THE LIFE CYCLE OF *ACTINOMYCES BOVIS*

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(With 2 Figures in the Text)

INTRODUCTION

The classification and nomenclature of the Actinomycetes have been confused since the time of their recognition.

The first species was described by Bollinger (1877) in sections of tissue from infected cattle. Wolff & Israel (1891) isolated an anaerobic actinomycete from 'lumpy jaw' of cattle. In the same year Bostroem (1891) isolated an aerobe of similar morphology from a similar source. Since the publication of these last two papers, the organisms isolated from 'lumpy jaw' of cattle and from actinomycotic lesions of man have been described as anaerobes, aerobes, or anaerobes capable of being trained to live under aerobic conditions. Nearly every character of these organisms has been reported in contradictory terms by different workers.

The author has only been able to trace two references to the cytology of *Actinomyces bovis*. Colebrook (1920) noted that a wet preparation of the organism, when stained with an alcohol-glycerine-fuchsin solution, showed deeply stained bodies distributed at regular intervals along the length of the filament. Bisset & Moore (1949) described the cell-wall arrangement and the type of branching, which description is in accordance with the author's findings in the 'A' phase of the life cycle, but not in the 'B' phase (see below).

MATERIAL

Ten strains were isolated from lesions in cattle; one strain was isolated from a human lesion, and one strain from a human lesion was obtained from the National Collection of Type Cultures.

All strains were capable of growing under anaerobic conditions, or in an atmosphere of 10% carbon dioxide under reduced oxygen tension. They could never be cultivated under completely aerobic conditions. All were filamentous and appeared to be branched, although it will be seen from later observations that this may be either true or false branching, according to the stage of development of the filament. Fragmentation, with club formation and spherical forms, was also seen. Beading was observed in smears stained by Gram's method. All were non-acid-fast and non-motile.

Two types of colony formation, similar to those described by Erikson (1940), were noted:

(1) Adherent to the medium, with ramifications into the medium beyond the limit of surface growth. Short filaments could be seen protruding out of the colony into the air, when viewed by oblique lighting.

(2) Non-adherent, without ramification or protruding filaments.

Continued subculture on the same medium often led to Type I changing to Type II. There was a marked difference in the morphology of the cells of the two types. Type I showed filaments composed of a number of individual cells, apparently branching, the whole colony forming a mycelium-like structure. In Type II the individual cells were usually separate; these were the so-called 'diphtheroid' forms of Negroni (1937), and Rosebury (1944). Both types of cells, however, pass through the same life cycle, and the difference between the two appears to be entirely due to the separation of the daughter cells of Type II, as soon as fission is complete. This is equivalent to the difference between rough and smooth variants of *Eubacteria* (Bisset, 1938).

METHODS

Impression colonies, as used by Klieneberger (1934), and teased-out colonies from Brewer's medium, were used for the preparation of specimens for examination. The staining techniques employed were the acid Giesma method for nuclear material, and the tannic acid-crystal violet technique for the cell walls (Robinow, 1942). Because the complexity of the cycle entails the examination of very many fields at varying depths of focus, composite drawings from actual preparations are used to illustrate this paper.

OBSERVATIONS

The spore is taken as the starting-point. This is a spherical, or occasional oval, cell with a central nucleus (Figs. 1, 1 and 2A, *a*). A germ tube grows out of the spore, the nuclear material divides, and a portion passes into the germ tube, grows and is set off by a cell wall (Fig. 1, 4). There may be only one germ tube produced, or there may be several developing successively. The nucleus in each tube is provided by the division of the nucleus in the spore and transference of a portion to each tube (Figs. 1, 4–8 and 2A, *a–d*).

Branches are formed by budding from the side of a cell and, as the branch enlarges, the nearest chromosal body moves towards it and divides into two, one part remaining in the cell, and the other part passing into a branch. As the branch enlarges, the nucleus within it divides and the daughter nuclei pass to the poles, while at the same time the branch is cut off by the formation of a cell wall (Figs. 1, 33–36 and 2A, *a*). This will be referred to as the 'A' phase.

