

STUDIES ON THE LIFE CYCLE OF SPIROCHETES

IV. THE LIFE CYCLE OF THE NICHOLS PATHOGENIC *TREPONEMA PALLIDUM* IN THE RABBIT TESTIS AS VISUALIZED BY MEANS OF STAINED SMEARS*

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PLATES 15 TO 17

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Evidence for the occurrence of a complex life cycle in the pathogenic and non-pathogenic strains of *Treponema pallidum* has been presented in previous reports of this series (1-6). The purpose of the present paper is to present observations of the pathogenic *Treponema pallidum* as it occurs in the rabbit testis visualized by means of a newly developed staining procedure.

It was felt that if adequate staining methods could be evolved, a confirmatory check could be made on the observations obtained by means of phase contrast microscopy and a further degree of confirmation of the studies, particularly by Meirovsky (7) and later by Leipold(8), could be obtained. The series of observations presented here confirm in essence those presented in an accompanying paper of this series (6) which were obtained by means of the phase contrast microscope.

Material and Methods

Material for study was obtained from syphilitic testes of the same type as those used in the previous study. Preparations for observation were made by sectioning the testis, drying the cut surface of the tissue on blotting paper, and making thin impression smears on clean coverslips. These smears were then stained by the technique previously described (2), and were then studied by means of an ordinary research microscope. The spirochetes stain a deep purple and, where extraneous material in the background does not interfere, the stained spirochetes stand out with sharp contrast. The use of blue-green and amber filters produces a jet black image which is optimal for photographic purposes. Photographs were made in the same manner as in past studies. The plates consist of composites of numerous photographic observations.

OBSERVATIONS

As visualized by this staining procedure, the spirochetes stain a deep purple and the surrounding coagulated protein and blood and tissue cells of the smear

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stain less densely. The nuclei of the blood and tissue cells likewise take up the stain avidly.

The observations presented here were for the most part made on smears produced from syphilomas ranging in age from 4 to 7 days. A few of the photographs were made from material taken from syphilomas 19 days old. In studies currently in progress an effort is being made to ascertain whether the changes observed in the life cycle may in any way be correlated with the phase in the development of the disease process produced. These studies will be presented in the future.

In Fig. 1 (Plate 15) are seen three characteristically spiral and twisted spirochetes each having delicately tapered ends. At the lower pole of the right-hand spirochete can be seen a delicate terminal continuation suggestive of a spiral terminal filament. In Fig. 2 (Plate 15) is shown an organism in an early stage of transverse division in which the narrow segment just below the upper twisted portion is the point of eventual separation. Fig. 3 (Plate 15) shows an easily recognizable spirochete within which can be seen a minute bleb from which there appears to be developing either a branch or a young spirochete. This figure is suggestive of those presented by Meirovsky which he interpreted as indicating the development of young spirochetes from such bodies while still attached to the parent spirochete. This is the only example of this that the present observers have seen up to the present time. Fig. 5 (Plate 15) shows two or three twisted and entwined, otherwise normal appearing spirochetes. Figs. 4 and 6 to 17 show stages in the development of gemmae comparable to those previously observed by means of phase contrast microscopy. Fig. 4 shows a dense body attached to the lower pole of the spirochete, while Figs. 6 to 8 show stages in the development of smaller bodies developing from the spirochete in an intercalary position. Two such bodies are being produced from a single spirochete in Fig. 7. In Fig. 9 a similar dense body is being produced from the spirochetes shown, and nearby is a dense granule which is interpreted as an undifferentiated gemma which has been freed from its parent spirochete. Figs. 9 and 10 show dense bodies attached to spirochetes, comparable to that shown in Fig. 4. In Fig. 10 in the central portion of the spirochete can be seen a delicate bleb-like structure which is interpreted as the remnant of the unispirochetal cyst from which this spirochete originated. In Fig. 12 the gemma being produced appears to be stipitate, while in Fig. 13 the gemma shows a dense basilar granule comparable to those seen in the photographs taken with the phase contrast microscope. In Fig. 14 a dense body is seen originating from a spirochete which is comparable to that shown in Fig. 9, within which no differentiation can be made out. In Fig. 15 is seen a tangled organism from which at least two such bodies are developing, and attached to the spirochetes shown in Fig. 16 is a larger round gemma within which a rather large dense body is to be seen. In Fig. 17 an irregularly shaped gemma is clearly attached to the

