginning of our study we considered these thickenings as the possible beginning of spore formation, but in further observation we could not detect real spores by several staining methods (examining up to 15 day old cultures either from surface growth on blood agar or in several kinds of liquid media). Surprisingly the club shaped rods are present mostly in young actively growing cultures. Staining young cultures with Robinow's method (after fixation of bacterial film directly on agar with osmium tetroxide vapors), we could detect in clubs described above a big accumulation of desoxyribonucleic acid, which in our opinion indicates that they are part of very active bacterial substance. (We wish to express here our thanks to Doctor Robinow for his kindly help.)

In older cultures numerous granules are observed, situated in the filaments or in their proximity, staining deeply with basic aniline dyes.

Besides, in unstained preparations from older culturẽs, oval yeastlike bodies are observed, yellowish, highly refractile, of different sizes with a maximum of that of a human red blood cell. In stained preparations these bodies remain practically colorless. As could be shown later, they are very likely the product of the growing culture and are of lipoidic nature (compare with oil droplets found by Klieneberger in cultures of Streptobacillus moniliformis, which have been shown by Patridge and Klieneberger (1941) to be composed from cholesterol).

The granulose reaction in filaments is always negative.

The optimal growth occurs at about 35° C. Broth culture first shows a slight turbidity for several hours only, after which a fluffy viscous sediment is formed with a perfectly clear supernatant. Deep agar colonies are relatively large (3 mm. or more) and are mostly lenticular, sometimes more oval and irregular without fine protuberances. The surface growth on blood agar plates is film formation with indefinite single colonies, whitish, of very viscous (waxy) consistency. No hemolysis of rabbit or human red blood cells occurs. All cultures have a putrid odor.

The biochemical activities of our microbe are slight (insignificant). The degree of acidity in liver broth (without any sugar) after 14 days of growth is relatively low, being between pH 6 and 6.2.

Gas production in very small amounts can be shown only under careful observation, mostly in shaking broth cultures.

Practically, none of the 21 carbohydrates or polyalcohols usually used in such work is fermented after incubation of at least 18 days. In broth with glucose, maltose, levulose, and arabinose there seems to be an extremely slight degree of acidity.

Milk is very slowly coagulated, mostly incompletely. Gelatine became soft after 14 days. There is no action on coagulated egg white or fibrin. Acetylmethylcarbinol is not produced and there are some traces of indol, NH_3, and H_2S. Reduction of nitrates is positive.

The thermoresistance of the microbe is relatively high. Heating of very rich broth culture (with many protective compounds, of course) during 30 minutes at 70° C. failed to kill the bacterium. After heating at 80° C. under the same conditions, no more growth in subcultures appeared.

On fresh isolation the organism was pathogenic for small laboratory animals causing abscesses on subcutaneous inoculation or fatal purulent meningitis after subdural inoculation of rabbits. The pathogenic properties were completely lost after several months of subculturing on artificial media.

As seen from these descriptions, the anaerobic microbe isolated by us from a vaginal discharge has many similarities with those in the genus Catenabacterium described by Prévot.

It cannot be any species of the genus Zuberella, because of its lack of very active locomotion. Genus Eubacterium is described by Prévot as having short chains only, with the exception of a new description of Eubacterium parvum (Choukevitch) by Prévot (1947/b) which is, however, strongly saccharolytic. Catenabacterium leptotricoides (Weinberg et al. 1937) is an obligatory serophilic microbe, while our microbe could be early adapted on ordinary media. Catenabacterium contortum (Prévot et al., 1947) differs by heavy gas formation, turbidity in broth and strong saccharolysis. Catenabacterium helminthoides (Prévot et al., 1947a) forms arborescent colonies in deep agar, does not reduce nitrates, is strongly saccharolytic, forms turbidity in broth. Catenabacterium catenaforme (Eggerth, 1935) grows in surface culture in closed colonies of very defined shape, does not change either milk or gelatine and is saccharolytic.

We found most similarities with Prévot's description (1947) of Jungano's Catenabacterium filamentosum (1909), which is also a poor producer of gas, forms disklike colonies in deep agar and forms fluffy sediment in broth mostly without any turbidity. In contradistinction with our microbe, C. filamentosum is definitely saccharolytic, does not reduce nitrates, produces acetylmethylcarbinol, and coagulates milk quickly. We miss also in Prévot's description of C. filamentosum the mention of yeastlike bodies (lipoid droplets), which are very typical and easy to find and seem to be characteristic of our microbe. We found less similarities with related anaerobic microbes described by Prévot, Eggerth, Weinberg, and other authors (Vincentini, Repaci, Gins).

Summarizing the morphology, growth characteristics and other chemical activities of our microbe, we decided to place it temporarily in the genus Catenabacterium as a species probably not yet described. In case the microbe under discussion is actually a new species, as we do suppose, we propose for it the name of *Catenabacterium rotans*.

Further studies concerning the morphology and metabolism of this microbe are under way (with special respect to the possibility of very late

spore formation) and the final description of all its properties and definite taxonomical position will be presented later.

For the study of colony fermentation of the organism in microculture a modification of Fortner's microculture was used throughout.

Large sterile cover slips are immersed into nutrient agar containing about 1 : 10,000 thioglycolic acid. After the agar has hardened on the cover slip it is removed with a sterile knife from one side completely and on the other side a square is left just large enough to fit over the cavity of a depression slide. From a 2 to 3 day old stock culture grown anaerobically on a blood plate, a loopful is transferred to the middle of the agar on the cover slip. Serratia marcescens is inoculated on opposite edges of the same preparation to the width of 1 mm. The cover slip is fitted onto a depression slide and the edges well sealed with a paraffin petrolatum mixture. It is necessary to work very quickly since the organism is very strictly anaerobic. Some water vapor usually condenses in the depression of the slide but this does not obstruct observation. It is possible to study this culture with low and high dry power of the microscope and if the layer of the agar is sufficiently thin, water immersion can be used.

On microscopic examination the inoculum consists of an amorphous sometimes coccobacillary mass, with single filaments visible on the margin only. Among the filaments very short forms can be observed which are highly refractile. In our opinion these forms may correspond to the single segments of the bacillary chain seen in stained preparations and very probably represent young organisms capable of the most vigorous growth. After from 9 to 17 hours at 35° C. growth begins. When the growth is sufficiently fast it manifests itself by the formation of very regular whorls resembling rings of different width. The whorls range in diameter from 50 to several hunderd microns. Thin rings consist of 3 or 4 filaments twisted into a ring, thick whorls consist of a whole mass of filaments. The most interesting fact observed was that the rings are not stationary, but rotate around ther axis with a velocity which is in relation to the size of the ring and the age of the culture. Small rings rotate faster than large ones. The whorls rotate usually counterclockwise, but sometimes movement in the opposite direction is observed. We have also noticed large whorls in which the center rotated counterclockwise and the outer part in the opposite direction.

