LXVI. THE MORPHOLOGY OF THE PLAGUE BACILLUS.

By SYDNEY ROWLAND, M.A., M.R.C.S.

Of the Lister Institute, London.

(With Plates XVII—XXIII.)

During the progress of my work upon plague vaccines it has been necessary to grow the bacillus upon various media under a variety of conditions, and some of the instances of pleomorphism of this organism seemed worthy of being placed upon record.

Probably no organism presents so marked a pleomorphism as the bacillus of plague. A glance at the photomicrograms which accompany this report, Plates XVII—XXIII, will demonstrate the truth of this statement. We find amongst them forms simulating micrococci, streptococci, bacteria, streptothriciae and even mould forms. The illustrations are from specimens either fixed and stained or from living specimens of the bacillus.

The dark ground illumination which was used to photograph some of the pictures was obtained by a central stop in an aplanatic condenser. By this means, a low-angled illuminating cone is produced in contrast to the excessively high angle of the rays emerging from a paraboloid. As has been pointed out already in these reports, this is a decided advantage as it eliminates light-haze from small or ultra-microscopic particles (see previous report, *Journal of Hygiene*, XII, p. 362).

If a broth culture of plague be examined by transmitted light it will be found that around a very small proportion of the bacilli a delicate halo can be observed. This is extremely faint and is visible in only a small proportion of the organisms. It is best seen when the edge or boundary line of the bacillus proper is in focus. So faint is it that unless some confirmatory evidence of its presence be forthcoming reliance could hardly be placed on the appearance as evidence of a definite structure.

If the culture be centrifuged, the bacilli washed in water, again centrifuged and taken up in a drop of Indian ink the hitherto faint aureole surrounding certain of the bacilli becomes strikingly visible.

Some idea of the appearance presented is given by the photomicrograms (figs. 18, 19 and 20) in Plate XXI.

The finest particles of ink impart a grey background to the picture and the larger particles show up black; all are in rapid Brownian movement, being small enough to respond to molecular bombardment. The limiting membrane of the bacillus can be sharply focused, and between it and the general black or dark grey background is a perfectly clear area free of all particles; the width of this area is often greater than the diameter of the bacillus. At the edge of this layer the Brownian movement is intense, the appearance suggesting that the particles of ink are prevented from bombarding the bacillus by some invisible envelope.

This envelope is a definite entity. It recalls the capsules of certain bacteria and more especially the slimy shell of *Bacillus tumescens*. Plague bacilli occasionally possess true capsules but the appearance I am describing differs from a capsule in having no definite outer limit. A typical capsule such as that possessed by the Pneumococcus is easily seen in an unstained specimen by reason of its sharp outer edge and its high refractive index. The layer around these plague bacilli is hardly visible in an unstained specimen mounted in a clear fluid and as seen in the ink preparation it possesses no sharply defined outer edge. If the cover-glass be tapped judiciously with a needle the layer is seen to possess little rigidity and, by lucky manipulation of the needle, can be drawn out into a streaming appendage resembling the tail of a comet. Its consistency is judged to be viscid. This observation reminds us of the well-known stickiness of plague cultures on solid media.

The envelope is insoluble in water but readily soluble in dilute alkalis.

To obtain nice preparations the bacilli should be centrifuged and washed in water. This is especially necessary when examining cultures growing in serum or serum broth, as Indian ink possesses to an extraordinary degree the property of adsorbing proteins, after which the particles no longer remain discrete, but flocculate and fail to afford the necessary Brownian movement to show the envelope clearly. Preparations made as above and sealed with paraffin remain for days unaltered.

The medium in which the bacilli are propagated exerts an influence upon the development of the envelope.

If the presence of the envelope be compared in two cultures, one grown in broth, the other grown in broth containing 10% serum (previously heated to 55° C. for half an hour), the number of organisms with a well-marked envelope is seen to be enormously increased in the latter case. In a broth culture it is present only in a minority of the organisms;

in the serum-broth culture practically every bacillus or chain of bacilli possesses it. In the case of chains, which are usual in serum-broth cultures, one chain is embedded in a common envelope.

