ON THE CLASSIFICATION AND NOMENCLATURE OF
SOME NONMOTILE AND COCCOID DIPLOBACTERIA,
EXHIBITING THE PROPERTIES OF
ACHROMOBACTERIACEAE

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SUMMARY. Five species and two subspecies of nonmotile and coccoid Achromobacteres are described, including the organisms hitherto known as Bacterium anitratum Schaub and Hauber and Alcaligenes metalcaligenes Castellani and Chalmers. They are Achromobacter mucosus (von Lingelsheim 1906) Mannheim and Stenzel 1962, A. conjunctivae Mannheim and Stenzel 1962, A. haemolyticus Mannheim and Stenzel 1962, A. haemolyticus subsp. alcaligenes Mannheim and Stenzel 1962, A. metalcaligenes (Castellani and Chalmers 1919) Bergey et al. 1925, and A. citroalcaligenes Mannheim and Stenzel 1962.

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The findings on a study of 175 strains of strictly aerobic, cytochrome - oxidase - negative, nonmotile Gram - negative, coccoid diplobacteria have recently been published (Mannheim and Stenzel 1962). The results of this examination are summarized and discussed here.

Most of the microorganisms described below are already known under various names, but since they either belong to ill-defined species, such as Alcaligenes metalcaligenes Castellani and Chalmers or lack a correct nomenclature, viz. Bacterium anitratum Schaub and Hauber, it seems necessary to arrange them into a reasonable system.

Five closely interrelated species were proposed and named (Mannheim and Stenzel 1962). Representative strains of the species concerned and of some of their subspecies, which were deposited in the National Collection of Type Cultures (NCTC), London, are designated below as type strains or proposed as neotype strains.

These species exhibit the following common properties: nonmotile, cytochrome-oxidase-negative diplobacteria which are coccoid as a rule but are frequently pleomorphic. They are strictly aerobic, grow well on simple media and do not produce pigments. If carbohydrates are attacked they are oxidized without formation of gas. Methyl red and Voges-Proskauer reactions are negative. Indole and hydrogen sulfide are not produced, and nitrates are not reduced. As a rule urea is not split but occasionally there may be delayed hydrolysis.

As stated previously (Mannheim and Stenzel 1962), these five species should be placed in the genus Achromobacter. They are:

1. Achromobacter mucosus (von Lingelsheim 1906) Mannheim and Stenzel 1962. Suggested neotype strain: No. 3516/60 (NCTC 10303). Mucoid colonies are produced on suitable media, e.g. pink, viscid, mucoid colonies on Endo's agar, and black zones, but no hemolysis, on sheep-blood agar. No growth occurs on Leifson's desoxy-cholate-citrate medium. Optimum temperature is near 37°, but good growth occurs at 22° and 45°. Glucose, galactose, xylose, arabinose and citrate are split promptly (within 24-36 hours). Maltose, lactose, sucrose, sorbose, rhamnose, salicin, inositol, mannitol, sorbitol, adonitol and dulcitol are not acidified within 30 days (fluid media). Ammonium salts are utilized as sole sources of nitrogen. Gelatin is not liquefied within 30 days, and urea is not split. When injected intraperitoneally in an amount of 0.1 to 0.5 ml of an 18-hour-old broth culture, it is pathogenic for mice.

A. mucosus is common, being isolated from wounds, abscesses, secretions of various mucous membranes, stools and urine, but it is rarely found naturally in pure culture. It may cause pyogenic infections in man.

Variations: Some strains do not grow at 45°. Urea (fluid medium) may occasionally show a delayed hydrolysis. Delayed acidification of maltose is rarely observed.

Synonymy: A xylose-negative, biochemically and serologically homogeneous group of strains conforming to the descriptions of A. mucosus in all other respects was clearly described by Schaub and Hauber (1948) and named by them Bacterium anitratum. This organism may be considered as a subspecies (possibly a biotype) of A. mucosus. Our investigations indicate that there is no justification for any

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separation or designation of infraspecific taxa in A. mucosus. Perhaps we may regard the name B. anitratum as a synonym of an unnamed variety of A. mucosus. As shown by other investigators, some strains, such as those designated by Piéchaud et al. (1951) as Moraxella lwoffii var. glucidolytica (see Brisou and Morichau-Beauchant 1952) and by De Bord as Herellea vaginicola (see Ewing 1949), are identical with or closely related to B. anitratum. These names are therefore probably to be regarded as synonyms of A. mucosus. Diplococcus mucosus (von Lingelsheim 1906, 1908), although originally poorly defined, essentially included strains closely resembling B. anitratum (Seeliger 1952, 1953), and the D. mucosus strains studied by Klinge (1959) show the properties of A. mucosus (especially growth at 44° and lack of hemolysis). Since A. mucosus is the only species in the group of microorganisms under consideration whose representatives are regularly isolated in a mucoid form, are regularly pathogenic for mice,¹ and are glucidolytic and nonproteolytic, there is, in our opinion, no doubt that D. mucosus von Lingelsheim is the earliest valid description of A. mucosus. We therefore regard the specific epithet "mucosus" as correct.

