TREPONEMA PALLIDUM BUDS, GRANULES, AND CYSTS AS FOUND IN HUMAN SYPHILITIC CHANCRES AND SEEN IN FIXED UNSTAINED SMEARS OBSERVED UNDER DARK-GROUND ILLUMINATION

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IN 1905 Schaudinn and Hoffmann discovered the spirochete of syphilis. Within three years most of the forms ascribed to its development or evolution had been described. Among the forms considered to be part of the developmental cycle of this microorganism were the so-called buds or granules, sporelike spherical bodies, described as small structures attached either laterally or terminally or occasionally separated from the spirochete by a stalk or a delicate filament and apparently originating from the cell wall (Buschke, 1906; Noguchi, 1912; Meirowsky, 1914; Levaditi, 1931; Manouélian, 1940; etc.).

The same type of granules have been reported from electron microscope studies of *T. pallidum* (Morton, 1942; Mudd, 1943; etc.). Some authors claim that they are particles of the medium accidentally attached to the microorganism; others that most sporelike bodies and end bodies are artifacts (Angulo and his colleagues[1]); and some contend that they are definite structures associated with the treponemal life cycle (Mudd).

In 1905 and 1906, Herxheimer[2,3] observed the development of masses on and within the body of *T. pallidum*. Other authors (Dutton, Leishman, Sergent, Nicolle, etc.) studying other spirochetes, reported observations suggestive of the development of new spirochetes from buds or granules. In 1914, Meirowsky[4] demonstrated the formation of granules from typical human *T. pallidum* and also the development of spirochetal forms from such granules both before and after their release from the parent microorganism. Leipold[5] confirmed these findings.

Later observations demonstrated that granular bodies, either dense or vesicular, may form at any point of the spirochetal body. Morton and Anderson[6] observed under the electron microscope dense spherical bodies protruding from the spirochetal body and attached by a stalk. Hampp, Scott, and Wyckoff,[7] using the same type of observation, noted the existence of bleblike structures containing a basilar granule attached to the spirochetal body. Gelperin[8] observed under dark-field illumination numerous minute rounded bodies within which

is constituted by apparently transparent spherules surrounded by a membrane containing a commalike body in the interior. This commalike body is frequently observed free without any surrounding membrane.

A fourth variety is represented by larger rounded bodies, which correspond to the so-called multispirochetal cysts described by some authors in rabbit syphilomas (DeLamater and associates). These cysts in some stages, probably the earliest, contain numerous round, oval, or commalike bodies. In other stages they contain fibrils. When overdistended they rupture and clusters or bundles of ropelike organisms may be seen emerging from them.

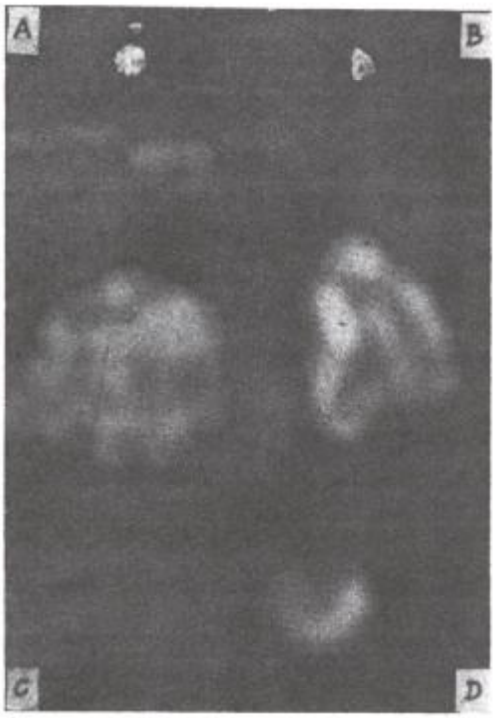

Fig. 5.—*A*, Multispirochetal cyst containing numerous rounded bodies; commalike body showing several zones of greater density. (Original × 900; enlargement 9½ diameters.) *B*, Coiled bodies, a more differentiated stage of commalike bodies. (Original × 900; enlargement 9½ diameters.) *C*, Commalike body showing polar and central zones of greater density. (Enlargement from × 900 original 9½ diameters.) *D*, Cyst containing well-developed commalike body. (Enlargement 9½ diameters from a × 900 original.) All forms in this plate from a human syphilitic chancre.

