STUDIES ON THE LIFE CYCLE OF SPIROCHETES

III. The Life Cycle of the Nichols Pathogenic Treponema pallidum in the Rabbit Testis as Seen by Phase Contrast Microscopy*

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Plates 11 to 14

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In papers published elsewhere (1–5) descriptions of the life cycles of two strains of non-pathogenic *Treponema pallidum* are given as they occur in culture and in the embryonated egg. In these studies the sequence of events was apparent from the study of cultures of successive ages, and in many instances sequences from one cell form to another in such processes as transverse division followed in single organisms. While recognizing the inherent danger of drawing analogies between different organisms and their basic biological processes, it still seemed justifiable to attempt to establish whether or not a sequence of events comparable to that observed in the saprophytic strains might not also occur in the pathogenic *Treponema pallidum* as it occurs in the syphilitic rabbit testis. In addition to the studies by the present authors, previous studies briefly summarized in one of the papers above mentioned further suggested the possibility that a complex life cycle also occurred in this organism. The purpose of the present paper is to present a series of observations made by means of phase contrast microscopy on preparations made from syphilitic rabbit testes. Interpretations of the structures observed are based, at least in part, upon the analogies which can be drawn between these organisms and the saprophytic forms, and also upon successive observations of the same preparations over periods of hours and even days. In these preparations definite changes in the organisms were observed to occur, and it now seems likely on the basis of work done since these initial observations were made, that these changes truly represent the proper sequence of events as they occur in the living testis as well as in cultures of the pathogenic spirochete.

Materials and Methods

The Nichols pathogenic *Treponema pallidum* used in these studies was kindly supplied by Dr. Robert Nelson, Department of Bacteriology, School of Public Health, Johns Hopkins University, and has subsequently been carried in animals in our laboratories.

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Material for study was obtained by removal of previously inoculated testes which were observed to develop early orchitis. These testes were sliced with a sterile scalpel or sterile razor blade, and small portions were macerated in thioglycollate medium with a mortar and pestle. The spirochetes were immobilized by adding a small amount of agar to the preparations, which facilitated photographing them by causing their partial or complete immobilization. A drop of this material was then placed on a sterile microscope slide, covered with a sterile coverslip, and sealed with vaseline. It was then studied directly by means of a phase contract microscope according to a technique already described (1). Photographic records were made of the findings. Such preparations were observed daily for as long as 31 days. They were maintained both at room temperature and at 37°C. Comparable preparations made with saline instead of thioglycollate medium showed activity of the spirochetes during the first few hours only, and subsequently no changes were to be observed in these preparations.

OBSERVATIONS

Spirochetes were readily visualized by phase contrast microscopy as characteristically spiral organisms which, when observed through this instrument, are typically a dense purple color with a light background. In most instances the organisms appear to be coiled as a spiral with a round transverse cross-section. In certain instances, however, organisms were observed which, when they rotated, appeared to be flat and the characteristic undulation appeared to be only a wave which went from one end of the organism to the other. When such organisms were rolled over they appeared flat instead of spiral. It seems likely, therefore, that not all the spirochetes to be observed are truly coiled or spiral, and that some of them at least have this other configuration. Characteristic spirals as observed by the phase contrast microscope are to be seen in Fig. 15 (Plate 11); Fig. 1 (Plate 12); and Fig. 6 (Plate 12).

In freshly prepared preparations most of the organisms seen are actively motile and proceed this way and that in the characteristic rotary motion described by others. A wave of undulations may occasionally be observed to progress from one end of the organism to the other and not infrequently the organisms are seen to bend and twist and coil in any direction with a lashing motion. Transverse division has been observed on numerous occasions and has been followed from its inception to its termination. It appears to follow the same sequence as observed in and described for the saprophytes (3–5). An organism about to divide is observed to bend rapidly at one point, forming a sharp angle upon itself. It then lashes back and forth, always bending at this point, until a break appears in the continuity of the spirochetal body. It then undergoes a short period of rest or quiescence after which a new motion is begun in which the two parts begin to rotate in opposite directions. By this process they literally twist themselves apart. As with the saprophytic forms, it appears likely that the terminal filament originates by this process of pulling apart and stretching of the periplast.

On a small percentage of the organisms observed bodies comparable to the gemmae described as originating in the saprophytes are seen to develop. Figs.