Development continues for 2–4 days, after which time the next stage begins to appear. This is characterized in the heat-fixed Gram-stained preparations by the appearance of clubs, and of pear-shaped and spherical forms. These clubs are, of course, entirely distinct from the acid-fast structures often found around the periphery of colonies in tissues from chronic actinomycotic lesions.

Nuclear material from the poles of cells in the 'A' phase moves to the centre and fuses, during which process the cell contracts and begins to swell at one end, becoming oval or pear-shaped (Figs. 1, 9–14 and 2B, *a–f*). If the adjacent cell remains unaltered, a drumstick appearance results. Two of these cells then conjugate, and a fusion cell is formed (Figs. 1, 14–17 and 2B, *f–j*), in a manner reminiscent of that described by Klieneberger-Nobel (1947) for the initial cell of *Streptomyces*, and observed by the author in Micromonospora.

The initial cell then buds. The bud is club-shaped. Its growth is rarely in a straight line, but is usually distinctly wavy. More than one bud may arise from an initial cell and, as they develop, the initial cell is absorbed. Thus a radiating mass of filaments arises, nearly twice the diameter of the 'A' filaments. This will be referred to as the 'B' phase. As in the 'A' phase, the nucleus in the initial cell

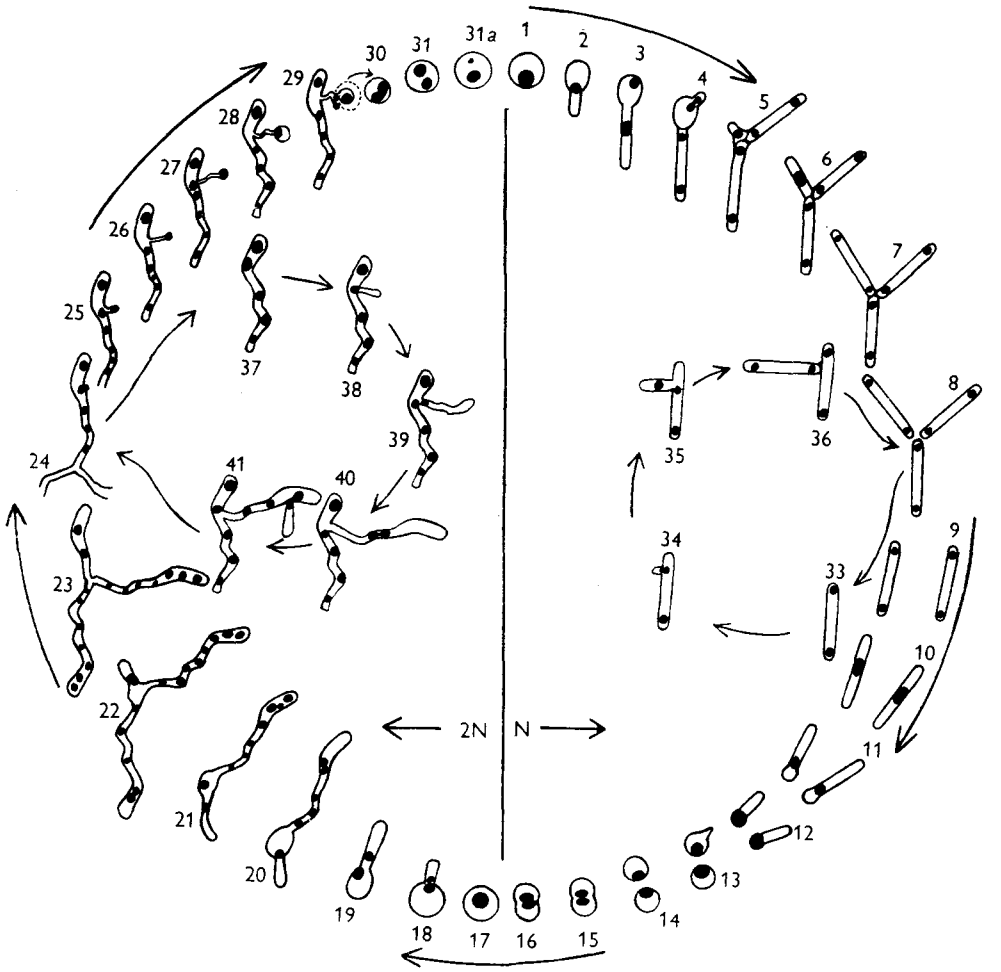

Fig. 1. Life cycle of *Actinomyces bovis*. 1–8, development of 'A' phase from germinating spore; 9–14, changes of 'A' cell before conjugation; 15–17, formation of initial cell of 'B' phase; 18–23, germination of initial cell; 24–29, formation of spore from 'B' phase filament; 30–31*a*, nuclear reduction in spore; 33–36, mode of branching in 'A' phase; 37–41, mode of branching in 'B' phase.

divides, and a portion passes into each bud as it forms. As the bud elongates the nucleus within it divides repeatedly, and the arrangement of the resulting nuclei is haphazard (Figs. 1, 17–24 and 2 C, *a–d*). Branching occurs by the budding of a filament adjacent to a nuclear mass, and the latter then moves to the growing bud, where it divides. One daughter nucleus remains in the parent cell, and the other passes into the bud. Within the bud, the nucleus divides repeatedly to give

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rise to another coenocytic filament (Figs. 1, 37–41, and 2 C, *e–g*). After 4–12 days, according to the strain and environment, the spore begins to form. A very slender tube is extruded from the 'B' filament, and the proximal nucleus moves towards it and divides, one part passing to the distal end of the tube. This terminal nuclear