In some human chancres, we have noted the absence of *T. pallidum* in fresh preparations observed under dark-ground illumination, but in fixed unstained smears from the same lesion we have been able to demonstrate the existence of free spherical bodies and commalike bodies as well as cysts, which warrants a diagnosis of syphilis. We have also found these structures in the aspirated lymph from enlarged satellite lymph nodes.

The disappearance of *T. pallidum* from human lesions has been attributed to attenuation (von Prowazek); others attributed this phenomenon to breaking up of *T. pallidum* into granules or coccoid forms, probably expressions of attenuation or of a life cycle (Dutton, Leishman, Balfour, Coutts, Manouélian, Gastinel, etc.).

DISCUSSION

Transverse fission of *T. pallidum* as a means of multiplication is accepted by all recent workers on the subject and has been observed in *T. pallidum* from both human lesions and rabbit syphilomas. Forms suggesting longitudinal fission have also been observed.

Accumulated observations up to the present strongly favor the possibility of a life cycle occurring in *T. pallidum*. The presence of buds, granules, and the variety in size of treponemes in human and experimental animal lesions soon led early investigators to believe in the existence of a possible life cycle in *T. pallidum* (Krzystalowicz, 1905; Bertarelli, 1905; Ciuffo, 1908; Fanthan, 1911; etc.).

Several theories have attempted to correlate the existence of these structures with a developmental cycle of the parasite. Meirowsky[13] elaborated a theory which held that from the bud a typical spirochete arose, from which by further budding multiplication in the number of spirochetes was achieved. This theory of evolution by budding was revised and extended by Antoni (1921), Saphier (1921), Szilvázi (1925), and Warthin (1930), among other authors.

McDonagh[14] classified the spirochete with the Protozoa and paralleled its development with that of the malaria parasite. Many investigators have observed the small intracellular granules not only in endothelial cells, but in red corpuscles, lymphocytes, fibroblasts, and giant cells (Ross, 1913; Lundie, 1919; Coutts[15]).

Levaditi[16] based his theory upon morphologic variations of *T. pallidum*. The atypical forms were considered developmental stages in the evolutive cycle and were preferentially found in fibroblasts, giant cells, and in other macrophages.

Among contemporary investigators DeLamater and his co-workers[17] accept the existence of a complex life cycle of *T. pallidum* (rabbit-testis Nichols pathogenic strain) with the production of gemmas or buds which eventuate into unispirochetal cysts, within each of which single spirochetes develop and differentiate, and from which they subsequently emerge. They also describe multispirochetal cysts within which numerous organisms develop and, when the cyst breaks up, emerge as tangled ropes. Subsequently these organisms undergo transverse fission and bud formation and so reproduce vegetatively.[18]

After our observation under dark-ground illumination of numerous fixed unstained slides of serum from human syphilitic chancres, we are firmly convinced of the existence of a *T. pallidum* life cycle. This cycle is apparently as complex as that of the malaria parasite and is multiphasic. However, up to the moment, it is practically impossible to establish an exact correlation between its different phases.

Among these cycle forms we find definite and characteristic dense or vesicular spheroid bodies closely in contact with or attached by short stalks to the cell body and which originate from the treponemal cell wall. As pointed out by several authors who have studied animal strains of *T. pallidum*, these recall conidia and chlamydospores of higher fungi. Some of them contain a denser granule in the interior. We also find free spheroid or ovoid bodies containing a denser granule in their interiors, which develop into a commalike body. This commalike body is liberated as such and eventually grows and spirals into a typical treponeme.

Another type of free structure may contain numerous dense rounded bodies, commalike bodies, or thin spiral organisms (spirochetal cysts). These spirals are liberated by rupture of the cyst owing to overdistention. All are phases of the same evolutive phenomenon.

Spirochetogenic granules are by far more numerous than the cysts. It may be that these represent a protective stage in the life of the parasite.

SUMMARY

In fixed unstained smears of serum from untreated human syphilitic chancres, observed under dark-ground illumination, numerous spheroid bodies containing commalike structures, commalike bodies, and cysts containing numerous dense granules, commalike structures, or coiled bundles of thin spiral bodies may be observed lying free in the medium. These structures undoubtedly represent phases in the life cycle of *T. pallidum*.