1 to 13 (Plate 11) demonstrate stages in the development of these gemmae or buds. They may occur at any point along the spirochetal body; that is, they may develop either in an intercalary position or in a terminal position. They may be sessile or stipitate; they may be single or multiple. Figs. 1 and 3 (Plate 11) demonstrate early minute vesicles developing from segments of spirochetes. The walls of these vesicles are clearly shown, and within them may be seen dense basilar granules. In Fig. 2 (Plate 11) a very delicate early vesicle can be seen to be developing from the outside surface of the bend of the organism. In Fig. 4 the granule in the gemma is located at the outer pole.

In Figs. 5 and 6 (Plate 11) are seen two terminal gemmae within which dense basilar granules are to be seen. Again, the walls of these minute cysts are clearly shown. In Fig. 7 is shown a typical spirochete from each end of which is developing a medium sized gemma, in each of which can be seen more highly developed or elongated masses; while in Fig. 8 the terminal gemma is seen to be stipitate. In Figs. 9 and 10 the gemmae, originating from the spirochetal bodies, are seen to be double. In Figs. 12 and 13 gemmae are developing from spirochetes within which later stages of development can be made out. In the smaller gemma shown in Fig. 12 a basilar granule is seen to be elongated while the body present in the terminal cyst of the upper pole is seen to be long and curved within the cyst. In Figs. 11 and 14 are seen two gemmae which have been freed from a parent spirochete and within which are the characteristic dense bodies. That seen in Fig. 14 has become an elongated curved rod. In Fig. 15 are seen two minute free gemmae lying in juxtaposition to a typical spirochete. Fig. 16 shows a tangle of spiral forms producing several gemmae. Figs. 17 to 29 demonstrate stages in the development of gemmae which have been freed from parent spirochetes and within which the developmental stages appear as granules, curved rods, or as delicate fibrils (Fig. 29), the exact configuration of which it is difficult to make out. The surrounding cyst membranes in each of these are apparent. In Figs. 30 and 31 can be seen coiled spirochetal bodies within such minute cysts. This is particularly apparent in Fig. 30. Figs. 2 to 5 (Plate 12) demonstrate stages in the emergence of spirochetes from such gemmae, Figs. 2 to 4 showing the unipolar type of emergence while Fig. 5 represents the bipolar type of emergence with the residual originating cyst persisting in the center of the organism. Figs. 1, 6, 7, and 8 show adult spiral forms.

Extremely short forms are not infrequently observed, as in Figs. 9 and 10, and these appear to be products of progressive transverse division. To the right of the upper pole of the spirochete seen in Fig. 9 can be seen the delicate shadow of the flagellum.

Meirowsky (6) in his extensive studies on *Spirochaeta pallida*, describes peculiar branching forms of this organism. In the preparations which have been studied in this work several instances of peculiar branching and straight forms

have been observed which have been not infrequently in direct continuity with spiral segments. Whether these constitute artifacts or are actually part of the organism, remains for future studies to decide. Such forms are shown in Figs. 11 to 15. In Fig. 11 a peculiar delicate branching structure which is bent in two places at sharp angles is seen to be in direct continuity with a recognizable spiral segment at the lower right. In Fig. 12 is a complex branching form, about in the center of which can be seen a spiral segment. From various branches can be seen the development of dark bodies suggestive of gemmule stages. A similar structure is shown in Figs. 13 and 14. In Fig. 15 a recognizable spirochetal segment at the lower right appears in direct continuity with a very delicate branching structure shown to the left.

In the type of preparations described and used in these studies changes in the activity of the organisms are observed to occur in from 9 to 12 hours. At this time actively motile spirochetes are seen to come together in pairs and complex clusters, as shown in Figs. 4 to 11 (Plate 13). The configuration of such aggregations varies widely in form and poses problems of interpretation. Figs. 9 to 11 are three optical sections at successively lower planes, showing two paired spirochetes which appear to be fused in their middle third, and at this point a dense mass can be seen to be developing. At the left lower pole, which comes into view in Fig. 11, is to be seen a grape-like cluster of bodies originating from one arm or branch of this pair of spirochetes. Fig. 8 (Plate 13) shows a comparable complex form from which clusters of such round bodies have also originated.

Figs. 1 to 3 (Plate 13) show organisms emerging from gemmae. In Fig. 1 the young spiral form is already producing a new gemma.