spirochete and shows denser masses within its matrix. In Figs. 1 to 10 (Plate 16) are seen structures which are interpreted as gemmae which have been freed from their parent spirochetes and are now free in the medium. In Fig. 1 the gemma is very minute, but within it can be seen a curved rod-like body similar to what was seen by means of phase contrast microscopy. In Fig. 2 the body is dense and as yet undifferentiated; while in Fig. 3 the round body is clear and contains a dense granule. In Figs. 4, 5, and 6 dark masses and delicate filaments or fibrils can be made out within these delicate cysts; while in Fig. 7, presented at about 5,000 diameter, is clearly shown a spirochete coiled within a delicate cystic wall. In Figs. 8 to 10 are clearly seen coiled adult spirochetes in late unispirochetal cysts.

Figs. 11 to 18 and Fig. 20 demonstrate stages in the unipolar emergence of spirochetes from their unispirochetal cysts. In Fig. 11 the parental cyst is still dense and little details can be made out within it, although the end of a recognizable spirochete can be seen to be emerging. Similar comment can be made about Fig. 13. Figs. 12 and 14 to 18 show progressively later stages in the emergence of the organism. In Fig. 20 at the upper pole can be seen the delicate remnant of the cyst wall from which the organism has been derived.

Fig. 19 demonstrates very clearly the development of a large cyst-like structure at the end of a spirochete. The spirochetal membrane is seen to be continuous with the membrane of this body and it suggests the type of structure observed in the saprophytic forms from which multispichoetal cysts appear to be derived. A considerable degree of differentiation, as indicated by the curved rods and bars present in this body, suggests that more than one organism may be developing within it.

Plate 17 presents stages of what are construed as phases in the development of multispichoetal cysts. Fig. 1 shows a delicate cystic body within which can be seen four dense granules. Fig. 2 is construed as an artifact caused by precipitation of stain on the slide, and represents the type of structure in stained preparations which must be differentiated from such structures as those shown in Figs. 1 and 3. Fig. 3 shows a later stage in the development of a multispichoetal cyst within which can be seen adult coiled spirochetes. This is particularly obvious in the right lower segment of this round body. Fig. 4 shows a later stage in the development of a multispichoetal cyst within which the spirochetal filaments are clearly defined. Figs. 5 to 7 show stages in the emergence of the organism from such bodies. This is particularly clear in Fig. 6 in which the emergence of a tangled cord of organisms at the lower pole can readily be seen and can be followed back into the tangle within the cyst.

COMMENT

The evidence presented, based upon stained material from rabbit testes, tends to confirm the observations made by means of phase contrast micros-

copy and adds to the accumulating evidence that a complex life cycle occurs in the pathogenic *Treponema pallidum* as well as in its saprophytic relatives. It can be seen that this life cycle appears to be essentially the same as that outlined for the saprophytes. Details of the processes involved remain for future study to elaborate.

SUMMARY

Further evidence for the occurrence of a complex life cycle in the pathogenic *Treponema pallidum* as visualized by means of stained preparations is presented.

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EXPLANATION OF PLATES

PLATE 15

FIG. 1. Photograph 721. Carbol-gentian violet stain of impression smear from 4-day-old syphiloma. Three adult spiral organisms. $\times 3750$.

FIG. 2. Photograph 695. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Spiral in early stage of transverse division. $\times 3750$.

FIG. 3. Photograph 709. Carbol-gentian violet stain of impression smear of 4 day old syphiloma. Adult spiral showing branch originating from bleb-like mass in spirochetal body. $\times 3750$.

FIG. 4. Photograph 697. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Adult spiral with dense granule or early gemma attached. $\times 3750$.