In a 30 hour old culture a dense ring with a diameter of about 150 μ makes one complete turn in $1^1/_2$ minutes to 2 minutes. (The whorl gives the impression of a watch-spring turning by its own elasticity.)

The whole whorl moves at the same time very slowly centrifugally from the inoculum. As far as we can distinguish from unstained preparations or stained impression smears, the ring is composed of a large number of filamentous bacteria or chains, each filament beginning with a highly refractile granule (corresponding to the pear or club shaped thickenings

described above). It appears that these granules play a leading part in the motility of the bacteria.

Apart from the whorls, single filaments are seen from time to time traveling very slowly by gliding movement in a large semicircle from the initial inoculum centrifugally, often leaving a visible path on the agar for several hours. Their barely distinguishable movement never lasts for long. Very likely, theirs is not only a forward movement but also a wavy and rotating movement, which can be deduced from the changes in the shape of the filament and from the fact that sometimes when two filaments meet they twist around each other. This interweaving of filaments may also be observed in the rings and whorls.

From the rotating rings some filaments are freed and continue to grow, whilst other free filaments combine with the whorl, rotate with it for some time and then leave it again. As soon as filaments of one whorl reach another whorl, a bridge is formed between the two whorls. This bridge of filaments becomes denser as the filaments grow, until both whorls are combined into one large irregular oval formation, no movement of which can be observed.

The above-described phenomena can be observed for the first 48 hours only. On the third or fourth day the culture loses its motility completely, and because of its increased density, the culture loses its flat character. The individual whorls change into three-dimensional, sometimes nearly convex bacterial colonies and the composition of individual filaments can no longer be distinguished. The growth is thereby completed and the culture has reached the maximum of its development. Apparently all the nutrients of the agar are used up by that time.

If the microculture is left in the incubator, obvious signs of degeneration may be observed after a week or later. The first symptom of this is the appearance of a larger number of small granules of slightly different aspect and staining quality than those found usually in the filaments. Thereafter, autolysis sets in, so that only the dim outline of the colonies and granules may be seen. In the center of the colony, an irregular drop of a yellowish fluid (probably a lipoid) is formed which is probably a product of the metabolism or autolysis of the microbes. When this drop is released by the complete dissolution of the colony, it becomes round and resembles a red blood cell or a yeast cell. These are obviously the same unstainable structures which we described above as being similar to (only less numerous than) cholesterol droplets found by Patridge and Klieneberger in the culture of Streptobacillus moniliformis.

In a series of experiments we have tried to follow the earliest stages of development, immediately after the lag-phase. This was not an easy task because the lag-phase lasts for from 9 to 11 hours and its length depends on the quality of the medium, its redox potential (which, besides the relative

humidity of the surface, is one of the main factors), and also on the age and quality of the inoculum as well as on the working speed. The longer the culture is exposed to air, the longer is the lag-phase.

In cases in which we were able to observe the culture in the initial growth phase, it was found that all whorls and most of the rings are formed already in the inoculum, most frequently in its margin, and leave the inoculum fully developed. The formation of rings outside the original culture is very rare and usually confined only to the formation of incomplete rings which usually merge into already formed rings.

The process of ring formation is very interesting and, as far as it can be observed at all, it occurs as follows: The above-described short and refractile structures, spaced irregularly among the filaments of the inoculum, which can be seen distinctly mainly on its periphery, and which form a considerable part of the inoculum, aggregate after 5 to 6 hours in the incubator into round lumps. This takes place without effecting any change in the appearance of the culture. The process closely resembles that of agglutination with one exception, i. e. that the lumps which are still inside the original inoculum become perfectly round until they adopt the shape of slightly convex disks, whose composition of short coccobacillary elements is still evident. After several hours the internal structure of the disks becomes indistinct. The first filament begins to form on the periphery and gradually becomes more distinct, growing like a spiral toward the center, and after about 4 to 8 hours a complete whorl is formed. This whorl begins to rotate and leaves the original inoculum. After wandering for a certain time it gives rise to a new colony.

In our opinion, the origin of the filaments is not a matter of complicated internal change. The coccobacillary forms arrange themselves into chains and then grow into filaments. The same force which governs this arrangement appears to be also the force causing the movement of the whorls.

The above-described process shows that the first phase of the development resembles the last phase, in which most filaments disintegrate into short rods, while the rings grow into three dimensional colonies.

It is interesting to ascertain that the growth in microcultures corresponds to the growth of the microbe on plates under anaerobic conditions. The analogy is complete. The fully grown culture appears on the blood agar as a film covered with whitish convex elevations, giving it the appearance of warty skin. If the cultivation is interrupted before the film reaches the edge of the plate, we find more or less dense ring colonies on the free part of the blood plate, which are sometimes connected by a filament with the edge of the film or sometimes remain without connection. This corresponds exactly to what may be seen in microculture. The elevations seen on the full film are the same rings, only afterwards they are overgrown by the filaments from which they originated.

The only question is what do the large granules, found in the growing filaments and also observed in the rotating whorls, correspond to. These granules were also found in some number on that end of the filament which is determining the direction of the movement. It almost gave the impression that they determined growth and motility. Very probably they are not real spores — because they are rather easily stainable with diluted aniline dyes and in our observations they have none of the usual characteristics of mature spores. Surprising is the large amount of desoxyribonucleic acid in most of them.

It is equally difficult to determine definitely the question of motility of the microbe, although it appears to be of principal importance. Very young cultures in liquid media (12 to 17 hours) under strongly anaerobic conditions did not produce locomotion movement in filaments. The staining of the flagella by 5 different modifications of flagellar staining methods showed negative results. We are aware of all weaknesses of these methods — particularly in anaerobes — but we are still convinced that the microbe has none of the normal motility which is usually attributed to the movement by means of flagella. The movement of individual filaments in very young cultures, outside the whorls, on agar plates was only gliding and too short, too slow and too rarely evident to be considered a permanent and regular characteristic of the microbe. For the sake of completeness, we have to add that Fortner's micromethod is particularly suited to the study of the motility of anaerobes. We have found it excellent for the study of Clostridium oedematiens, Cl. septicum, Cl. tetani and other motile anaerobic organisms.