The temperature at which the culture is incubated has also considerable influence on the presence of the envelope.

Thus a broth culture propagated at 20° C. presents organisms all of which are naked, whereas in the case of propagation at 36° C. about 50 % of them are clothed with this envelope.

The envelope is also well marked in those organisms which I have examined, taken straight from the spleen of a rat dead of plague.

If a serum-broth culture be prepared and the organisms washed and transferred to perfectly fresh normal rat serum or immune horse serum and incubated at 37° C. for half an hour the envelopes that were practically universal in the case of the serum-broth culture are found to have disappeared almost entirely.

Under certain conditions the plague bacillus develops a capsule which has a sharp, regular outline; which can be distinctly seen in unstained specimens and is coloured by the usual capsule stains. This structure has been described by several observers; Kitasato (1894), Yersin (1897), Zettnow (1896), Albrecht and Ghon (1900), Wherry (1905), and Lohlein (1906), both in the body and culture media. All observers agree, however, that the presence of capsules is inconstant and generally difficult to demonstrate. From the descriptions and figures given I have no doubt that in many cases the authors were dealing with the slimy envelope I have described above. There are, however, often intermediate appearances when it is difficult to decide whether capsules exist or not.

I have only met with a definite capsule in bacilli:

(1) At the site of inoculation in experimentally infected rats.

(2) In bacilli that have been grown in a serum medium.

Some normal sera, notably that of the horse, have the property of lysing certain strains of living bacilli. Under these circumstances I have sometimes noticed that many of the bacilli develop a definite capsule which is plainly visible under dark ground illumination and can be stained with the usual capsule stains.

The following technical data refer to the series of microphotograms illustrating the pleomorphism of the plague bacillus, and the existence in some cases of a layer of viscid material surrounding the bacilli.

A. Stained preparations.

Magnification. 1000 diameters.

Illuminant. Open arc 4 amps. D.C.

Condenser. Zeiss aplanatic.

Objective. 2 mm. 1, 40, 10″ tube.

Ocular. Projection No. 4.

Screen. Wratten and Wainwright B screen transmitting light from 6000 A.U. to 4600 A.U. with screen G transmitting from red end to 5100. The two screens combined transmit a monochromatic band from 5100 to 6000 A.U.

Stain. Carbol thionine. This stain has a strong absorption band extending from 5500 to 6800.

B. Dark ground preparations.

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DESCRIPTION OF PLATES XVII—XXIII.

(Magnification 1000 diameters.)

Figs. 1 and 2. Stained preparations from growth on agar and in broth. The bacillus stains for the most part uniformly.

Figs. 3 and 4. Growth in broth to which had been added 10 % horse serum previously heated to 55° C. for half an hour. Growth in chains with well-marked bi-polarity.

Figs. 5 and 6. Growth in the spleen of a rat dead of pest. Note the pseudo-capsules (envelopes).

Fig. 7. Smear preparation from spleen of plague infected rat showing pseudo-capsules (envelopes).

Figs. 8, 9, 10 and 11. Mould forms.

Fig. 8. From an old abscess at the seat of inoculation in a rat dead of pest.

Figs. 9 and 10. From an old growth in serum broth. Living specimens photographed with dark ground illumination.

Fig. 11. From an old abscess at seat of inoculation.

Figs. 12 and 13. Pfaundler's Balls, a form observed when growth takes place in immune serum.

Figs. 14 and 15. Another form of growth occasionally observed in rat serum that has been heated to 55° C. for half an hour.

Figs. 16 and 17. Yeast-like forms often observed at the site of inoculation in rats.

Figs. 18 and 19. The plague bacillus, 18 hours' culture in 10 % serum broth, as seen in unstained specimens by the aid of the ink process. The organisms were centrifuged down, washed in distilled water, again centrifuged and the residue taken up in Indian ink, a sufficiently fine layer for examination being obtained by pressing a flat cover-glass on a flat slide with a drop of the ink emulsion between.