FIG. 5. Photograph 711. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Two or more entwined spirochetes. $\times 3750$.

FIG. 6. Photograph 709. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Adult spiral form with very young gemma attached. $\times 3750$.

FIG. 7. Photograph 723. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Adult, irregularly coiled spiral with two young gemmae attached. $\times 3750$.

FIG. 8. Photograph 707. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Spiral with larger gemma attached. $\times 3750$.

FIG. 9. Photograph 715. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Spiral with dense small gemma developing within it, and older free gemma nearby. $\times 3750$.

FIG. 10. Photograph 689. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Dense gemma developing on spiral which still has remnant of its own cyst wall attached. $\times 3750$.

FIG. 11. Photograph 699. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Spiral with dense young gemma attached. $\times 3750$.

FIG. 12. Photograph 645. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Spiral form with stipitate gemma attached. $\times 3750$.

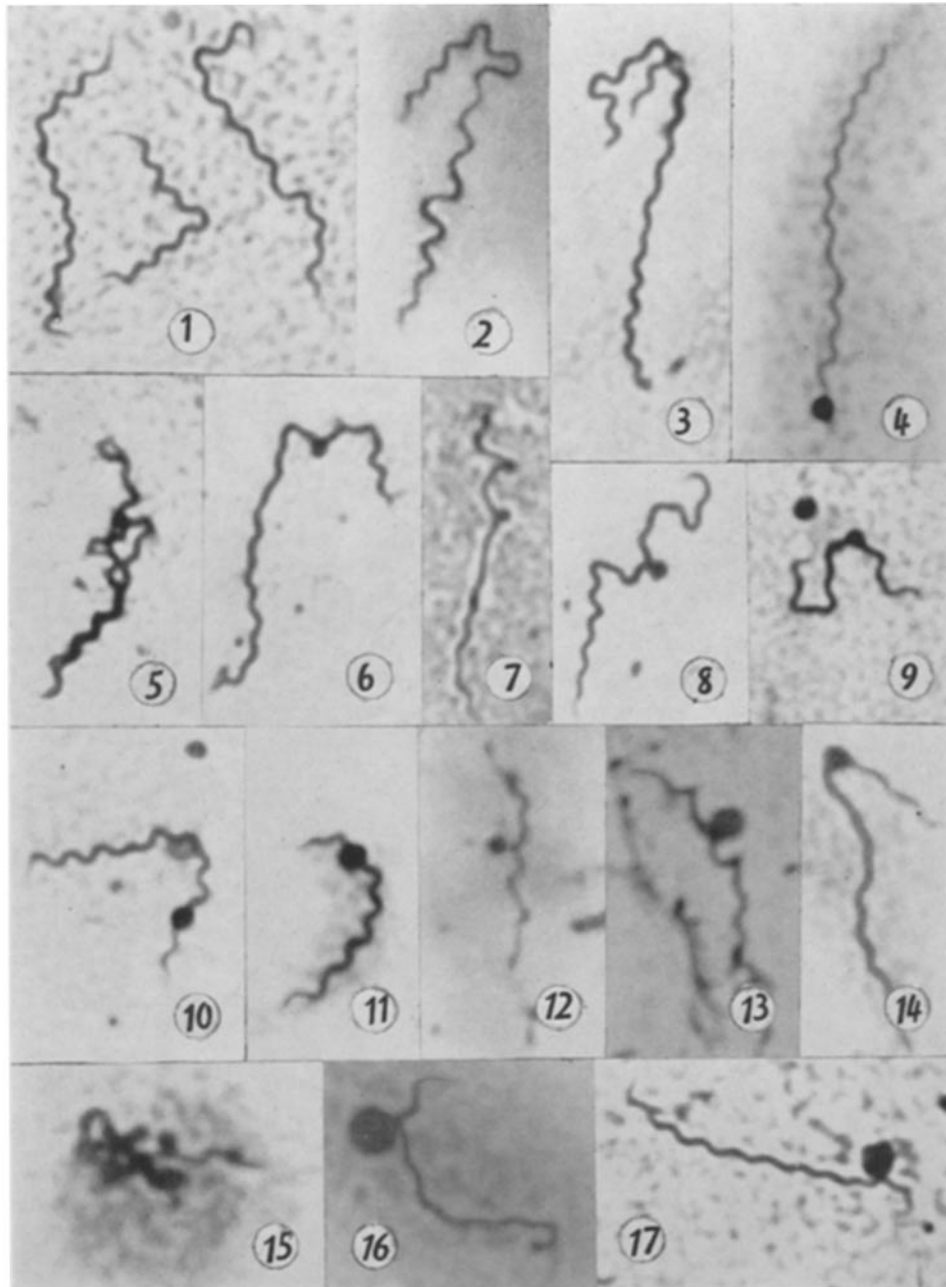
FIG. 13. Photograph 607. Carbol-gentian violet stain of impression smear of 3 to 4-day-old syphiloma. Spiral form with gemma attached within which a basilar granule is visible. $\times 4067$.