In the last series of experiments, we tried to confirm the influence of some physical and chemical factors on the formation of motile colonies of the described filamentous micro-organism as far as such experiments were feasible.

A slight surface humidity of the medium is essential, as in all methods of anaerobic cultivation.

A second factor of equal importance is the speed of the growth. This is again contingent on the degree of the redox potential. Only if that degree is sufficiently low and, consequently, the growth quick, can we get the typical picture as described. If agar without thioglycolic acid (but with prodigiosus) is used, whorls are very rare, although the culture is growing. If agar with thioglycolic acid but without prodigiosus is used, the growth is still slower and there is never any formation of rotating colonies. In both cases, filaments spread from the inoculum in all directions but they remain connected with the inoculum. A part of the filament turns back to the inoculum in the shape of nooses, so that the growing inoculum will resemble an anthrax bacillus colony.

Racker considers tho glutamic acid as one of the main reasons for the characteristic growth of B. proteus (so-called "swarming"). On a medium

without glutamic acid B. proteus grows in closed colonies although the individual bacteria remain equally motile.

Our microbe could not be cultivated under completely defined conditions. However, we have tried to add glutamic acid in excess to the medium and have arranged a series of experiments so as to keep the growth of the culture relatively slow. While the formation of whorls was only slightly indicated in control microcultures, the formation of ample and very motile round microcolonies was observed in all cases in which glutamic acid was used. The favorable influence of glutamic acid on this phenomenon, therefore, seems to be indisputable.

The formation of actively motile colonies is therefore proportional to the speed of the growth and we assume that the quick growth of rods (to or in chains or filaments) in length may be the reason for their temporary gliding motility and for the elasticity forces in the whole system of whorl colonies which are also moving, however only for the duration of the growth.

Discussion.

We have described the formation of rotating motile whorls in cultures of an anaerobic filamentous bacterium isolated from the vagina, whose formation and development could be followed under the microscope.

Similar phenomena have been described for other organisms. Muto in 1904 described it for Bacillus helixoides (helicoides) which is an aerobic nonspore-forming microbe from the soil which, according to Lehmann, is related to the Bacillus proteus. In 1935, Roberts observed whorl formation and motility of colonies on agar plates in cultures of Bacillus rotans which is an aerobic motile spore-forming rod. Ford, in his book, quoted Jordan's observation on Bacillus circulans (aerobic, gram-positive, spore-forming motile rod). The colonies of this organism display rotating movement rather than linear motility. Smith and Clark (1938) studied the same phenomenon in detail on Bacillus alvei and on another gram-negative nonspore-forming rod, isolated from the intestinal tract of an angleworm. Colonies of Bac. alvei display a motility resembling that of Bac. rotans, while those of the latter organism show a movement similar to the movement of Bac. circulans. The authors ascribed the motility of colonies to the motility of the individual cells and to the properties of the medium. Shinn (1938) presented a detailed cinematographic analysis of the movement of colonies of Bac. alvei. This movement consists of five phases. At the same time he expressed surprise at the fact that this interesting phenomenon had been overlooked for so long. Russ-Münzer observed the same phenomenon on an unclassified bacterium which was later identified by Smith as Bac. alvei. Clark (1939) found a nonmotile variant of Bac. alvei, which did not form motile colonies.

Turner and Eates (1941a) isolated a soil bacterium, spore-forming, motile and aerobic, forming wandering colonies. The authors maintained that this organism is different from those previously described.

All those cases dealt with aerobic motile micro-organisms, and the movement of colonies could be observed directly on agar plates. The latter authors (1941b) tried to study motility of colonies in bacteria of the genus Clostridium. Since, in their opinion, a direct observation was not feasible, they tried to deduce the presence of this phenomenon from the presence of remote daughter colonies and traces leading to them, and from microscopic impression preparations. They postulated migration and rotation of small colonies in numerous strains of Cl. oedematiens from the presence of these colonies outside the main colony. The double margin of the mother colony is probably the remainder of the path by which the daughter colonies moved out of the mother colony. The same observation was made on some strains of Cl. septicum, Cl. botulinum and Cl. tetani. We agree with Turner's assumption, although we could never observe this phenomenon in Fortner's microcultures of above-mentioned strains.

It is our opinion that a rudiment of the same phenomenon exists in the formation of the curved projections of the colonies of Bac. mycoides. The origin and growth of these dextro- and levorotatory projections have been studied and described by many authors (de Jong, 1933; Hastings, 1932). Similar phenomena possibly occur in spirochetes (Dyar, 1947). The formation of rotating rings was also observed in Bac. proteus (Friedemann). The same was once observed by us.

All microbes hitherto described as producing motile colonies were themselves actively motile. However, this does not seem to apply to the filamentous bacterium described by us in the present communication. We observed only a slight gliding movement of few filaments in the first stages of the development.

In 1950, Mandia described motile colonies of W. perfringens in semi-solid culture medium though the microbe itself was quite nonmotile. His paper confirms our observation that the formation of motile colonies need not be connected with individual motility of the single microbes.

In this connection it might be interesting to recall Pijper's views on motility of bacteria (1946, 1947). According to Pijper (observations on motility in highly viscous media) the so-called "flagella" are the result of motility and not its cause. The motility itself is caused by screwlike and wavy movement of the body of the bacteria. It is noteworthy to mention here that in the big class of Schizomycetes the motility without flagella has been certainly proved by gliding movements in rigid cells of some species in Ordo III, Chlamydobacteriales, and creeping motility in nonrigid cells with swarming of whole culture in all of Ordo IV, Myxobacteriales. The swimming motility by flexion of cells in Ordo V, Spirochaetales, has been

known of for a very long time (Bergey, 1948). If Pijper's assumption is correct it could apply to certain microbes only in some phases of their development, i. e. when they are most active, as for instance at the beginning of their growth.

Our microbe could serve as an example of this type of movement as twisting of filaments around each other can frequently be observed. In this case, however, it is impossible to explain the rotation of colonies by co-ordinated movement of individual bacterial cells but rather by tension and pressure originating during growth. The wandering of whorls to the periphery by some centrifugal force results from rotatory movement of each whorl. This explanation is also corroborated by the fact that the phenomenon disappears with the slowing-up of growth. The rotation of colonies can only be observed in very fast-growing colonies. A further corroboration of our explanation can be found in the work of Racker, on the so-called "swarming" of B. proteus. He has shown that "swarming" is not dependent on motility of the individual micro-organism, but is a function of metabolism and requires glutamic acid for its appearance.