In Fig. 1 (Plate 14) is seen a twisted aggregate of organisms from which minute bodies appear to be developing and from the upper pole of which there is a single large body within which some degree of complex differentiation appears to be proceeding. Fig. 2 (Plate 14) is a second optical section through the same mass shown in Fig. 12 (Plate 12) and again shows a second recognizable spirochetal segment in the center and the apparent origination of numerous dense bodies from this complex. This picture is repeated in this place because it is not yet possible to interpret whether it belongs in this series or in the series with which it was previously described. In Fig. 3 a cystic structure at the upper pole is seen to be derived from two delicate fibrils; within this can be seen three dense granules. In Fig. 4 (Plate 14) is shown a complex cluster of dense spherical bodies originating from a branching structure. It is thought that these may be later stages of the grape-like clusters shown in Fig. 11 (Plate 13) originating from aggregating organisms, and in Figs. 5 to 7 and 10 (Plate 14) in the upper right-hand section of each of these photographs.

Figs. 5 to 7 and 10 represent successive optical planes through another complex aggregate of organisms derived from which at the left of each pic-

ture can be seen a large spherical body which can be seen to be derived from two spirochetes in Figs. 5 and 6. Within this body dense masses and delicate filaments can be made out. As previously stated, attached to one branch of this, complex grape-like clusters of dense granules can be seen to be developing at the right upper pole.

In preparations made in the manner described, not infrequently larger spherical bodies lying free in the medium are to be observed which appear to be surrounded by a delicate wall and within which delicate fibrils and filaments can be made out, as seen in Fig. 8 (Plate 14). These are interpreted as being multispirochetal cysts, and it is suggested that these are derived from the bodies that have just been described as occurring in the complex aggregates of organisms. They have been observed on numerous occasions since these initial observations have been made, and their presence has been confirmed and photographic records and stained smears made. They will be further described in a succeeding paper.

In a manner comparable to what was seen in the emergence of organisms from multispirochetal cysts in the saprophytic forms, twisted cords or ropes of organisms can be seen to emerge from such multispirochetal bodies. A late stage in the emergence of such a complex cord of organisms is seen in Fig. 9 (Plate 14). Unfortunately the cyst from which they originate is just out of focus, but can be surmised from its defraction pattern in the right lower corner of this photograph. Just to the left of this shadow can be seen a gemma developing from one of the young spirochetes as it emerges from this cyst.

COMMENT

The series of observations presented in this paper is in a sense preliminary, but it is felt because of the parallelism which they demonstrate between the pathogenic *Treponema pallidum* and the saprophytic and non-pathogenic strains of this organism that presentation as observations is justifiable. They indicate that a complex life cycle probably also occurs in this organism and that the details of this process must be worked out by future studies. Studies in progress indicate that the concept of the occurrence of a complex life cycle in *Treponema pallidum* is correct, and it is suspected on the basis of these studies that the presence of this life cycle may form a part of the basis of the latency problem as it occurs in syphilis.

It should be stated that the structures which have been observed and presented in this report form but a small percentage of the organisms that are to be seen in the type of perparation described. The major vegetative method of reproduction is by means of transverse division, at least during the most active stages of development of the organisms and the lesions that they produce. It should be emphasized, however, that the structures reported here do occur, though in relatively small percentages, and that they are to be found if looked for.

SUMMARY

A series of observations with the phase contrast microscope on the occurrence of a complex life cycle in the pathogenic *Treponema pallidum* as it occurs in the syphilitic rabbit testis has been presented and it seems likely from these observations that there are two means of vegetative reproduction, consisting of (1) transverse division (the most important under usual conditions); and (2) the production of gemmae or buds which eventuate into unispirochetal cysts comparable to those described for saprophytic forms, within each of which single spirochetes develop and differentiate, and from which they subsequently emerge.

In addition preliminary evidence is presented which suggests that a more complex process is involved in which multispirochetal cysts develop following aggregation of two or more organisms. Within each of these larger cysts numerous organisms develop and subsequently emerge as tangled ropes. Following emergence, they subsequently undergo transverse division and gemmae formation, and so reproduce vegetatively. Subsequent papers will elaborate upon these processes.

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EXPLANATION OF PLATES

PLATE 11

FIG. 1. Photograph 296. 9-hour-old slide preparation of macerated rabbit testis in thioglycollate medium. Segment of spirochete showing development of minute gemma with basal granule. × 4850.

FIG. 2. Photograph 291. 72-hour-old slide preparation of macerated rabbit testis in thioglycollate medium. Young bleb-like gemma attached to spiral form. × 4850.

FIGS. 3 and 4. Photograph 552. 5-day-old slide preparation of macerated rabbit testis in thiglycollate medium. Lateral gemmae with dense granules within. × 4850.