FIG. 14. Photograph 665. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Spiral with early dense attached gemma. $\times 3750$.

FIG. 15. Photograph 659. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Completely coiled spirochete with two dense gemmae attached. $\times 3750$.

FIG. 16. Photograph 641. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Spiral form with larger gemma attached within which a dense granule can be seen. $\times 4067$.

FIG. 17. Photograph 717. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Spiral form with large gemma attached within which a dense granule can be seen. $\times 3750$.



(DeLamater *et al.*: Life cycle of spirochetes. IV)

PLATE 16

FIG. 1. Photograph 713. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Section of spiral. Minute free gemma within which some differentiation is apparent. $\times 3750$.

FIG. 2. Photograph 721. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Adult spiral form; dense free gemma. $\times 3750$.

FIG. 3. Photograph 693. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Typical spiral form; young gemma with dense granule within. $\times 3750$.

FIG. 4. Photograph 691. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Irregular long spiral form with larger differentiating gemma nearby. $\times 3750$.

FIG. 5. Photograph 747. Carbol-gentian violet stain of impression smear of 19-day-old syphiloma. Later differentiating gemma. $\times 3750$.

FIG. 6. Photograph 723. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Differentiating free gemma. $\times 3750$.

FIG. 7. Photograph 634. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Later gemma which has developed into a unispirochetal cyst within which is a single spiral organism. Cyst wall is clearly evident. $\times 5083$.

FIG. 8. Photograph 719. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Spiral form tightly coiled into ball within unispirochetal cyst. $\times 3750$.

FIG. 9. Photograph 713. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Spiral found tightly coiled within unispirochetal cysts. $\times 3750$.

FIG. 10. Photographs 713 and 711. Same preparation as Fig. 9. Two spiral forms coiled within unispirochetal cysts. $\times 3750$.

FIG. 11. Photograph 671. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Young spiral emerging from small unispirochetal cyst. $\times 4067$.

FIG. 12. Photograph 747. Carbol-gentian violet stain of impression smear of 19-day-old syphiloma. Unipolar emergence of spiral from unispirochetal cyst. $\times 4067$.

FIG. 13. Photograph 669. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Unipolar emergence of young spiral from cyst. $\times 4067$.

