THE CLASSIFICATION OF THE PLEUROPNEUMONIA
GROUP OF ORGANISMS (BORRELOMYCETALES)

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The general properties of the organisms of the pleuropneumonia group (pleuropneumonia and pleuropneumonia-like organisms (PPLO)) may be summarized as follows:

1. Grow in cell-free culture media. Exacting nutritional requirements for most of the species.
2. A peculiar mode of reproduction characterized by breaking up of filaments (with more or less pronounced tendency to true branching) into coccoid elementary bodies.
3. A marked tendency to pleomorphism depending on cultural conditions.
4. A characteristic appearance of the minute colonies on solid media.
5. Filterability of the smaller reproductive units.
6. Poor affinity for the ordinary bacterial stains.
7. A high resistance to penicillin and sulfathiazole.

Nutritional requirements. With the exception of the saprophytic species, Mycoplasma laidlawii, all other species require enrichment with serum or ascitic fluid for growth on artificial media. Both protein and lipid components appear to be necessary (Edward and Fitzgerald (1951), Edward (1953), Smith and Morton (1951, 1953)). The ability of rabbit serum agar (low concentration of cholesterol) to support a poor or good growth can be used as a mark of differentiation between different species (Edward, 1954). The addition of a filtrate of a staphylococcal culture or fresh extract in a small concentration has been found to improve the growth of some strains (Edward, 1947). Desoxyribonucleic acid appears to be necessary for certain strains, at least at their first isolation (Edward, 1954).

Morphology. It has proved difficult to interpret properly the significance of the morphological details and the mode of growth of these organisms. The morphology of the type species, the organism of bovine pleuropneumonia (Mycoplasma mycoides), has been studied most thoroughly by various techniques. In fluid as well as on solid media the minute elementary bodies extrude one or more filaments of considerable length that ramify and form an apparently unicellular,

branching mycelium. At a later stage of growth tiny endomycelial corpuscles develop in the filaments by a process of successive condensation and constriction, no preceding formation of septa being demonstrable. As a result, the homogeneous filaments are transformed into chains of close-set spherical bodies. New free elementary bodies are released by fragmentation of these chains. The essentials of this mode of development and multiplication were described with various modifications by Ørskov (1927), Nowak (1929), Hosakawa and Kawamura (1931), Wroblewski (1931), Ledingham (1933), Klieneberger (1934), Tang et al. (1935), Turner et al. (1935), and Freundt (1953).

The elements of the pleuropneumonia organism are extremely plastic and under certain growth conditions develop into peculiar forms, among which are the so-called "large bodies", often containing granules that have been a subject of special interest. Some of the authors cited above, especially Turner and Wroblewski, also claim to have observed various deviating growth forms, including multiplication by simple budding. But above all, an important role in the life cycle of the organism was ascribed to the "large bodies" by Klieneberger and Smiles (1943), Dienes (1945), Dienes and Weinberger (1951), and Tulasne (1951). According to these authors, minute granules multiply within the large bodies and after rupture of the surrounding membrane start the "life cycle" again as free elementary bodies. The long filaments are considered by Klieneberger and Smiles mainly as artefacts. On the other hand, Ørskov (1938) and Freundt (1952) regard the "large bodies" as representing a stage of involution and degradation. Experimental support to this concept was given by Freundt.

In the opinion of the author, the mycelial mode of growth and multiplication by development of endomycelial bodies as described for the type species should be regarded as fundamental morphological criteria of the group as a whole. The description merely of very pleomorphic elements consisting of granules, globules, rings, and filaments, does not appear to be sufficient for the morphological identification of pleuropneumonia-like organisms.

Branching filamentous forms breaking up into new elementary bodies were also described in the following species or strains: in Mycoplasma agalactiae by Wroblewski, by Ledingham and by Ørskov; in M. arthritis (L4) by Preston;

BACTERIOLOGICAL NOMENCLATURE
AND TAXONOMY

in Asterococcus canis Shoetensack, M. pulmonis (L3), and M. laidlawii by Ørskov, and in strains from human sources by Beveridge and by Freundt. The above authors will agree that except for the smaller size of the filaments the morphology of these species is essentially similar to that of the bovine pleuropneumonia organism. On the other hand, Dienes (1953) and Morton et al. (1954) were not able to demonstrate anything but free elementary bodies in electron micrographs of PPLO isolated from man and poultry. Elementary bodies and small rod-shaped and filamentous forms were demonstrated by Klieneberger-Nobel and Cuckow (1955) in M. agalactiae, M. mycoides var. capri, and M. pulmonis.