It follows from our observations (exceptional forming of rotating colonies in. B. proteus, formation of filaments in microculture) that the forming of motile colonies is likely to be just a particularly intensive case of the growth phenomenon which we characterize as the so-called "swarming" and which is typical of Bacterium proteus and numerous anaerobic clostridia.

The phenomenon of swarming is, to our judgement, coupled with a definite phase of microbial growth only.

This phase is probably the end of the lag-phase and the beginning of the logarithmic growth of the microbe. As is known, all microbes are in this phase of their development metabolically most active and have by this time the largest sizes, too. In B. proteus and swarming clostridia this phase may be easily followed by the formation of long bacterial forms, whose metabolic activity manifests itself in quick gliding movements. This active metabolic phase does not last long. The long, serpentine filaments disintegrate into short flagellated rods which, then, by means of flagella, acquire locomotion without co-ordination and polarity (corresponding to an advanced phase of logarithmic growth).

Catenabacterium colonies rotate only as far as they are composed of long filaments growing terminally and forming spirals.

The metabolic activity of Catenabacterium towards the end of the lag-phase is manifested by a rapid growth of the spiral; this being held together by means of a special capsular substance, a tension develops which puts the whole microcolony into rotatory movement. The rotation of the whole microcolony gives rise to centrifugal powers that are, subsequently, the cause of the centrifugal movement of the colonies.

The lag-phase and the initial phase of logarithmic growth having expired, the metabolic activity of Catenabacterium decreases. The filaments rapidly disintegrate into shorter rods, forming by that time a three-dimensional colony. The internal tension of the colony is reduced by that and the movement stops.

The formation of motile colonies, as it has been observed by us in the described species of the genus Catenabacterium, is, consequently, nothing but a special growth phenomenon corresponding — as to stage — to the end of the lag-phase and the beginning of the logarithmic phase of bacterial growth. It is not connected with the motility of individual bacterial cells by means of flagella, but is related to the so-called swarming which is a typical growth phenomenon in some bacteria.

Summary.

The authors describe a filamentous anaerobic Bacterium, isolated from a purulent vaginal discharge, gram-positive, and, according to findings made so far, non-sporebearing and nonmotile, which they tried to place in the genus Catenabacterium as probably a new species. The microbe was in the first cultures slightly but distinctly pathogenic for laboratory animals; subsequently, it lost this property. The bacterium had some interesting properties as it formed big large bodies in the course of the filaments, was, sometimes, subject to autolysis, and produced, in a similar way as Streptobacillus moniliformis, droplets of lipoids. Its most interesting property, however, was its forming, under anaerobic conditions, rotating and centrifugally moving microcolonies, whose rise, movement, and further development it was possible to follow in microcultures directly under the microscope.

The formation of motile colonies is a very rare phenomenon in microbes and has been described so far only in about 6 species of aerobic sporebearing bacilli and bacteria, among which rank, e. g., Bacillus helicoides, alvei, and circulans. The Catenabacterium described by the authors is the very first of anaerobic bacteria, in which this interesting growth phenomenon actually has been proved directly. Subsequently, by a different method, it has been most probably ascertained (as a rare exception only) in some strains of Welchia perfringens. As probably existing (on the basis of indirect cutivation phenomena) it is adduced also in some anaerobic Clostridia, such as Cl. septicum and Plectridium tetani.

Since the microbe in question is nonmotile, some other explanation of the mechanism of rotating colonies is necessary than that current for the above-mentioned species, which — except Welchia perfringens — belong altogether to microbes actively moving by means of flagella.

In the authors' opinion, the formation of rotating colonies is but a special and extreme case of the socalled swarming of bacterial cultures which is typical of e. g. Bacterium proteus. The pursuit of the development of the swarming culture and the rotating colonies evinced the probability of the coincidence of both with the end of the lag-phase and the beginning of the logarithmic phase of bacterial growth. One of the manifestations of a large quantum of energy the microbe is endowed with in this phase, is the movement of the whole mass, polarized and of a nature different from that of the current locomotion of a fully developed microbe. It may be similar to the locomotion by which Pijper explained the motility of bacteria in general.

The mass of fast-growing filaments of Catenabacterium, tending to spiral growth and held together by a mucous substance of unknown character, incontestably produces in the whole system of the round and flat microcolony a tension, whose final resultant is the power bringing the whole colony into rotatory movement.

According to this, the rise of the rotating colonies seems to be a particular instance of a growth phenomenon. Finally, the possible influence of other factors, such as redox potential and glutamic acid, on the rise of motile colonies, is discussed in the paper.

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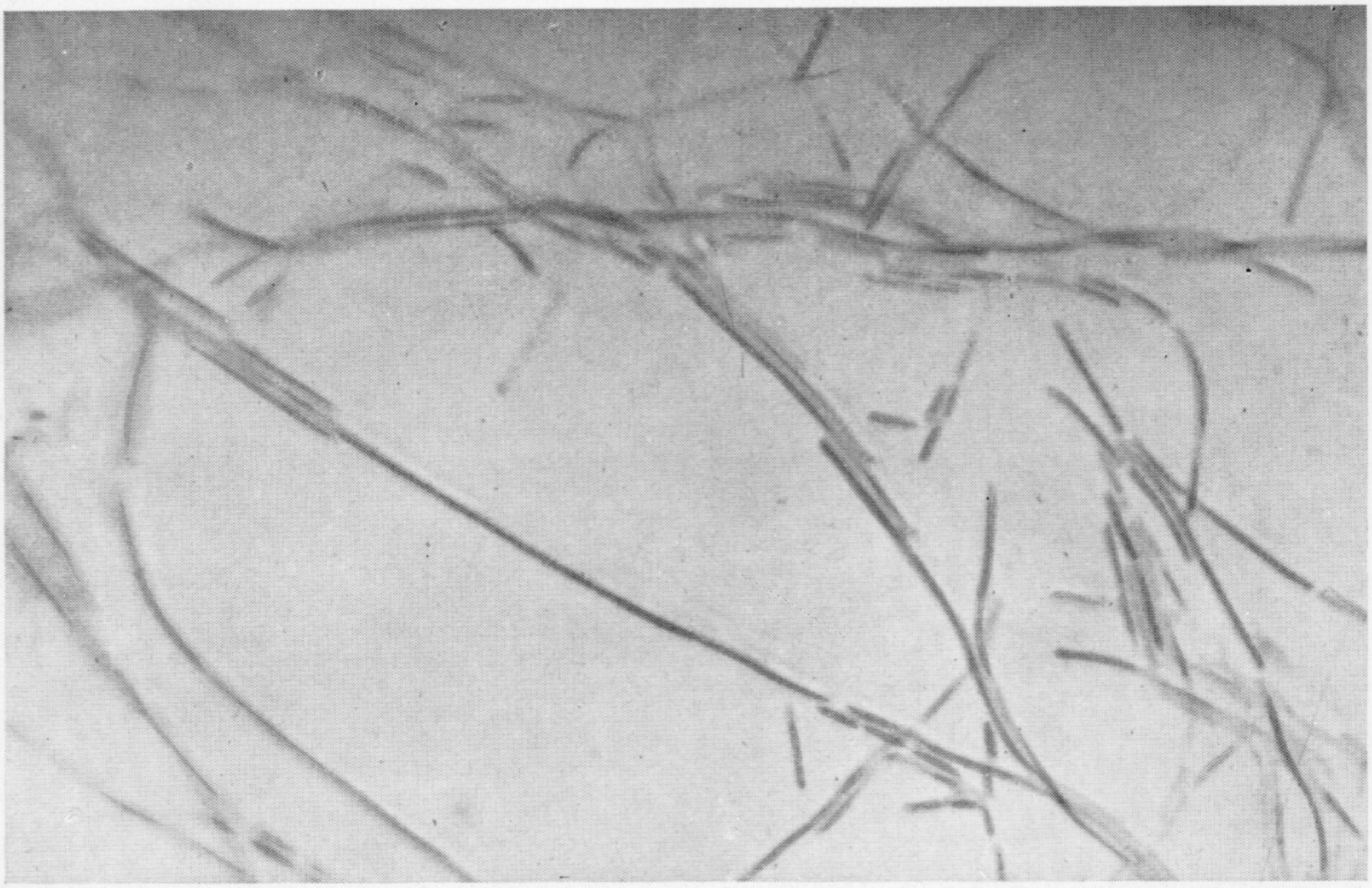