Fig. 20. A specimen similarly prepared to those illustrated in Figs. 18 and 19. In this case the centrifuging has been more energetic, forcing several previously discrete organisms provided with envelopes into fusion.

Figs. 21, 22, 23, 24, 25 and 26. Involution forms seen when the plague bacillus is grown in broth containing 2 % sodium chloride. Two days growth. Photographed unstained and alive with dark ground illumination. These well-known involution forms are very difficult to stain as they contain so much water in the bladder-like swellings that fixing is almost impossible.

Figs. 27, 28 and 29. A short chain growing in fresh horse serum. The chain is surrounded with a well-marked capsule. MacConkey's capsule stain was used for these and the following two figures.

Fig. 30. Three short chains lying close together. In the lowest chain one individual appears normal, in the remainder the bacillus is undergoing lysis and of the chromatin a few dots alone remain.

Fig. 31. Two individuals. One unprovided with a capsule the other capsulated and the chromatin reduced to two dots.

Figs. 32, 33, 34, 35 and 36. Living bacilli from a culture in spleen juice showing well developed envelopes surrounding each chain. The preparation was made by the Indian ink method (see description to Figs. 18 and 19 above) but in this case illuminated by means of a paraboloid condenser. The ground between the bacilli and outside the enveloping layer is bright as the particles of ink scatter the light.

Figs. 37 and 38. The wet preparation from which Fig. 36 was made was ringed round with gold size. When this was dry a small hole was made in the ring of size which cemented the coverslip to the slide. This allowed of a very slow desiccation of the preparation. The preparation was dry in 6 days. This was evident by the cessation of the Brownian movement in the preparation and by the adhesion of the ink particles to the coverslip. The coverslip was then carefully lifted so as to disturb the film of dry ink and bacilli as little as possible. The film was then stained with MacConkey's capsule stain. The picture shows that the bacilli alone take the stain, the layer remaining colourless. Compare these figures with Figs. 27, 28 and 29 above showing the true capsules which stain well.

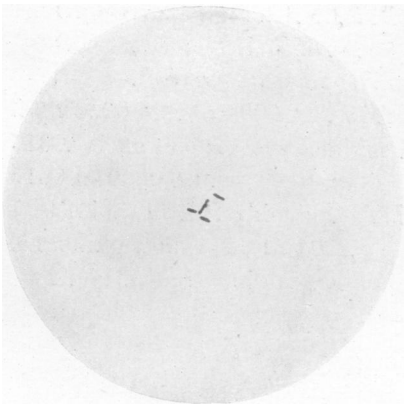


Fig. 1.

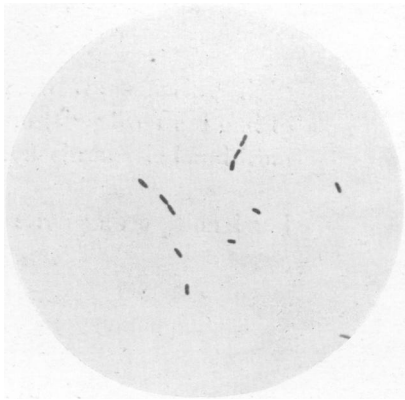
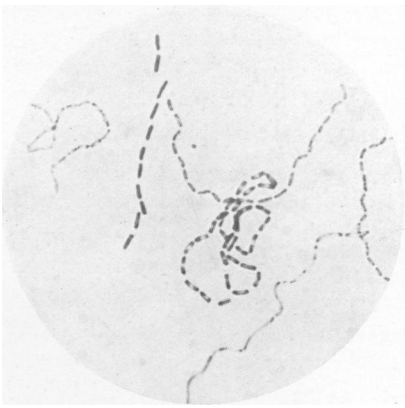


Fig. 2.