FIGS. 5 and 6. Photographs 539 and 544. 5-day-old slide preparation of macerated rabbit testis in thioglycollate medium. Terminal gemmae with dense basilar granules within. × 4850.

FIG. 7. Photograph 572. 7-day-old slide preparation of macerated rabbit testis in thioglycollate medium. Slightly older terminal gemmae, one at each end with granules present. × 4850.

FIG. 8. Photograph 339. 3-day-old slide preparation of macerated rabbit testis in thioglycollate medium. Spiral with stipitate terminal gemma. × 4850.

FIGS. 9 and 10. Photographs 348 and 330. 5-day-old slide preparation of macerated rabbit testis in thioglycollate medium. Spiral forms with two terminal gemmae attached at same end. × 4850.

FIG. 11. Photograph 563. 5-day-old slide preparation of macerated rabbit testis in thioglycollate medium. Spiral form with small terminal gemma with basilar granule at lower pole, and a recently detached larger gemma near upper pole. The granule present in free gemma is seen to be elongating. × 4850.

FIG. 12. Photograph 574. 7-day-old slide preparation of macerated rabbit testis in thioglycollate medium. Spiral form with two attached gemmae showing degrees of elongation of included granules into curved rods. × 4850.

FIG. 13. Photograph 533. 4-day-old slide preparation of macerated rabbit testis in thioglycollate medium. Spiral form with three gemmae of different sizes within which early differentiation is occurring. × 4850.

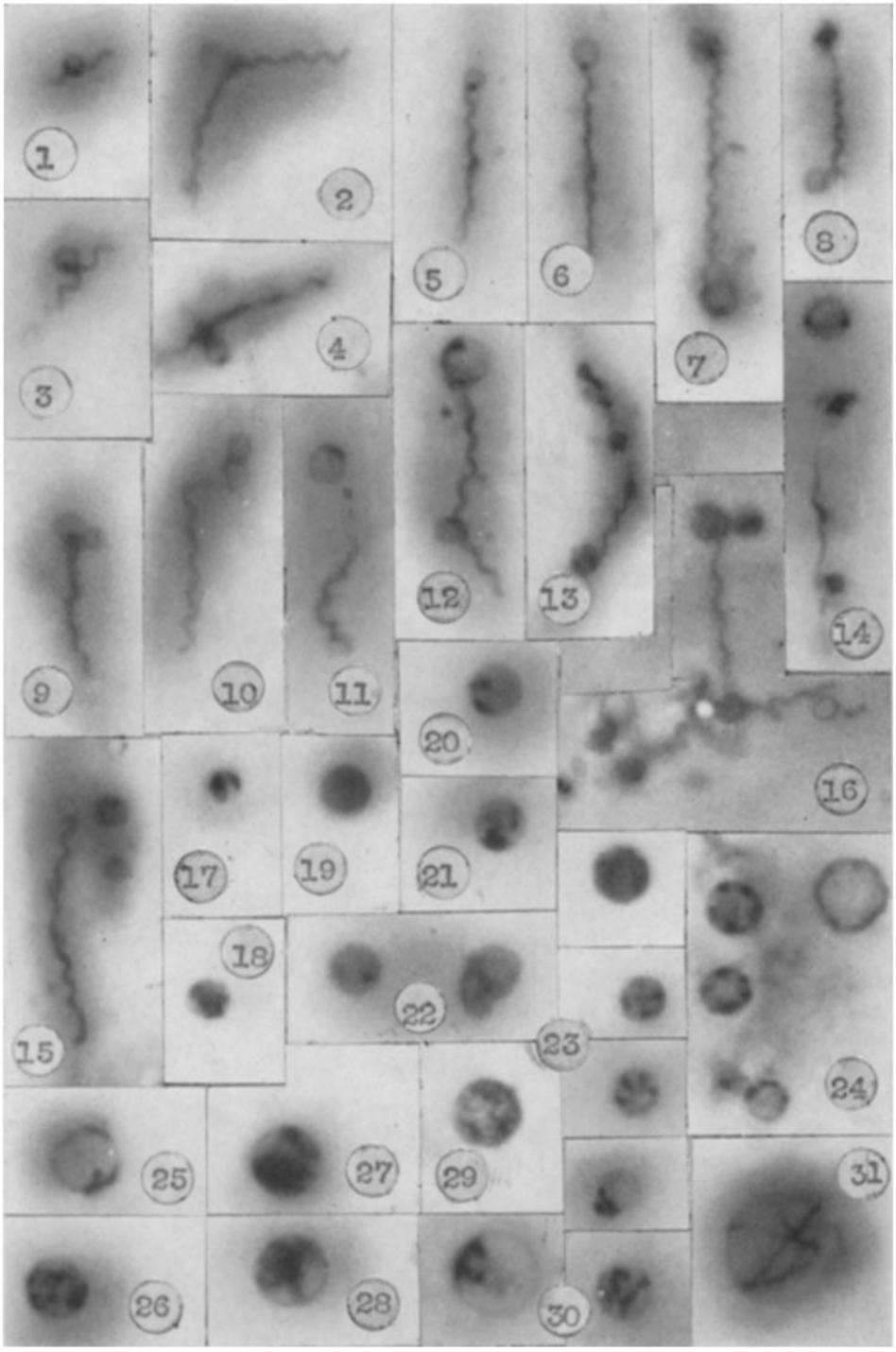
FIG. 14. Photograph 561. 5-day-old slide preparation of macerated rabbit testis in thioglycollate medium. Spiral form with young gemma and free gemma in which curved rod forming young spirochete is evident. × 4850.