Fig. 1· Catenabacterium. — Broth culture stained with fuchsin. — Some filaments showing capsules.

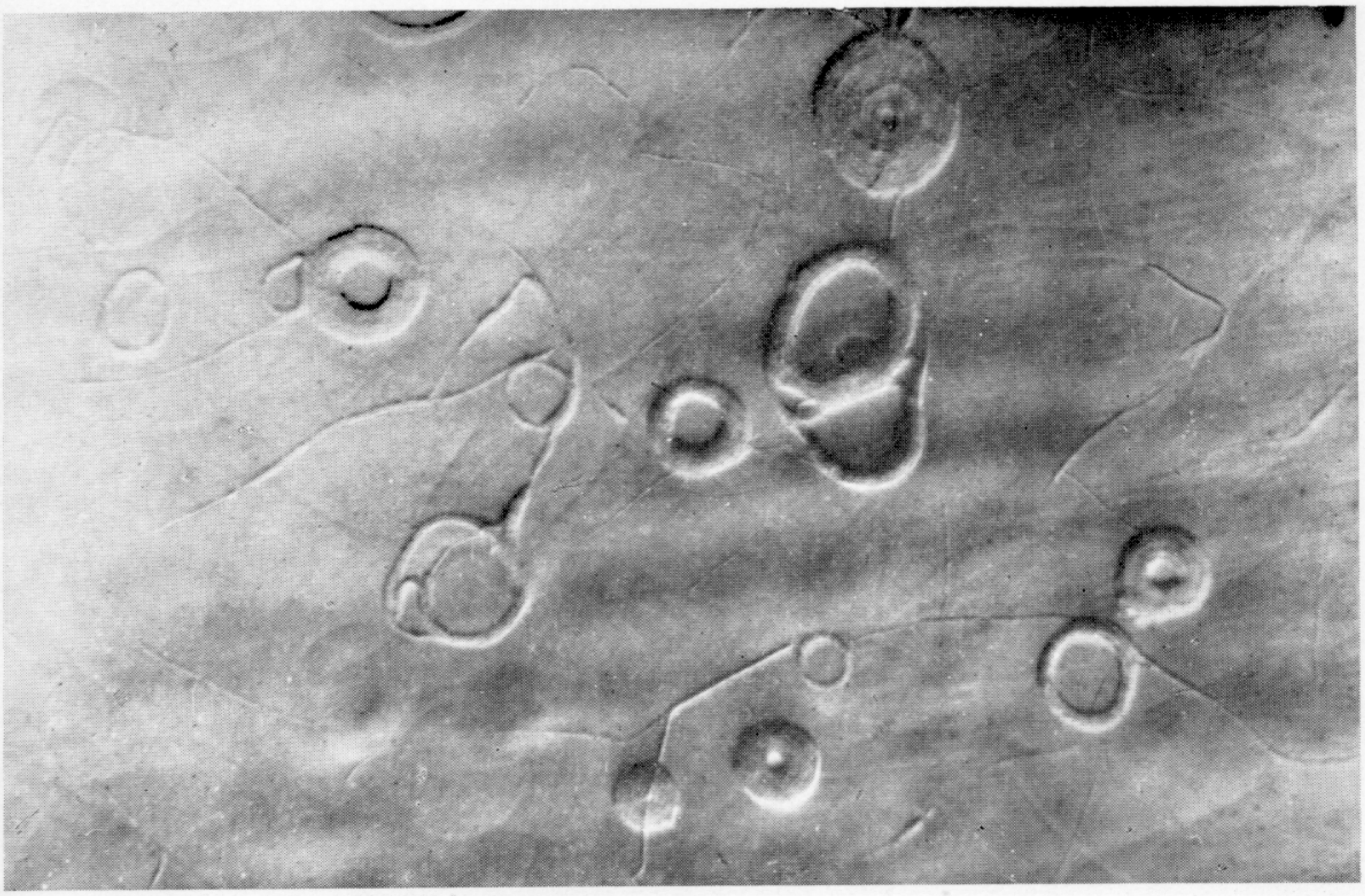

Fig. 2. Photograph of microculture under anaerobic conditions showing rotating whorls and rings. Gliding filaments leave a visible path on the agar.