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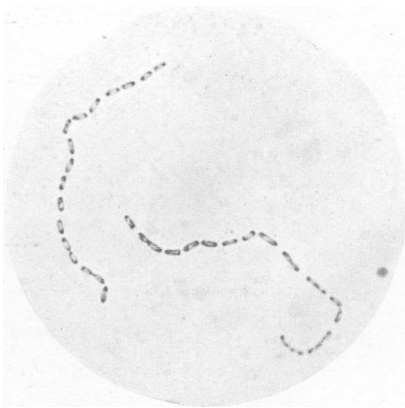

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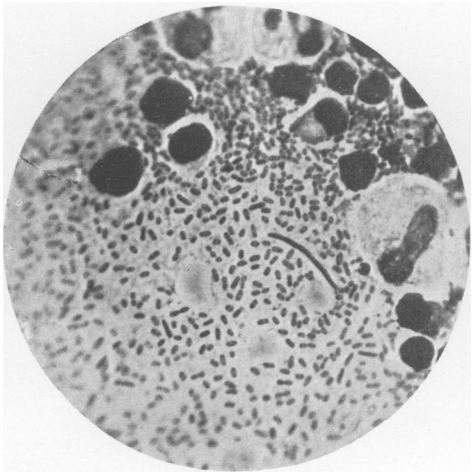

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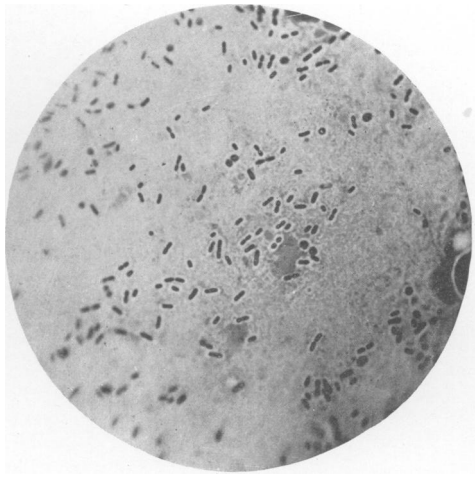

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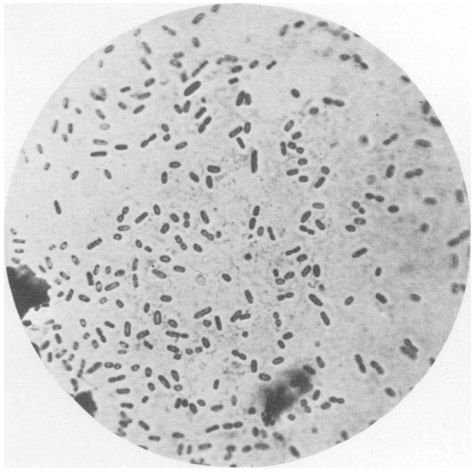

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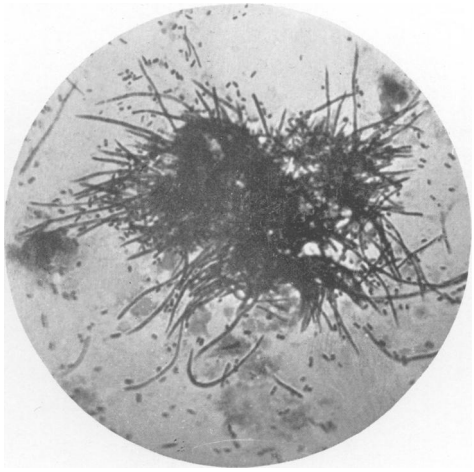