Available representatives of most of the various species were investigated by the present author. Branching filaments could be demonstrated as a constant feature in all species, though the frequency of branching was admittedly low in some organisms. On the whole, each species could be classified within one of three morphological groups. Morphological differences between various strains belonging to one species may possibly occur, but in cases where the observation included more than one strain of each species these differences were of minor importance only. The organisms of Group I are characterized by an unstable, sparsely branching mycelium with very short, almost bacillary filaments (usually 2-5 μ). Gradual transitions are found to the relatively stable mycelium of Group II with a moderate length of filaments (10-30 μ). Group III is distinguished by a quite regular, fully developed, richly branching mycelium with filaments attaining a length of 100 to 150 μ or even more.

Colonial morphology. The appearance of the minute colonies on solid medium is very characteristic: an opaque, granular, brown, or yellowish central area growing down into the agar, surrounded by a translucent, flat peripheral zone of variable size. Slight variations in the colonial morphology are found in various species and on different media.

A peculiar phenomenon was observed by Edward (1950, 1954) in plate cultures of certain species. Small black dots consisting of deposits of calcium and magnesium soaps are produced by these species beneath and around the colonies, together with a crinkled, greyish film of a more complex composition on the surface of the medium, especially on horse serum agar incubated for 6 days. Growth in fluid and semi-solid media is granular or smooth and fluffy.

Size. Ultrafiltration experiments indicate a particle diameter of the basic reproductive units, the elementary bodies, of 125-150 m μ , i.e., the same order of size as that of the viruses of herpes simplex and vaccinia.

Staining properties. The organisms are Gram-negative. They stain poorly with ordinary bacterial stains, but fairly well with Giemsa.

Classification and Nomenclature

No general agreement has as yet been obtained as to the nomenclature and classification of the pleuropneumonia group. The variety of names suggested for the type species, the organism of bovine pleuropneumonia, reflects the confusion of nomenclature.

Ledingham (1933) emphasized the resemblance of the organisms of bovine pleuropneumonia and of agalactia to the actinomycetes and consequently proposed that these species be incorporated in the existing order Actinomycetales, family Actinomycetaceae. Undoubtedly there are conspicuous morphological similarities between the pleuropneumonia group and the actinomycetes. Thus the organism of bovine pleuropneumonia bears some resemblance to the actinomycetes within Ørskov's Group I, while the small-mycelial pleuropneumonia-like organisms may correspondingly be paralleled with the genus Nocardia (Ørskov's Groups IIa and IIb). However, these similarities do not necessarily suggest that a phylogenetic relationship exists between the two groups of organisms. The similarities may probably be interpreted only as an evidence of a parallel morphological differentiation within two large groups of mycelial micro-organisms.

Turner (1935) erected a new order Borrelomycetales, with family Borrelomycetaceae and genus Borrelomyces.

The term Borrelomycetales was rejected by Sabin (1941) since Bordet and not Borrel was the first to describe the morphology of the pleuropneumonia organism. Sabin proposed a new class, Paramycetes, order Paramycetales or Anulomycetales, family Parasitaceae or Anulomycetaceae. A sub-grouping into genera and species referring to the animal host of the various organisms was also drawn up by this author. Recently Tulasne and Brisou (1935) proposed the ordinal name Pleuropneumoniales.

BACTERIOLOGICAL NOMENCLATURE
AND TAXONOMY

Clearly contrasting with the above attempts for a classification, Dienes in the last edition of Bergey's Manual suggested a close relationship with the species included in the genera Pasteurella and Haemophilus, thus adhering to the classification of Buchanan (1918). This classification appears to be inconsistent with the true mycelial nature - also admitted by Dienes - of the type species of the group, the organism of bovine pleuropneumonia.

Much confusion has arisen from an incorporation in the pleuropneumonia group of the so-called L-forms isolated from various bacterial cultures (e.g. the L₁ colonies isolated by Klieneberger from Streptobacillus moniliformis), especially under the influence of penicillin. Though it was stated by Dienes (Bergey, 1948) that these are "variant forms of the bacteria and should be classified with the parent organisms," Dienes and Weinberger later (1951) stressed the similarities between the pleuropneumonia organisms and the L-type variants, being inclined to regard the pleuropneumonia organisms as originally descended from ordinary bacteria. The same theory was advanced by Tulasne (1951). However, there appears now to be a growing feeling that the L-phase variants should be separated from any connection with the pleuropneumonia group (Edward, 1954).