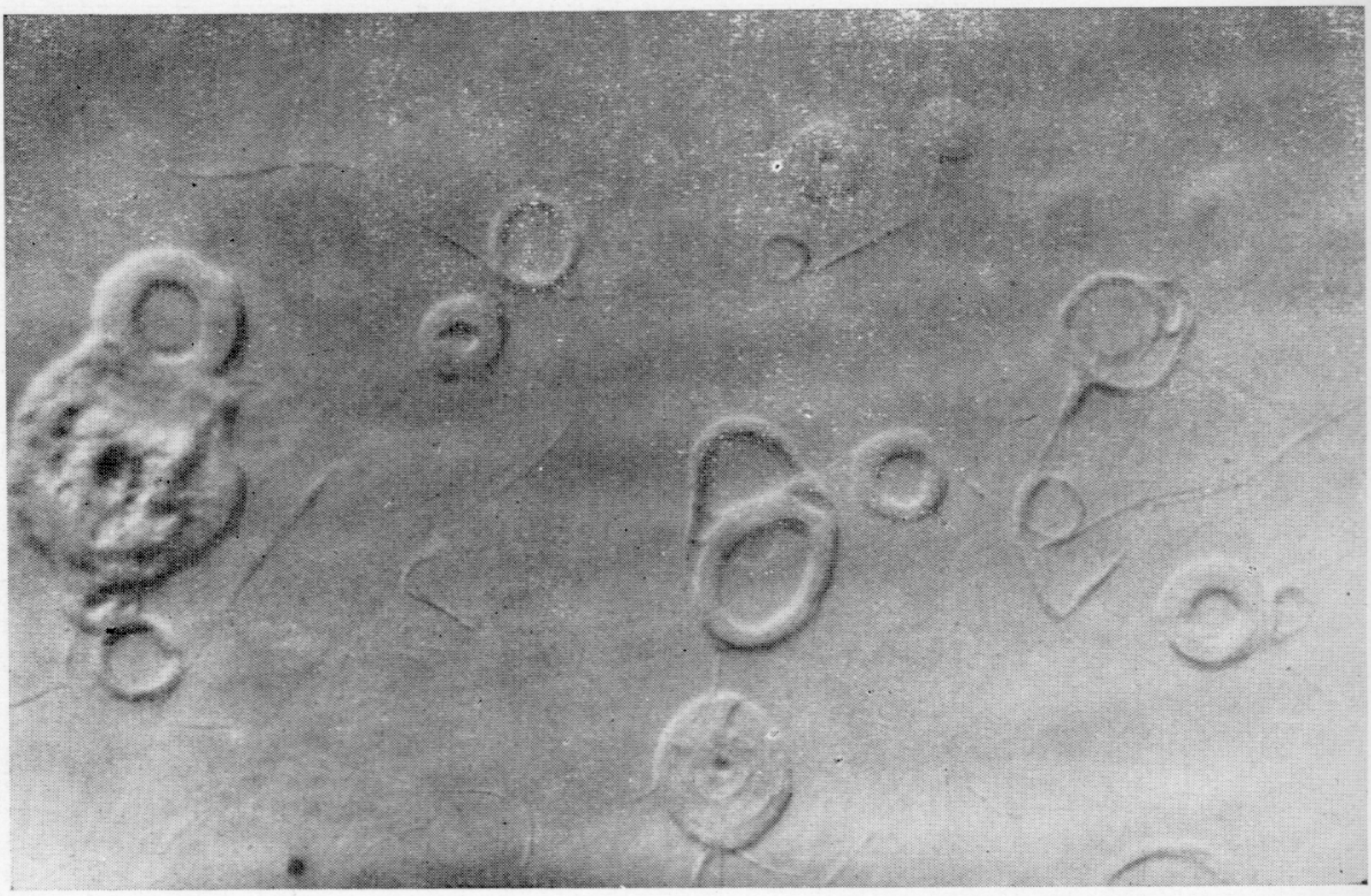

Fig. 3. More advanced stage of rotating whorls and rings.

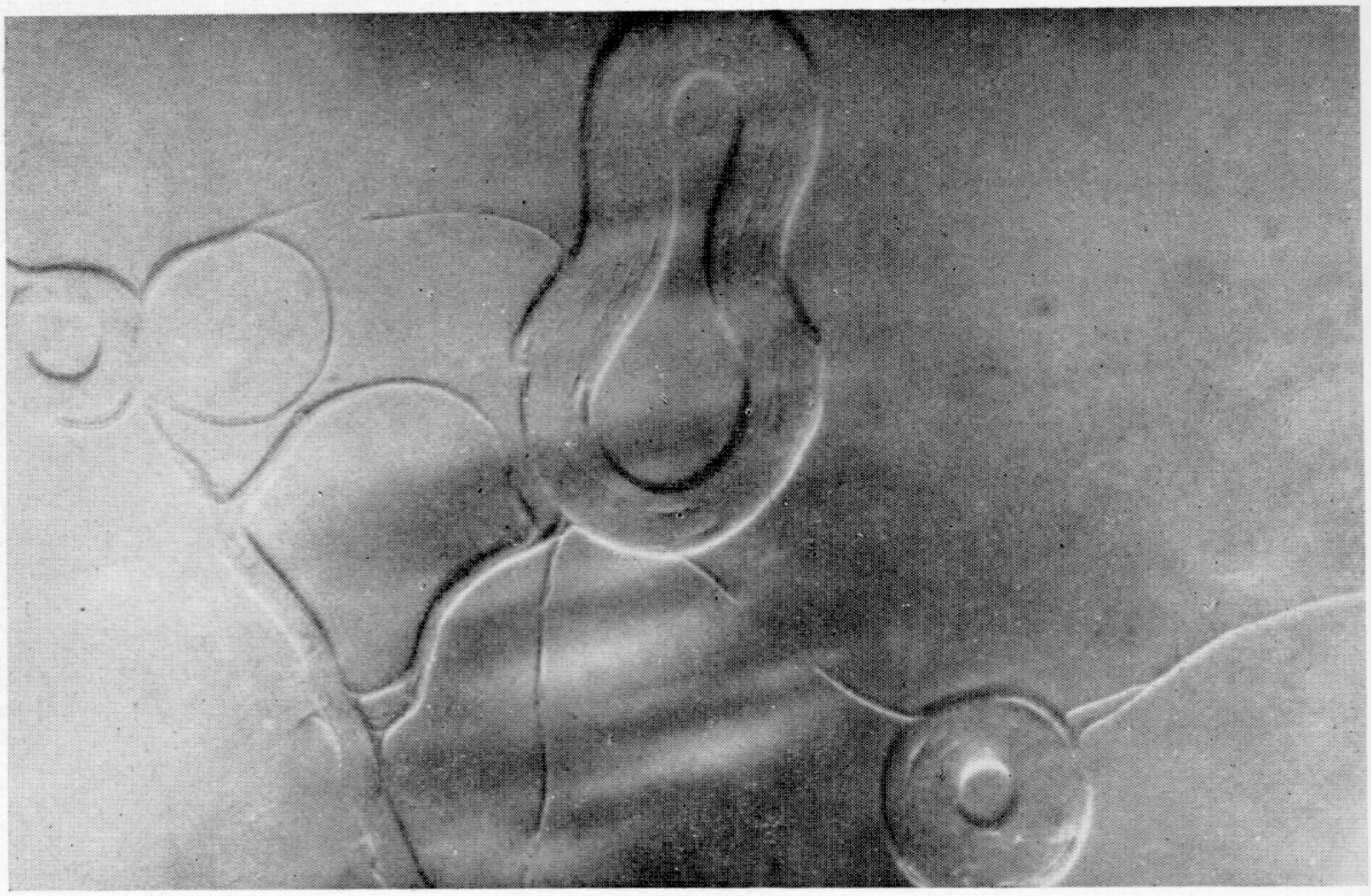

Fig. 4. Typical whorl (big magnification). Oval formation originated by fusion of two rings.