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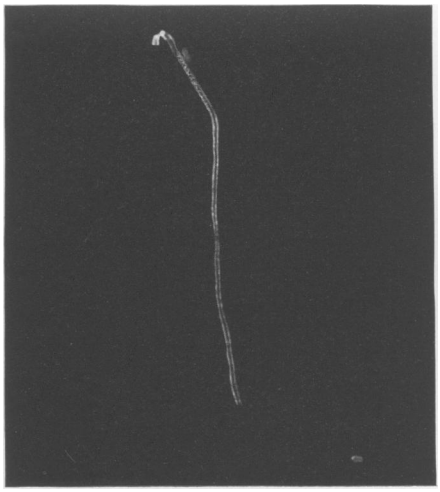

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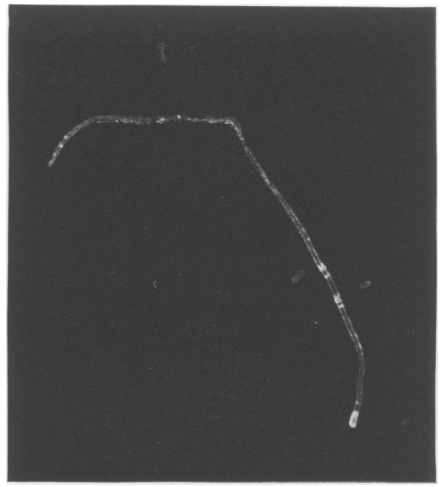

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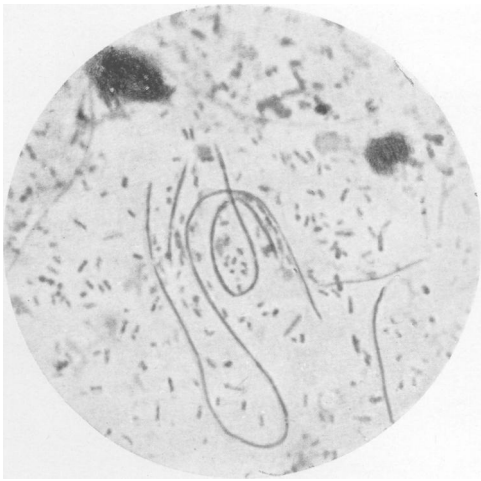

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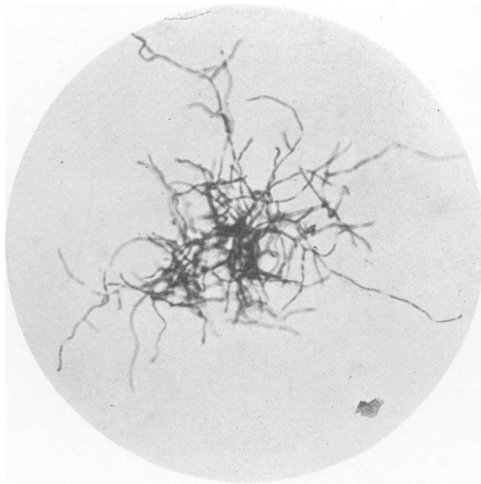

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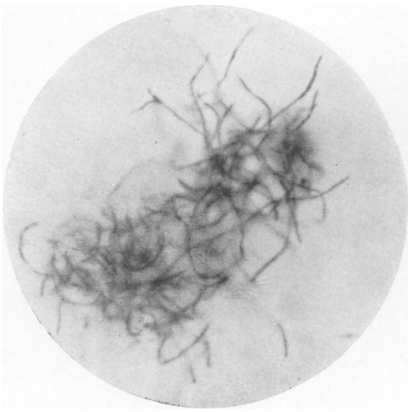

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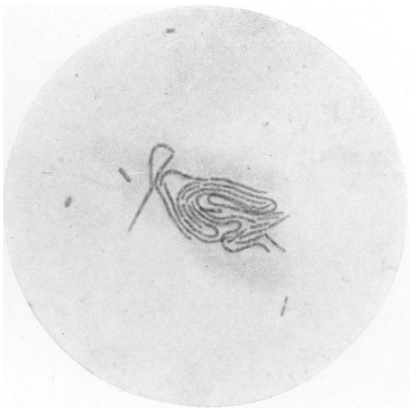

