THE GENUS VEILLONELLA

I. GENERAL CULTURAL, ECOLOGICAL, AND BIOCHEMICAL CONSIDERATIONS

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Abstract

ROGOSA, M. (National Institutes of Health, Bethesda, Md.). The genus Veillonella. I. General cultural, ecological, and biochemical considerations. J. Bacteriol. 87:162-170. 1964.—Arguments are presented for excluding Veillonella discoloides, V. reniformis, V. orbiculus, and V. vulnovaginitidis from the genus, and for restricting it to aerogenic organisms such as V. parvula and V. alcalescens. The genus Veillonella thus would comprise species which are anaerobic and nonmotile; are small, spherical, gram-negative cocci appearing as pairs, masses, and short chains; and are cytochrome-oxidase- and benzidine-negative. Veillonella would be further characterized in that glucose or any other carbohydrate is not fermented; indole is not produced; gelatin is not liquefied; nitrate is reduced; H₂S is produced; propionic and acetic acids, CO₂, and H₂ are produced from lactate during growth; and pyruvic, oxaloacetic, malic, fumaric, and succinic acids are metabolized by resting cells, but citric, isocitric, and malonic acids are not. In addition to the above, a number of cultural, ecological, and biochemical characteristics are described. At present, V. parvula (the type species) and V. alcalescens would be retained as valid species. Errors in the descriptions of V. parvula and V. alcalescens are corrected by amended statements. These species are differentiated serologically. Also, V. alcalescens differs from V. parvula in having an absolute requirement for putrescine or cadaverine and in decomposing H₂O₂.

The entire comment on the genus Veillonella by Topley and Wilson (Wilson and Miles, 1955) is restricted to the following short paragraph: “We should perhaps refer to a group of anaerobic gram-negative cocci found mainly in the mouth and intestine of man and animals to which the generic name Veillonella was given by Prévot after Veillon and Zuber (1898) who described the first species. The organisms are small spherical cocci about 0.3 microns in diameter, usually arranged in pairs. They grow under anaerobic conditions on the usual media, having limited fermentative powers, and appear to be harmless saprophytes, though they may at times invade the blood stream after operations on the mouth (see McEntegart and Porterfield, 1949). Two species are recognized—Veillonella parvula and Veillonella gazogenes. It is very doubtful whether these organisms, some of which are gram-positive, form a homogeneous group, and we should for the present not regard Veillonella as a valid genus.”

Reviews of the earlier literature are available in papers by Hall and Howitt (1925), Branham (1927, 1928), and Prévot (1933). The scheme of Prévot (1933) was followed by the 5th and 6th editions of Bergey’s Manual (Bergey et al., 1939; Breed, Murray, and Hitchens, 1948), which placed the genus Veillonella in the family Neisseriaceae and named two species, V. parvula and V. gazogenes. Both species were described as aerogenic; the gases produced by V. parvula were mentioned as CO₂, H₂, and H₂S. V. gazogenes was differentiated from V. parvula as follows. “Distinctive characters: Differs from Veillonella parvula in that it does not ferment sugars, does not produce H₂S nor indole, is not hemolytic, does not produce nitrites from nitrates, and does not develop fetid odors.”

Fouquet and Douglas (1948a, b) described an oral organism, otherwise having the characteristics of a Veillonella species (Bergey et al., 1939; Breed et al., 1948), as gram-positive in the early logarithmic growth phase. For this reason, they allocated the organism to the genus Micrococcus and named a new species, M. lactilyticus. Langford, Faber, and Pelczar (1950) contested this conclusion and insisted that the Veillonella organisms isolated from the human mouth were gram-negative diplococci. In a general study of
anerobic cocci, Hare et al. (1952) recognized their group V as members of the genus Veillonella, as described in *Berger's Manual* (Berger et al., 1959; Breed et al., 1948).

The 7th edition of *Berger's Manual* (Breed, Murray, and Smith, 1957) recognizes four new species, and introduces a new heterogeneity by creating two divisions in the genus; three of the species are described as producing gas in culture media, and three species as not producing gas. In addition, four of the species are incompletely described, unavailable for study, or based on the examination of single strains isolated infrequently before modern techniques were available.

The evidence to be presented will show that, in all probability, the descriptions of *V. parvula* and *V. alcalescens* (*V. gazogenes*) in the 5th, 6th, and 7th editions of *Berger's Manual* and that of Prévot (1933) are erroneous, particularly in respect to the pertinent differentiating characteristics, i.e., the fermentation of sugars, indole and H₂S production, and nitrate reduction.