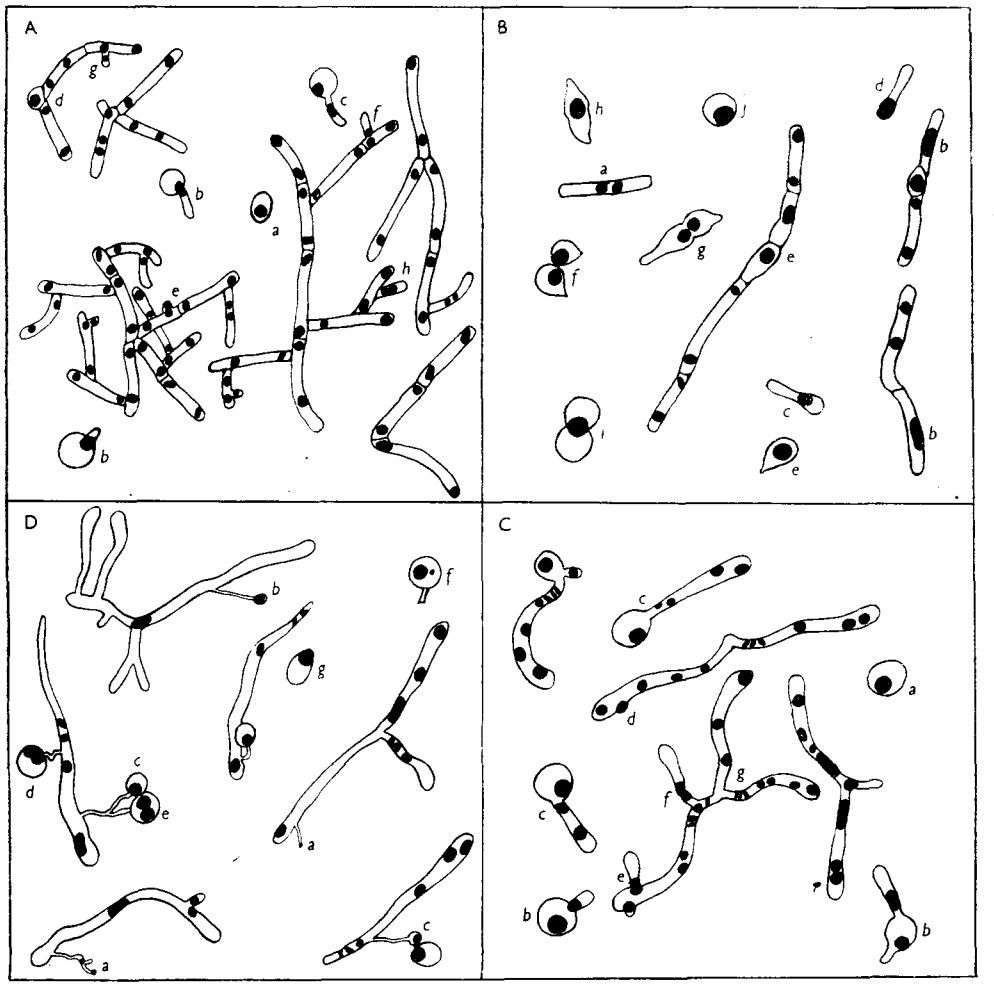

Fig. 2. Stages in life cycle of *Actinomyces bovis*. A, germinating spores and young 'A' phase cells: *a*, spore; *b*, germinating spore; *c*, migration of nuclear material to the germ tube; *d*, spore with 'A' cells and division of nucleus to form bud; *e–g*, division of nucleus in parent cell near bud; *h*, bud complete and daughter nucleus dividing. B, formation of the initial cell of the 'B' phase: *a–e*, re-arrangement and fusion of nuclear material in mature 'A' phase cells; *f–j*, conjugation and formation of the initial cell. C, early stages of 'B' phase: *a–c*, germination of initial cell; *d–g*, stages in the development of branches. D, development of the spore: *a–c*, spores arise from 'B' phase filaments; *d–f*, maturation of the spore, reduction process; *g*, free, mature spore.

mass expands to nearly twice the diameter of the tube, and the rind of cytoplasm around it begins to grow rapidly to form a spherical body 2–3 times the diameter of the 'B' filament. The nuclear material divides and one daughter nucleus forms the spherical nucleus of the spore, while the other degenerates. This can be assumed

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to be a reduction division, making the spore haploid. Sometimes two spores may form from a single germ tube, but each is borne at the end of a separate short filament (Figs. 1, 25–31 *a*, and 2 D, *a–g*).

When the formation of the 'B' phase is well advanced, smears from a culture show Gram-positive 'B' forms lying among degenerate 'A' forms, which become Gram-negative.

DISCUSSION

It appears from the above observations that *Actinomyces bovis* has a haploid generation, the 'A' phase, from which arises the diploid 'B' phase.

The initiation of the 'B' form by the fusion of two haploid cells is analogous to the process observed by Klieneberger-Nobel (1947) in the case of *Streptomyces*. The changes in the 'A' phase cell, just prior to the conjugation, resemble in many respects the changes seen in *Eubacteria* and *Myxobacteria* during microcyst formation (Bisset, 1950).