On the basis of present knowledge this life cycle cannot be defined in terms of certainty, but from evidence accumulated by others and from our own observations it is probable that the nature of this cycle is complex and includes a form of multiplication from uni- and multispirochetal cysts.

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The smear is placed on the microscope and a cover glass laid over the material to be examined. If an oil immersion lens is used, a drop of cedar oil is placed on top of the cover glass. When the immersion lens is lowered and contacted with the oil, the cover glass adheres to the lens. The slide when displaced for observation slips under the cover glass. By simply raising the lens the unsoiled smear can be removed and kept for future observation.[12] We have smears nine months old that are in perfect condition.

Our observations have been carried out only with material obtained from untreated human syphilitic chancres. As demonstrated in the accompanying photomicrographs, buds, granules, or cysts, as found by former authors in human and experimental lesions may likewise be observed in chancre serum.

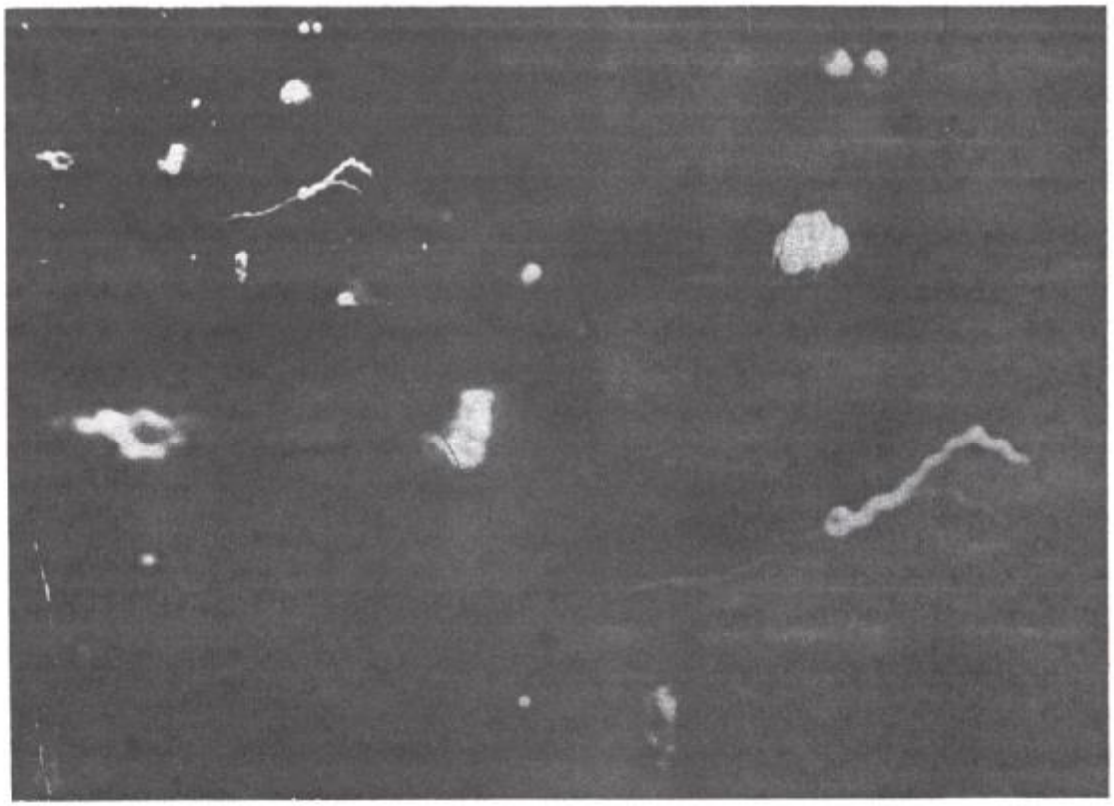

Fig. 4.—*T. pallidum* from human chancre. Different aspects of buds, uni- and multispirochetal cysts and commalike bodies. (Original × 900; enlargement 3½ diameters.)

Buds, apparently in intimate connection with or attached to any part of the treponemal body, are not uncommonly observed. Their true significance is still disputed, but undoubtedly they represent some phase of the treponemal life cycle as emphatically upheld by several investigators in whose opinion we participate.

Lying free in the serum a variety of brilliant spherical bodies can be found. They are far more numerous than the number of spirochetes found in the same microscopic field. Some are homogeneously very dense; others are less dense and show in their interiors a denser ovoid or spherical granule. A third variety

spirochetes seemed to be coiled; also cystlike structures appearing to contain numerous spirochetes.