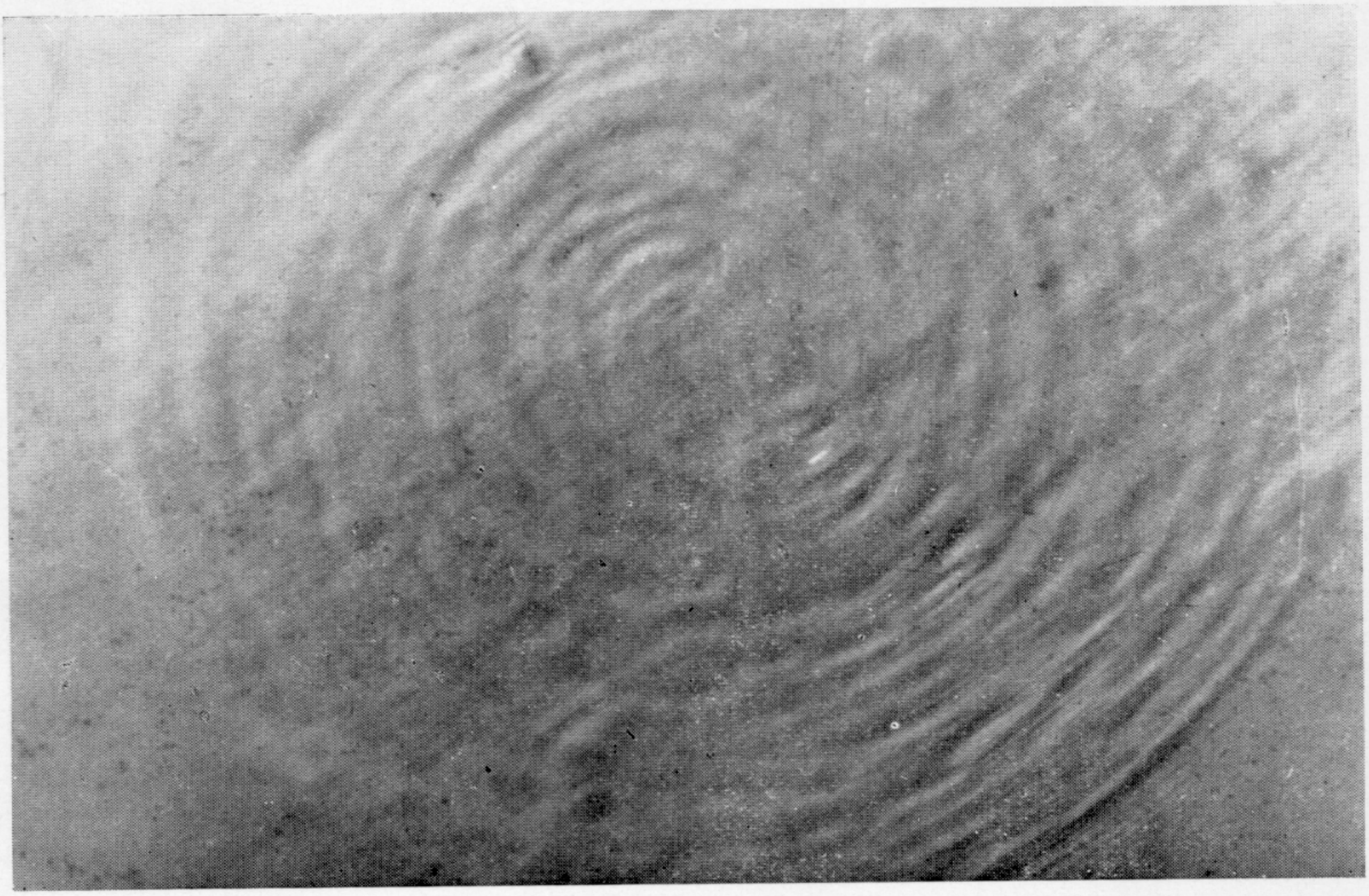

Fig. 5. Rotating whorls (big magnification) composed of filaments forming spirals. The picture gives impression of a watch-spring.