Name of the Order and Family. Turner (1935) was the first to suggest a new order for the pleuropneumonia group of organisms. His name for this order, Borrelomycetales, and the family name, Borrelomycetaceae, therefore clearly have priority, and appear to be in accordance with Rule 1 of the International Bacteriological Code of Nomenclature. Some may argue that these names are unfortunate because they allude to a morphological character ("mycelial") which has not so far been generally accepted. It if be preferred to propose a new ordinal name based on an outstanding morphological property which all workers agree upon the name Molliparietales (lat. mollis soft + lat. parietalis wall) proposed by H.E. Morton in a personal communication to the author deserves consideration. Another possibility would be to derive the order and family name from the generic name to be proposed in the following: Mycoplasmatales, Mycoplasmataceae (cf. the second alternative of Rule 1).

Name of the Genus. As far as it appears from our present knowledge there is hardly any valid basis for the erection of more than one genus within the family. Any generic sub-

division founded on morphological and biochemical characters etc., would probably be rather arbitrary and the more so as there is no obvious constant correlation between these properties.

The status of the various generic names that have been applied to the type species has been fully summarized by the Editorial Board (this BULLETIN 5:13-20. 1955). It appears from this that Asterococcus Borrel et al. (1910) is illegitimate because it is a later homonym of the algal genus Asterococcus Scherffel (1908). Coccobacillus Martzinovski (1911) ted by Ørskov (1938) and Freundt (1952) is also invalid because it was used in 1888 by Dangeard for a fungus and in 1891 by Grüber for an organism which was later placed in the genus Actinomyces. The first name that is valid is Mycoplasma Nowak (1929) which is set up by the Editorial Board as an alternative to Borrelomyces Turner (1935).

The Editorial Board suggested that Borrelomyces might perhaps be preferred to Mycoplasma on the assumption that Borrelomyces is the only generic name that has been proposed to replace Asterococcus "which has found any general acceptance". This supposition, however, will hardly appear to be quite correct. In fact, as far as I can see, none of the generic names proposed to replace the illegitimate Asterococcus, including Borrelomyces, has ever found any general usage by bacteriologists. Therefore, though the present author originally felt inclined to conserve Borrelomyces there is scarcely any real justification to prefer this name to one which has priority. The conservation of Mycoplasma is formally correct and hence unimpeachable and less liable to meet with criticism. The possible objection by some workers that the syllable myco- involves an allusion to a disputed morphological character can in this connection be refuted with a reference to Rule 23 of the International Bacteriological Code of Nomenclature: "A name or epithet must not be rejected, changed or modified merely because it is badly chosen or disagreeable or because another is preferable or better known".

The following tentative list of species is proposed for the pleuropneumonia group. Two new species (Nos. 2 and 10) are described and named.

BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY

Order Mycoplasmatales ordo nov.

Genus Mycoplasma Nowak 1929.

Species 1. Mycoplasma mycoides (Borrel et al.) comb. nov. (Asterococcus mycoides Borrel, Dujardin-Beaumetz, Jeantet and Jouan 1910).

Subspecies 1. Mycoplasma mycoides var. mycoides comb. nov.

Subspecies 2. Mycoplasma mycoides var. capri comb. nov. (Asterococcus mycoides var. capri Edward 1953)

Species 2. Mycoplasma bovigenitalium sp. nov.

Species 3. Mycoplasma agalactiae (Wroblewski 1931)

comb. nov. (Anulomyces agalaxiae Wroblewski 1931)

Species 4. Mycoplasma spumans Edward 1955.

Species 5. Mycoplasma canis Edward 1955 (not Asterococcus canis Shoetensack 1934).

Species 6. Mycoplasma maculosum Edward 1955.

Species 7. Mycoplasma pulmonis (Sabin 1941) comb.

nov. (Murimycetes pulmonis Sabin 1941).

Species 8. Mycoplasma arthritidis (Sabin 1941) comb.

nov. (Murimycetes arthritidis Sabin 1941).

Species 9. Mycoplasma neurolyticum (Sabin 1941) comb.

nov. (Musculomyces neurolyticus Sabin 1941).

Species 10. Mycoplasma gallinarum sp. nov.

Species 11. Mycoplasma hominis Edward 1955 (in part

Micromycetes hominis Freundt 1954).

Species 12. Mycoplasma fermentans Edward 1955.

Species 13. Mycoplasma salivarium Edward 1955.

Species 14. Mycoplasma laidlawii (Sabin 1941) comb.

nov. (Sapromycetes laidlawii Sabin 1941).