FIG. 15. Photograph 547. 5-day slide preparation of macerated testis material in thioglycollate medium. Adult spiral form with two young gemmae lying free near by. Granules present in each. × 4850.

FIG. 16. Photograph 305. Fresh slide preparation of macerated testis material in thioglycollate broth medium. A tangle of spiral forms producing several gemmae, single and in clusters. × 4850.

FIGS. 17 to 29. Photographs 124, 542, 286, 549, 534, 288, 541, 558, 140, 565, 537, 324, and 123. Slide preparations of different ages of macerated testis material. Stages in the development of freed gemmae into unispirochetal cysts. × 4850.

FIGS. 30 and 31. Photographs 569, 537, and 529. Slide preparations of macerated testis material. Unispirochetal cysts with coiled young spirochetes within. × 4850.



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

PLATE 12

FIG. 1. Photograph 296. 9-hour-old slide preparations of macerated testis material in thioglycollate medium. Long delicate spiral form. × 4850.

FIGS. 2 to 4. Photographs 322, 347, 286. Slide preparations of different ages of macerated rabbit testis material in thioglycollate medium. Unipolar emergence of spiral forms from unispirochetal cysts. × 4850.

FIG. 5. Photograph 329. 3-day-old slide preparation of macerated rabbit testis material in thioglycollate medium. Spiral form showing bipolar emergence from a unispirochetal cyst. × 4850.

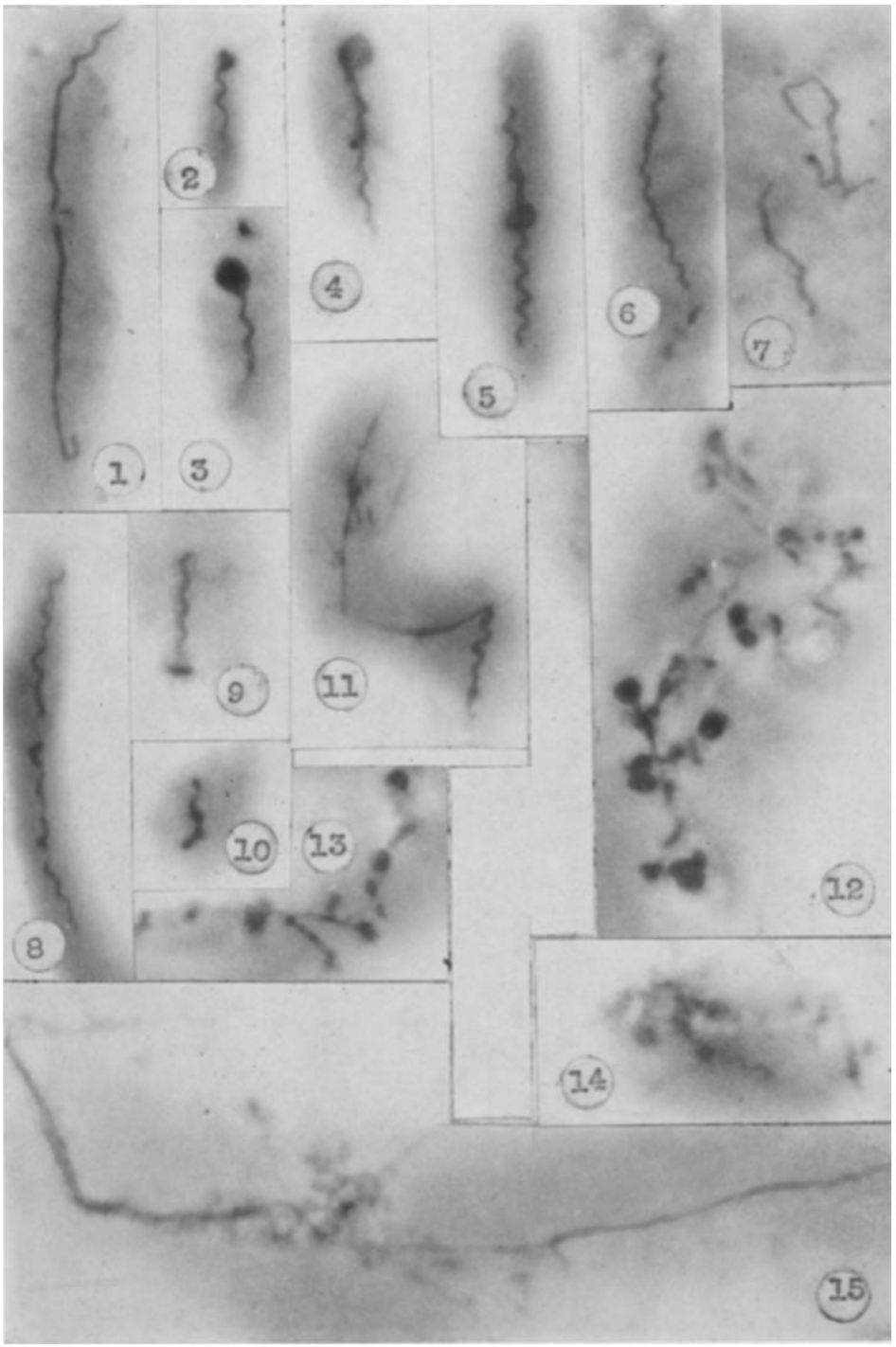
FIGS. 6 and 7. Photographs 136 and 133. Slide preparation of rabbit testis material cultured 2 weeks in thioglycollate medium. Adult spiral forms. × 4850.