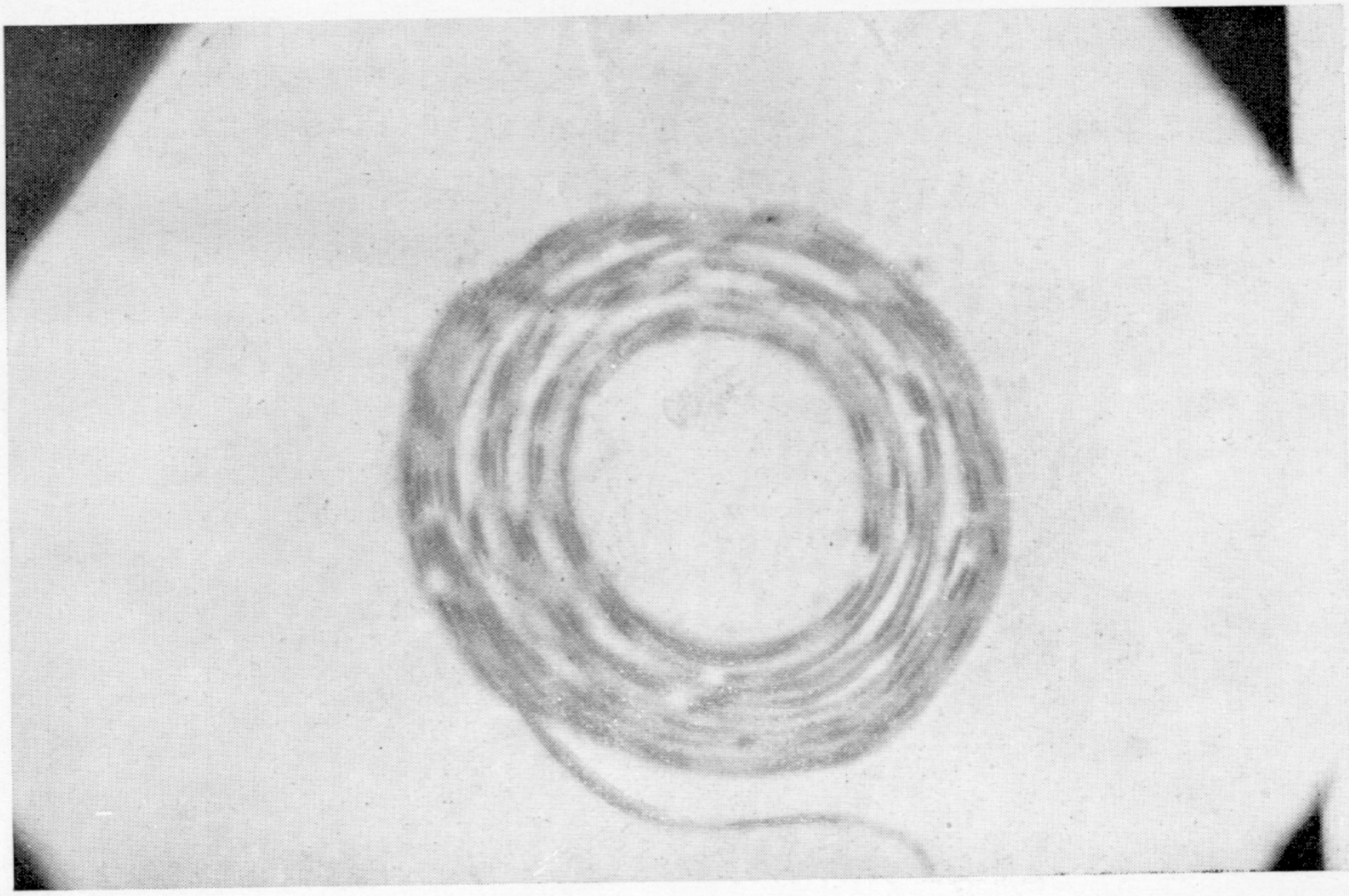

Fig. 6. Impression smear of rotating ring stained with fuchsin. Single filaments showing large round bodies are distinctly visible.

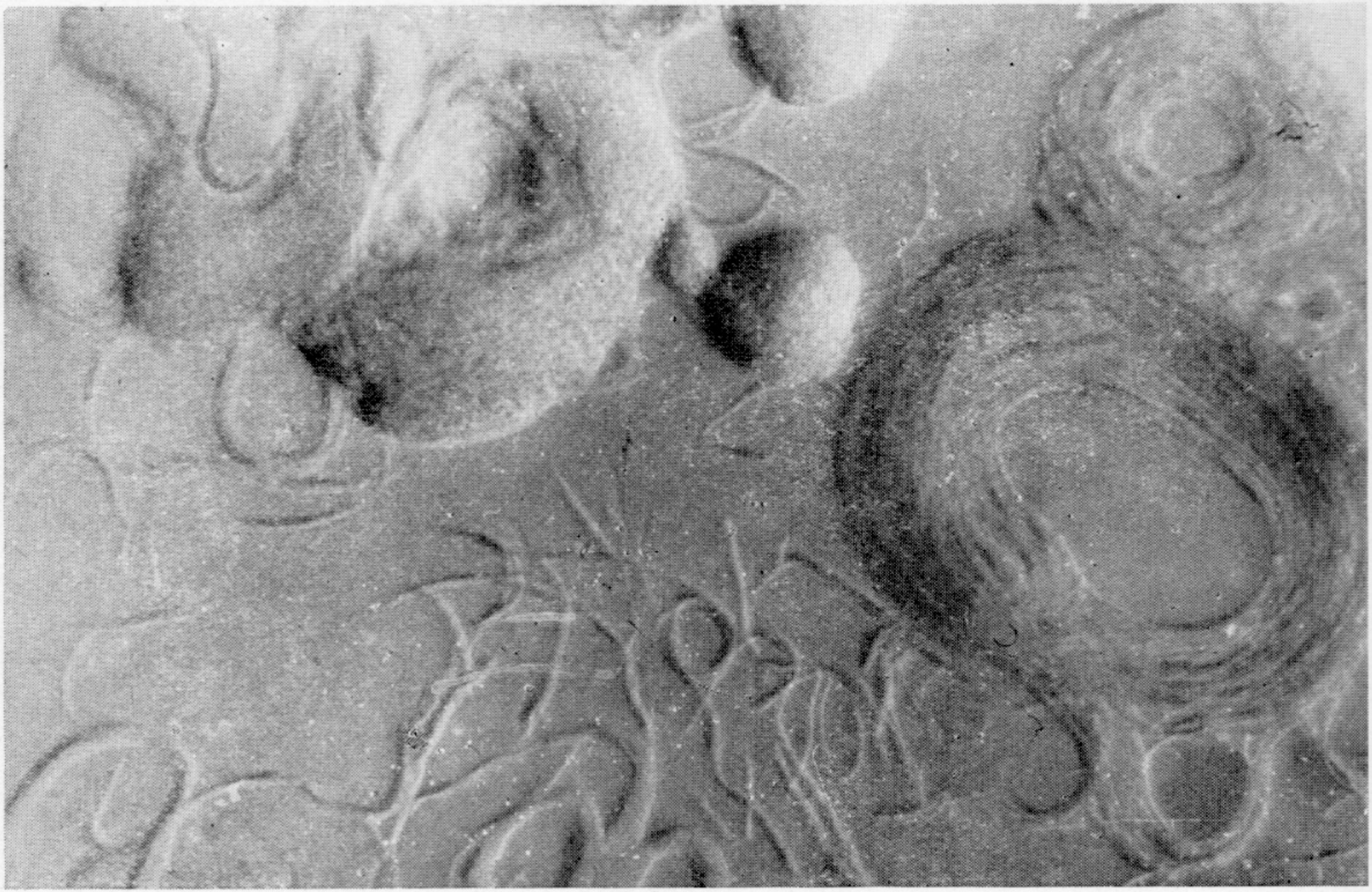

Fig. 7. Ustained preparation of the fully grown microculture. Whorls stopped their movements and changed into three-dimensional nearly convex bacterial colonies.