Cystlike structures at the ends of *T. pallidum* (rabbit-testis syphiloma, Nichols strain) were observed in specially stained smears (carbol-gentian violet) by DeLamater, Haanes, and Wiggall.[9] The spirochetal membrane is seen to be continuous with the membrane of this body. They believe multispirochetal cysts are derived from them.

Most of the recent investigators who have worked with different strains of animal-adapted *T. pallidum* contend that buds, granules, and cysts are definite structures associated with the treponemal life cycle. Rose and Morton,[10] after their recent studies on this aspect, obtained no evidence that makes necessary the hypothesis of a true life cycle.

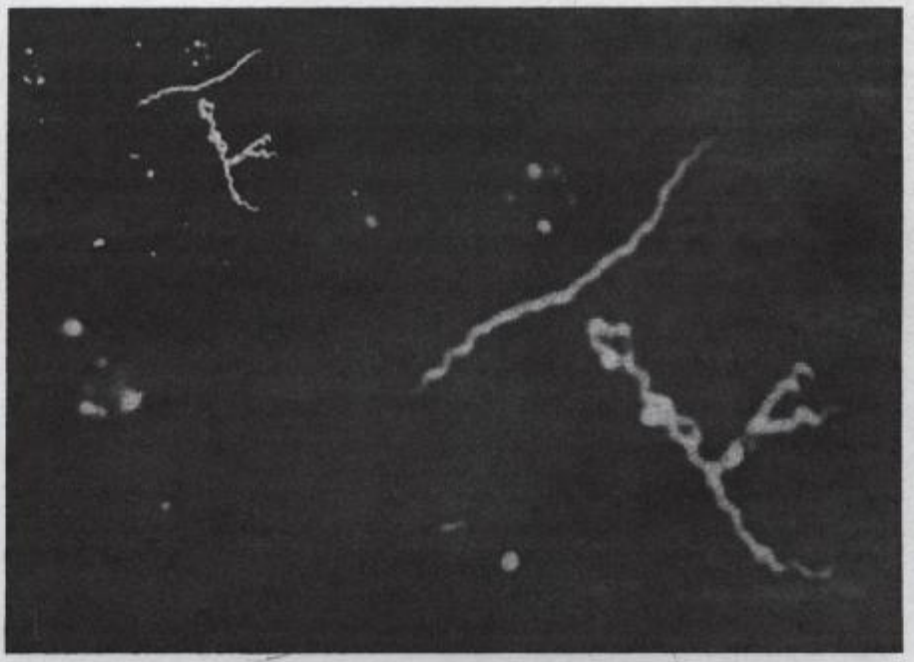

Fig. 1.—*T. pallidum* from human chancre. Transverse fission, closely attached buds and free spirochetal granules. (Original × 900; enlargement 3½ diameters.)

More than 30 years ago Hoffmann[11] emphasized the advantages of observing fixed stained and unstained smears and sections under dark-ground illumination for the study of spirochetes. Based on this principle and using the following technique, we initiated the investigation of a possible life cycle in *T. pallidum*. Buds, granules, and cysts can be observed also in fresh preparations but their continuous displacement does not allow a detailed study of these structures.

Chancre serum is tested for its ionic acidity (pH). A drop of this serum is spread in a very thin film on a glass slide and immediately covered with a 10 per cent acid or alkaline solution according to the result obtained from the ionic-acidity test. After 10 minutes' contact with this solution the slide is washed with distilled water and dried under filter paper.