FIG. 8. Photograph 332. 3-day-old slide preparation of rabbit testis in thioglycollate medium. Two adult spirals, the upper showing early gemma with enclosed granule at lower pole. × 4850.

FIG. 9. Photograph 557. 5-day-old slide preparation of rabbit testis in thioglycollate medium. Spiral with flagella visible at upper pole. × 4850.

FIG. 10. Photograph 88. Slide preparation of 4 weeks culture of rabbit testis material in thioglycollate medium. Short irregular spiral form. × 4850.

FIGS. 11 to 15. Photographs 105, 101, 93, 121, 86. Slide preparations of different ages of macerated rabbit testis material in thioglycollate medium. Peculiar irregularly branching forms observed to be attached to or continuous with spiral segments. On several of these dense granules or gemmae are developing. × 4850.



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

PLATE 13

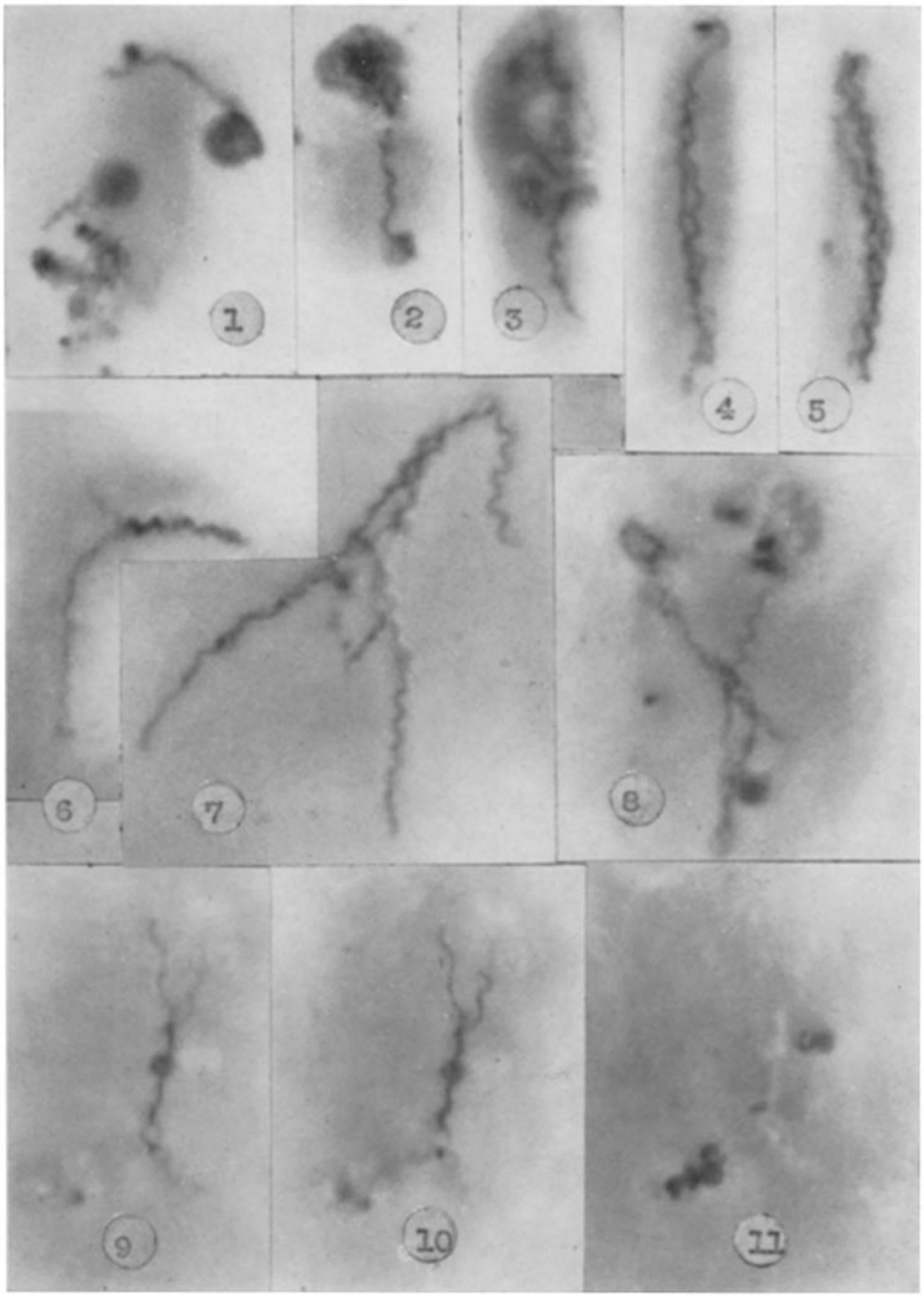
FIG. 1. Photograph 303. 9-hour-preparation of macerated rabbit testis material in thioglycollate medium. Spiral with large and small gemmae attached. × 4850.

FIG. 2. Photograph 299. Same preparation as Fig. 1. Spiral with large irregular mass at upper pole with dense irregular material in center and smaller gemma developing at lower pole. × 4850.

FIG. 3. Photograph 339. 3-day-old slide preparation of macerated rabbit testis material in thioglycollate medium. Dense mass from which irregular spiral is coming. × 4850.

FIGS. 4 to 8. Photographs 95, 301, 350, 342, and 319. Slide preparation of different ages of macerated rabbit testis material in thioglycollate medium. Six to 91 hours after preparations are made organisms come together in clumps or in pairs as shown. × 4850.

FIGS. 9 to 11. Photographs 80, 81, and 85. 5-day-old slide preparation of rabbit testis material in thioglycollate medium. Three optical planes. Three paired spirochetes showing origin of dense mass along point of contact of organisms (Figs. 9 and 10) and grape-like cluster of round bodies on left lower branch (Fig. 11). × 4850.



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

PLATE 14

FIG. 1. Photograph 141. Slide preparation of 3½-week-old culture of rabbit testis material in thioglycollate medium. Entwined clump of two or more spiral forms from which many dense bodies are developing. At upper pole is large cyst within which some differentiation can be made out. × 4850.

FIG. 2. Photograph 100. Same object as Fig. 12 (Plate 12) at higher optical plane. Complex cluster of entangled branching forms with a spiral segment evident. Numerous dense granular forms are being produced. × 4850.