The club shape of the newly germinated 'B' form, and the altered shape of the 'A' phase cells, when seen in heat-fixed preparations, undoubtedly represent the pleomorphic forms described by Wright (1905) and Crowley (1941), and probably are the structures which Gibson (1934), Naeslund (1929), Bosworth (1923), Baynes-Jones (1925) and Erikson (1949) have observed in media containing a high concentration of serum, and which these workers believe to be of a similar nature to those acid-fast clubs seen in many tissue sections. Wright (1905) and Ørskov (1923) believed the club forms seen in tissues to be different from those seen in cultures. The development of the spore, although differing in detail, agrees in general principles with that described for the *Streptomyces* by Klieneberger-Nobel (1947).

The round or oval forms of the spore and initial cell probably account for the coccal contaminants noted by many workers, among whom are Lignières & Spitz (1902, 1903) and Magnusson (1928), because these workers failed to isolate the 'cocci' in pure culture. Wright (1905), Bosworth (1923), Gibson (1934), and Negroni (1937) suggested that these forms might be reproductive stages of the organism.

The arrangement of the nuclei in the individual cells of the 'A' phase and their mode of division are similar to that seen in the smooth forms of the *Streptococcus*, *Bacillus* and *Bacterium* (Bisset, 1950).

This life cycle is in general accordance with the scheme of bacterial cytology suggested by Bisset.

SUMMARY

Actinomyces bovis passes through a complete life cycle. The spore germinates to produce a haploid generation, from which a diploid generation arises by conjugation of two specialized haploid cells. A haploid spore is produced from the diploid generation by reduction division.

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