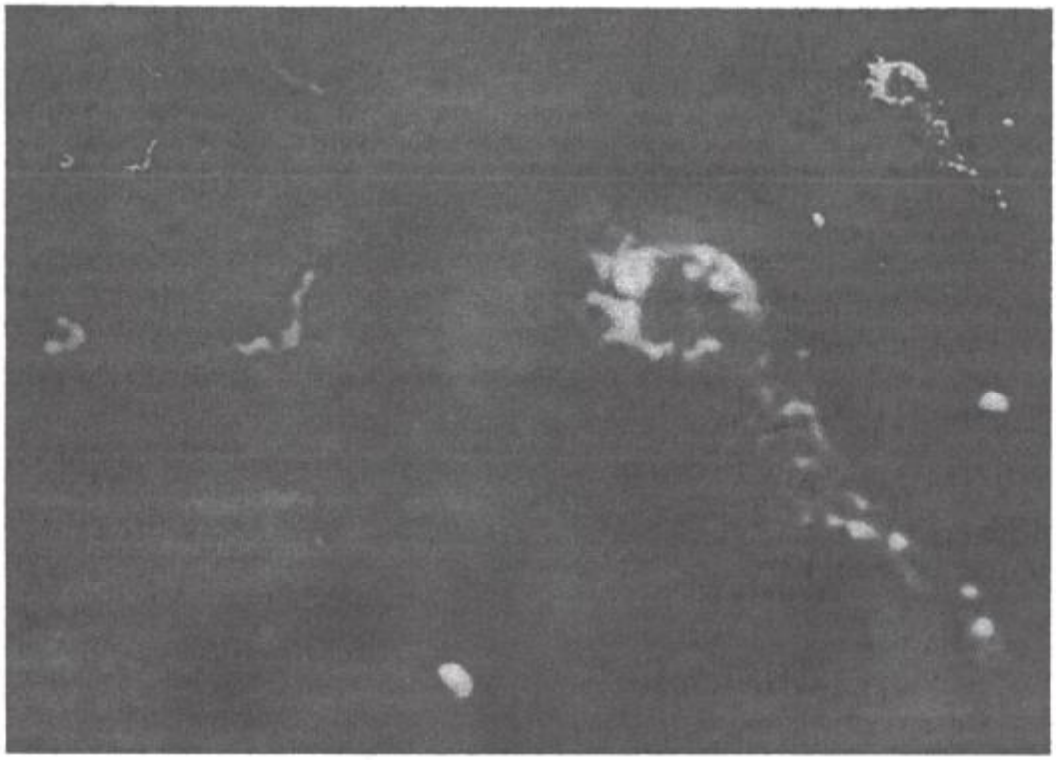

Fig. 2.—*T. pallidum* from human chancre. *A*, short treponemes and unispirochetal cysts. *B*, ruptured treponemal cyst showing coiled and bundled flexed filaments and spiraled bodies. (Original × 900; enlargement 3½ diameters.)

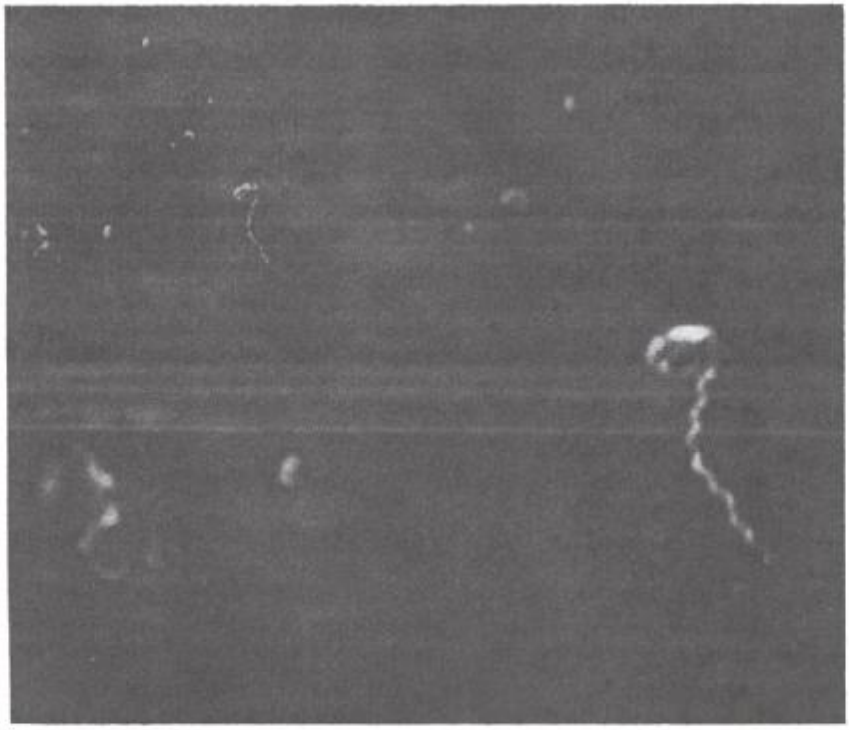

Fig. 3.—*T. pallidum* from human chancre.