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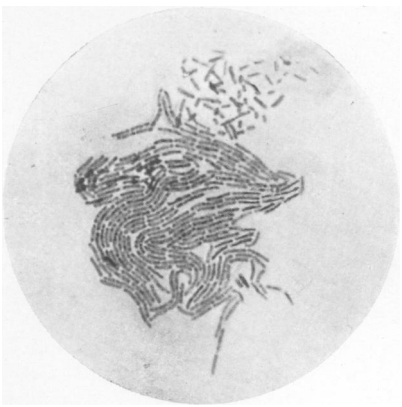

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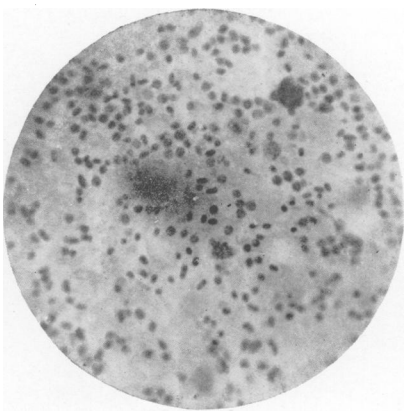

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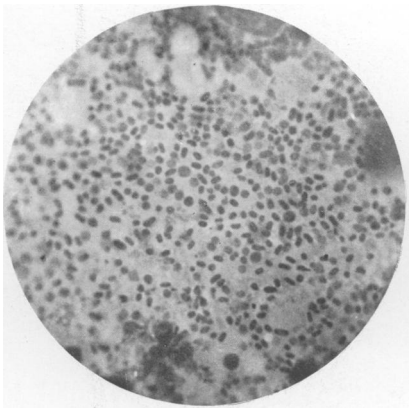

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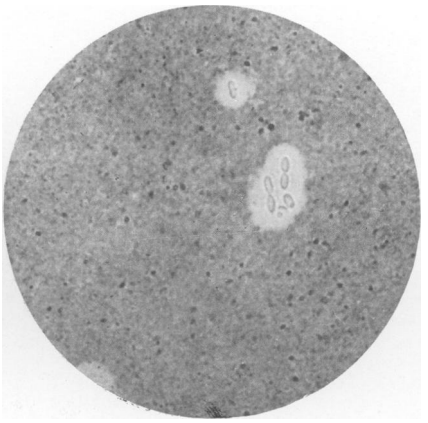

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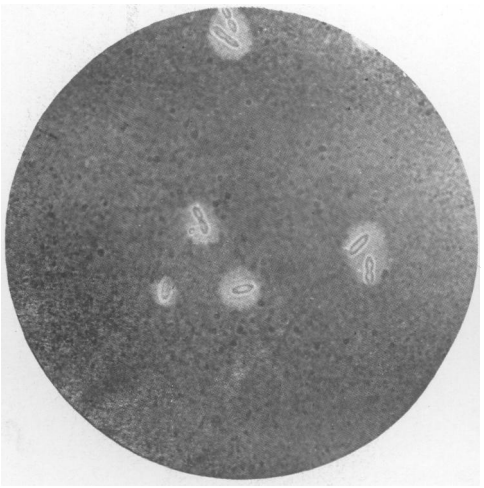

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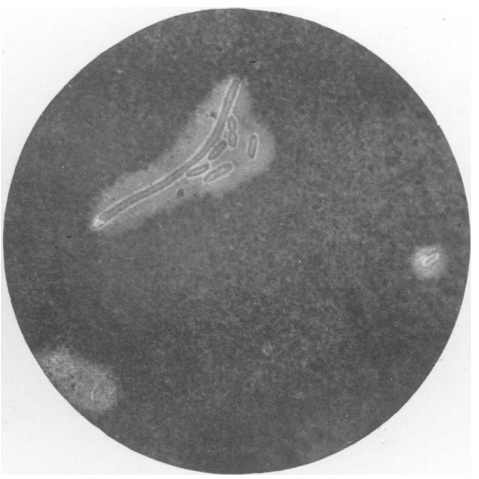