2. Achromobacter conjunctivae Mannheim and Stenzel 1962. Type Strain: No. P544/60 (NCTC 10304). Slightly raised colonies, with hemolysis, on blood agar. No growth on Leifson's medium. No growth at 45°. Glucose, galactose, arabinose, xylose and citrate are split quickly. Ammonium salts are utilized. Urea is not split. Gelatin is liquefied within 1 to 3 weeks. Not pathogenic for mice.

Variations: Delayed acidification of lactose and delayed hydrolysis of urea are occasionally observed. As a rule, nonpathogenic for mice.

A. conjunctivae seems to be the specific causative agent of subacute conjunctivitis in man. Therefore its distinctive characters, which differentiate it from the likewise hemolytic A. haemolyticus, are of some importance: A. conjunc-

¹ The arguments of Véron et al. (Ann. Inst. Pasteur 97:497 (1959), to place D. mucosus in the genus Neisseria, are not convincing, since N. mucosa is toxic but not pathogenic for mice (Mannheim, Zbl. Bakt. I Abt., Orig. 183:180 (1961).

tivae is isolated without exception from the human conjunctivae, does not grow in an ammonium-ethanol medium (Mannheim, unpubl.) or on Leifson's agar, and possesses a specific major K-antigen by which it can be identified serologically (Mannheim 1962).

3. Achromobacter haemolyticus Mannheim and Stenzel 1962. Two subspecies are recognized:

a) Type subspecies: A. haemolyticus subsp. haemolyticus nom. nov. (Syn: A. haemolyticus subsp. glucidolytica Mannheim and Stenzel 1962). Type strain: No. 2446/60 (NCTC 10305). Resembles A. conjunctivae in colony form, hemolysis and inability to grow at 45°, but grows on Leifson's agar and in an ammonium-ethanol medium. Glucose, arabinose, xylose and citrate are split promptly, but there is delayed action on galactose, lactose and rhamnose. Gelatin is liquefied. Urea is not split.

Variation: Some strains do not attack rhamnose within 30 days.

b) A. haemolyticus subsp. alcaligenes Mannheim and Stenzel 1962. Type strain: No. 2181/60 (NCTC 10306). Differs from the type subspecies in that carbohydrates and sugar alcohols are not attacked.

A. haemolyticus is usually isolated from the respiratory tract. As a rule it is nonpathogenic for mice. With respect to pathogenicity for man, nothing is known.

Possible synonyms: Achromobacter anitratum var. saproniphilum Billing 1955 and probably also the proteolytic cultures of Moraxella lwoffii, Moraxella glucidolytica Piéchaud et al. (1956) seem to be closely related to or identical with A. haemolyticus.

4. Achromobacter metalcaligenes (Castellani and Chalmers 1919) Bergey et al. 1925. Suggested neotype strain: No. 3865/60 (NCTC 10308). Small, flat colonies, without hemolysis, on blood agar. No growth on Leifson's medium. No growth at 45°. Gelatin is not liquefied within 30 days. Urea is not hydrolyzed. Carbohydrates and sugar alcohols are not acidified within 30 days. The type strain, as strains of A. metalcaligenes occasionally do, shows delayed and scanty growth in an ammonium-citrate medium without essentially changing its pH and attacks ethanol in an ammonium-salts medium with slow acidification. Not pathogenic

for mice. Nothing is known concerning its pathogenicity for man. Very common.

Variations: Some strains are able to attack glucose, galactose, arabinose and xylose after a marked delay (representative strain: No. P 790/60 (NCTC 10309)).

Synonymy: Apparently cultures resembling A. metalcaligenes were described by Audureau (1940) as Moraxella lwoffii and by DeBord (1941) as Mima polymorpha (typical variant). As previously stated (Mannheim and Stenzel 1962), the name Alcaligenes metalcaligenes Castellani and Chalmers has priority, even though there may have been some confusion with true Moraxellae in the original description.

5. Achromobacter citroalcaligenes Mannheim and Stenzel 1962. Type Strain: No. 2723/59 (NCTC 10307). Resembles A. metalcaligenes in its cultural behaviour. Gelatin is not liquefied. Urea is not hydrolyzed. Carbohydrates and sugar alcohols are never split within 30 days, but ammonium citrate is attacked promptly with a strong production of alkalinity. Nonpathogenic for mice. With regard to pathogenicity for man, nothing is known. Isolated from urine and from sputum. Due to its lower sensitivity to antibiotics and to other minor properties, A. citroalcaligenes is distinct from A. metalcaligenes and must therefore be classified as a separate species.

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