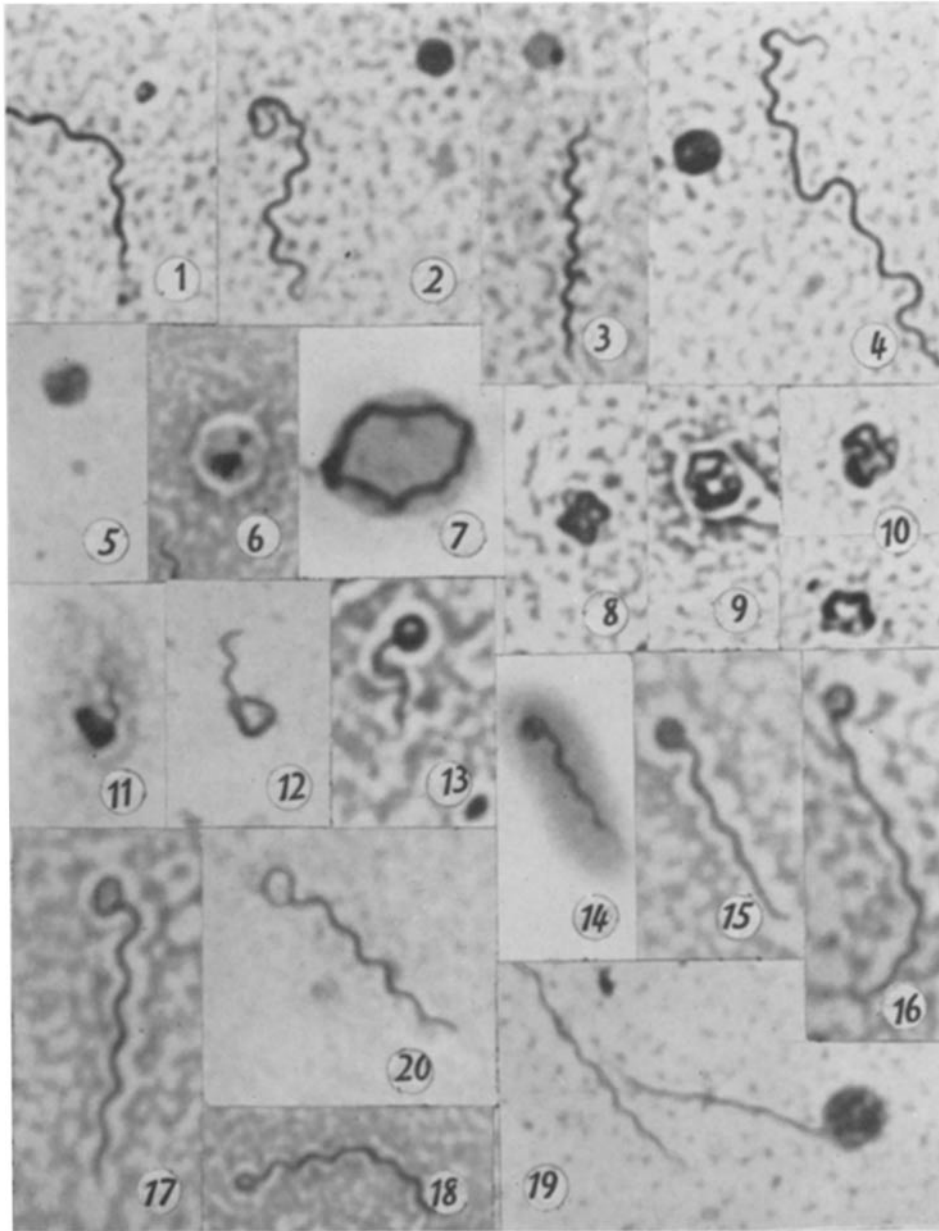
FIG. 14. Photograph 621. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Later stage in unipolar emergence from cyst. $\times 4067$.

FIGS. 15 to 17. Photographs 654, 652, and 657 respectively. Carbol-gentian violet stain of 4 to 7-day-old syphiloma. Three progressively later stages in emergence of spiral forms from unispirochetal cysts. $\times 4067$.

FIG. 18. Photograph 723. Carbol-gentian violet stain of 4-day-old syphiloma. Late stage in emergence of spiral from unispirochetal cyst. $\times 4067$.

FIG. 19. Photograph 742. Carbol-gentian violet stain of 19-day-old syphiloma. Spiral form with large terminal mass attached showing origin from within spiral cyst wall continuous with spirochete. $\times 3750$.

FIG. 20. Photograph 749. Carbol-gentian violet stain of 4 to 7-day-old syphiloma. Spiral showing later stage of emergence. Cyst wall still evident at upper end surrounding terminal twist. $\times 4067$.