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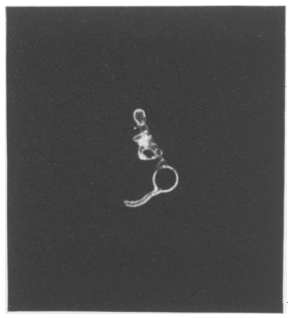

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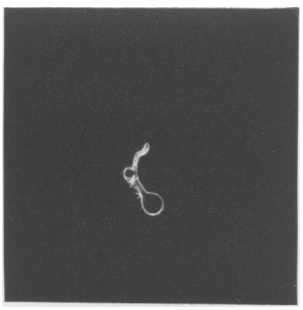

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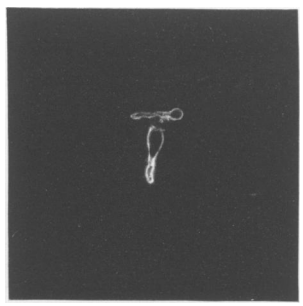

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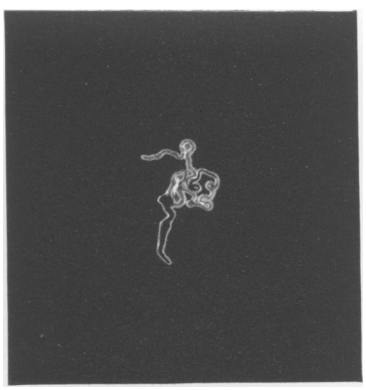

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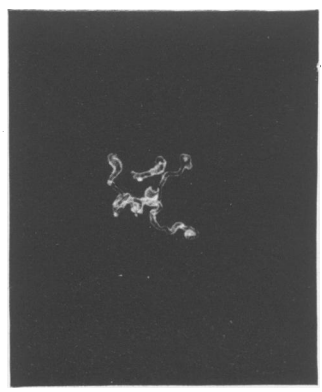
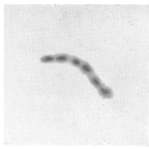


Fig. 26.

Fig. 27.



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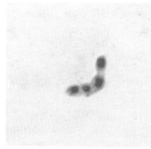

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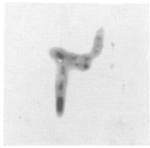

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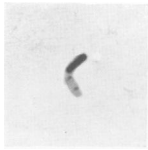

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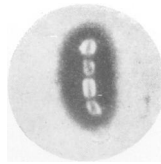

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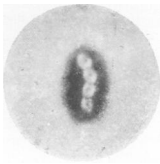

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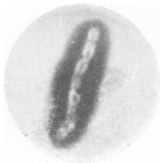

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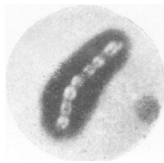

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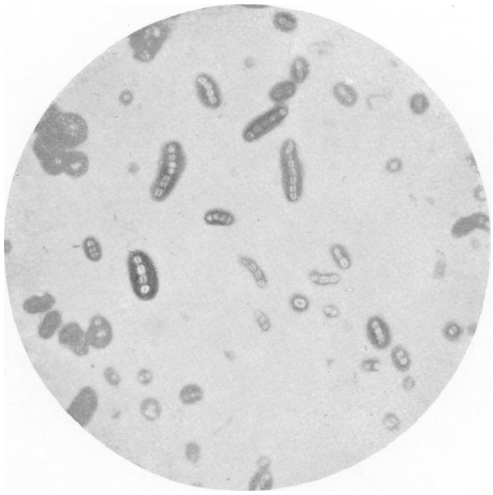

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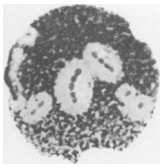

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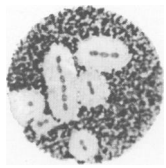

Fig. 38.