Certain groups of strains of PPLO described in the literature undoubtedly deserve rank of species, but have from various causes not been listed above. Asterococcus canis Shoetensack I and II cannot from Shoetensack's descriptions be identified with any of the dog strains isolated in recent years, and the original strains are lost. Good descriptions were published of Musculomyces arthrotropicus and Musculomyces histotropicus Sabin (1941). But though very likely they really are independent species, the relationship to the species isolated from mice and rats by the English workers and based on still existing strains, still seems obscure. Sabin's strains are lost, but sufficient quantities of antisera for comparison and possible identification with other mice

strains still exist (Sabin, personal communication). But until this work has been done it will probably be more wise to make some reservations.

Pleuropneumonia-like organisms isolated in recent years from different sources, e.g. from swine (Carter and McKay 1953, 1954), from bronchopneumonia of cattle (Carter 1954) and from the eyes of a chameleon (Klinge 1954) need further study before they can be named as independent species.

Comments: The above list of proposed species in part results from informal discussions between Dr. D.G. ff. Edward and the author. A similar, but not quite identical list, is published by Edward in this BULLETIN (5:85-93. 1955). While the present author originally intended to set up one species for the organisms isolated from dogs, and one for those isolated from humans, each covering three or four types respectively, Edward - with one exception probably more correctly - proposed and named three species for each of them (from dogs: Nos. 4, 5, 6; from humans: Nos. 11, 12, 13). In the opinion of the present author the two groups of organisms isolated from dogs, named M. spumans and M. canis, should be included in only one species (M. canis) covering two types or subspecies, as the properties that separate them seem to be relatively insignificant and partly variable. However, in order not to create undue confusion Edward's nomenclature for the dog strains also on this point has been followed here.

The following two species are described and newly named.

Borrelomyces bovigenitalium nov. sp.

(P strains of the bovine genital tract, Edward)

Etym. bo.vi.ge.ni.tá lium. L. noun bos, bovis the ox; L. pl. n. genitalia the genitalia; M.L. adj. bovigenitalium of bovine genitalia.

Unstable, sparsely branching mycelium with very short, almost bacillary filaments which usually measure 2 to 5 microns in length. Gram-negative.

Horse serum agar: A film and spots are produced.

Horse blood agar: Alpha hemolysis.

Rabbit serum agar: Poor growth.

Semi-solid media: Fluffy growth throughout.

Broth: Dense, uniform opalescence.

BACTERIOLOGICAL NOMENCLATURE
AND TAXONOMY

Carbohydrates not attacked.

Methylene blue is reduced.

Ten strains investigated serologically shared common antigens, but at least three different serological types appear to exist.

Pathogenicity: Suggested as a cause of inflammation of the genital tract, predisposing to infertility, although inoculation of cultures into the uteri of heifers has so far been unsuccessful.

Source: Isolated from the bovine genital tract.

Habitat: Frequent inhabitant of the bovine lower genital tract, both in males and females.

Borrelomyces gallinarum nov. sp.

Etym. gal.li.ná rum. L. fem. n. gallina a hen; L. fem. gen. pl. n. gallinarum of hens.

Description taken from Edward (1954, pp. 52, 53).

Horse serum agar: A film and spots are produced.

Horse blood agar: Hemolysis.

Rabbit serum agar: Good growth.

Semi-solid media: Smooth growth throughout.

No acid from glucose.

Aerobic, facultative.

Pathogenicity: Does not produce lesions after experimental inoculation.

Comments: Herick and Eaton (1945) isolated a pleuropneumonia-like organism as a contaminant of a pneumonia virus which was being passed in chick embryos. Report of pleuropneumonia-like organisms from egg-passage material containing microscopically visible agents which were regarded as viruses and which caused upper respiratory catarrh in fowls and turkey sinusitis was made by Markham and Wong (1953); following a series of thirteen successive subcultures in artificial media, the organisms produced specific lesions in embryonated eggs. Further work is needed before the significance of these pleuropneumonia-like organisms and their relationships to the viruses associated with these infections and the coccobacillary bodies of fowl coryza (Nelson 1935, 1937, 1940) can be determined.

Relationships to the L-phase of bacteria: Reversion of cultures of so-called pleuropneumonia-like organisms from chronic respiratory disease and turkey sinusitis to an organ-

ism resembling Haemophilus gallinarum has been reported by McKay and Taylor (1954); the significance of this finding cannot be evaluated as yet.

Source: Isolated from the upper respiratory tract of a fowl.

Habitat: From the normal and diseased upper respiratory tracts of fowls; presumed to be the etiological agent of chronic respiratory diseases of chickens and of sinusitis of turkeys although this has not been proven experimentally.

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