(DeLamater *et al.*: Life cycle of spirochetes. IV)

PLATE 17

FIG. 1. Photograph 687. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Early stage in development of multispirochetal cyst. Cyst wall and enclosed granules clear and sharp. $\times 3750$.

FIG. 2. Photograph 638. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Artifact dried droplet of stain. Notice fuzzy outline and contents. $\times 3750$.

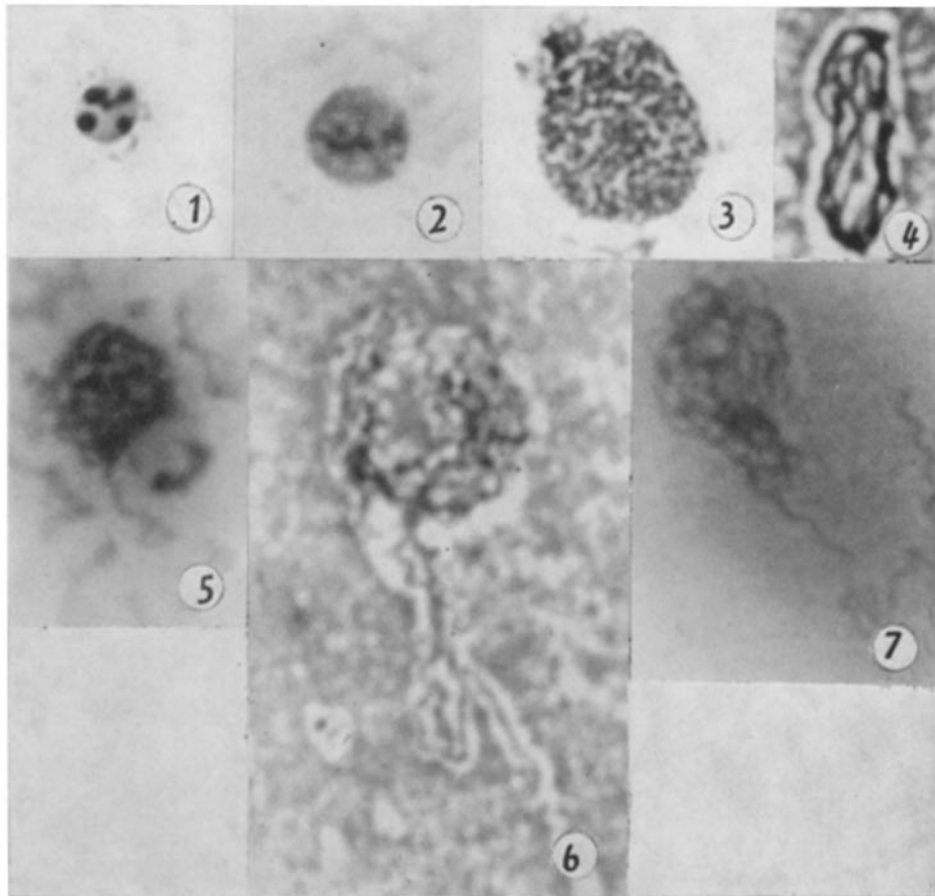
FIG. 3. Photograph 632. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Late multispirochetal cyst showing spirals coiled within. One spiral particularly clear. $\times 2541$.

FIG. 4. Photograph 667. Carbol-gentian violet stain of impression smear of 4 to 7-day-old syphiloma. Late multispirochetal cyst with adult organisms within. $\times 4067$.

FIG. 5. Photograph 621 *a*. Carbol-gentian violet stain of impression smear of 4-day-old syphiloma. Multispirochetal cyst with spiral forms emerging. $\times 4067$.

FIG. 6. Photograph 614. Carbol-gentian violet stain of impression smear of 6-day-old syphiloma. Late multispirochetal cyst with spirals emerging in cords. $\times 4067$.

FIG. 7. Photograph 617. Same preparation as Fig. 6. Spirochetes emerging from multispirochetal cysts. $\times 4067$.



(DeLamater *et al.*: Life cycle of spirochetes. IV)