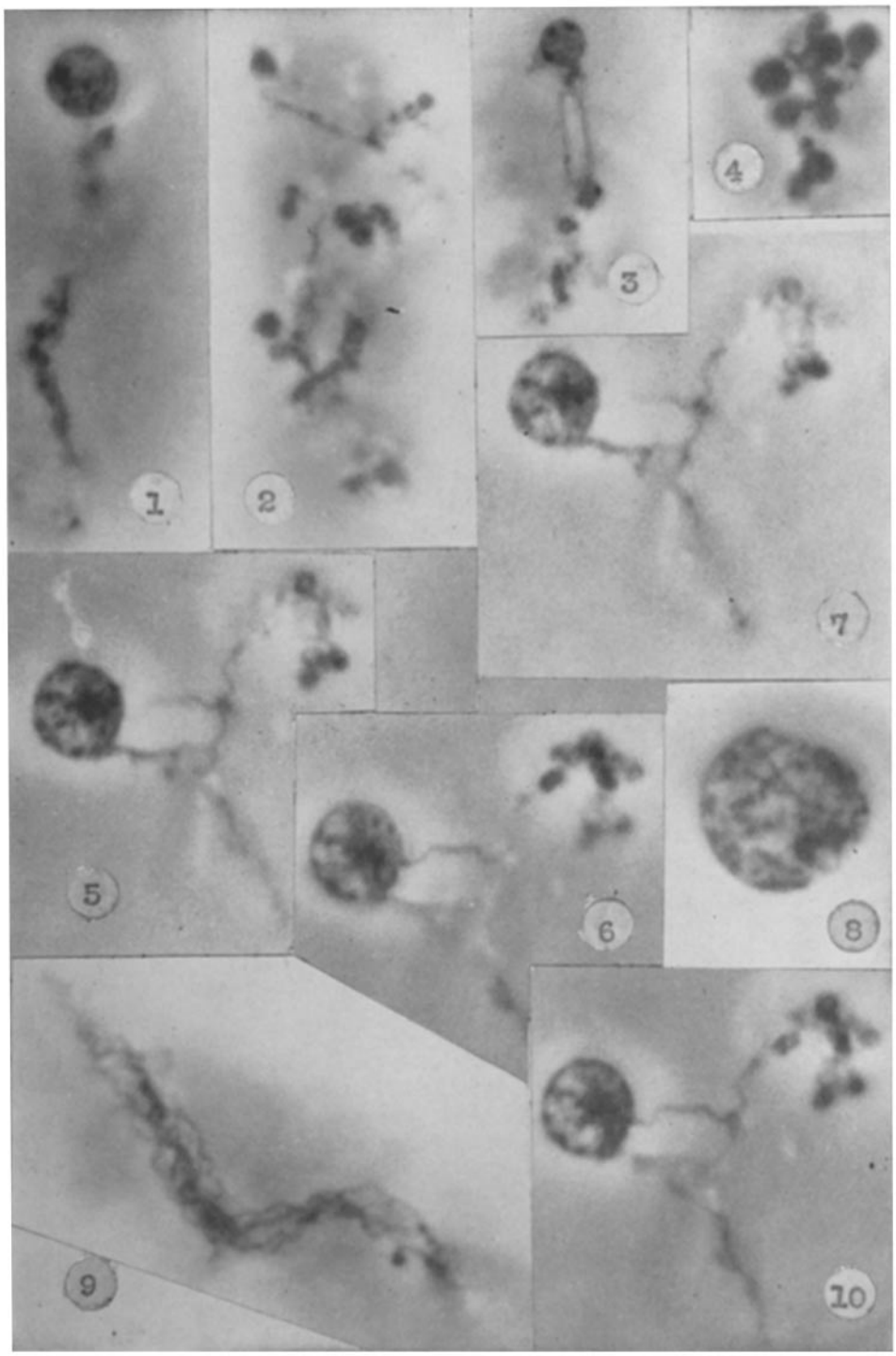
FIG. 3. Photograph 145. Slide preparation of 3½-week-old culture of rabbit testis material in thioglycollate medium. Small differentiating cyst attached to two delicate spiral fibrils. × 4850.

FIG. 4. Photograph 102. Slide preparation of 6-day-old culture of macerated rabbit testis material in thioglycollate medium. Dense grape-like cluster of bodies attached to branched spirochetal structures. × 4850.

FIGS. 5 to 7, and 10. Photograph 114. 6-day-culture of macerated rabbit testis material in thioglycollate medium. Four optical sections through the same complex cluster of spirochetes, to which are attached (1) a large cystic mass within which differentiating spiral forms can be made out, and (2) grape-like clusters of smaller bodies. × 4850.

FIG. 8. Photograph 344. 3-day-old slide preparation of macerated rabbit testis material in thioglycollate medium. Large multispirochetal cyst within which delicate filaments of young spirals can be made out. × 4850.

FIG. 9. Photograph 96. 6-day-old slide preparation of macerated rabbit testis material in thioglycollate medium. Tangled mass or cord of adult spirochetes emerging from cyst at right. A gemma is already being formed. × 4850.



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