Except for some fragmentary studies of small numbers of strains from human sources (Hall and Howitt, 1925; Branham, 1928), no systematic serological study of the genus has been conducted. A preliminary report on serological and other studies of the genus *Veillonella* was presented by Rogosa, Hampp, and MacKintosh (1961). The present paper and later reports will describe more extensive serological, biochemical, and cultural experiments with significant numbers of strains from man, the rat, the hamster, and the rabbit, in an attempt to contribute toward a better understanding of the genus.

### Materials and Methods

**Source of cultures.** The first experiments were performed with organisms isolated from the human mouth by Langford et al. (1950), and also with the reference cultures listed by them. The cultures were revived from the lyophilized state in V16 or V17 medium (described below), and were replated repeatedly for purity in the V15 medium and under the conditions described by Rogosa (1956) and Rogosa et al. (1958). Later, more isolates from the human mouth and also from bacteremias after certain dental operating procedures (Rogosa et al., 1960) were included. In all, 38 human strains were studied.

Samples from the rat, rabbit, guinea pig, and hamster were obtained by swabbing the oral cavity of 25 animals of each species. The swabs were placed into tubes of fresh V17 broth containing either vancomycin (7.5 μg/ml) or streptomycin (5 μg/ml), then diluted 1:100 and 1:1000, and pour-plated in V15 medium containing either of the above antibiotics. Plates were incubated 3 days at 36°C in an atmosphere of 95% N₂ and 5% CO₂. Isolates from the rat (19), from the hamster (24), from the guinea pig (11), and from the rabbit (15) remained viable throughout this study and comprised only 1 isolate per animal.

**Maintenance of cultures.** All cultures were lyophilized from skim-milk suspensions, and remained viable at 5°C for at least 5 years. They were also grown in medium V22A consisting of Tryptoase (BBL), 1%; yeast extract (BBL), 0.5%; 85% lactic acid, 1%; sodium thioglycolate, 0.075%; Tween 80, 0.01%; agar, 0.1%; adjusted with solid K₂CO₃ to pH 6.6. This medium was dispensed in 14-ml amounts into screw-capped tubes (15 by 125 mm) and autoclaved at a pressure of 15 psi for 15 min. Each tube was inoculated with ca. 2.5 ml of culture, incubated at 36°C for 24 to 48 hr, and then stored at 5°C. Transfers of stock cultures were made monthly.

**Media.** All media were routinely boiled and cooled to an appropriate temperature immediately before use. The V15 medium was the solid agar medium used as a selective medium for direct plating of specimens by Rogosa (1956) and Rogosa et al. (1958). The V16 medium was a modification of V15 medium only in the reduction of the agar content to 0.1%. The V17 medium was simply a broth medium with agar and basic fuchsin omitted, but otherwise having the same composition as the V15 medium. The V23 medium consisted of Tryptoase, 1%; yeast extract, 0.5%; sodium thioglycolate, 0.075%; Tween 80, 0.1%; 85% lactic acid, 1%; adjusted with solid K₂CO₃ while stirring, to pH 6.5 to 6.6.

**Carbohydrate fermentation tests.** Fermentation of carbohydrates was tested in V17 medium modified by the omission of sodium lactate. Arabinose, galactose, fructose, maltose, mannose, rhamnose, sorbose, and xylose were sterilized by filtration and added aseptically. Inulin was always autoclaved in the medium. Adonitol, amygdalin, cellobiose, dulcitol, glucose, glycerol, inositol, lactose, mannitol, melibiose, melezitose, α-methyl-d-glucoside, α-methyl-d-mannoside, raffinose, salicin, sorbitol, sucrose, and trehalose...
were sterilized by filtration. Separate portions were also sterilized by autoclaving. The final concentrations of carbohydrates were 0.5 to 2% in different experiments. Tubed media were arranged in racks allowing direct contact of steam with each tube, and were autoclaved by quickly raising the temperature to 121 C and by immediately beginning the exhaust cycle. Cells from 24- to 48-hr V17 broth cultures were washed twice in freshly sterilized distilled water containing either 0.01% NaS·9H2O or 0.0125% sodium thioglycolate, and diluted to the original volume; 1-drop inocula were then made. Inoculated tubes of basal medium without added carbohydrate were always included. Anaerobiosis was effected within 10 min by repeated evacuation and flushing with 95% N2 plus 5% CO2 (at least four times) of McIntosh and Fildes aluminum jars. Slight positive pressure (100 mm of Hg) was maintained, and no results were accepted as valid unless positive pressure was still present after the usual 2-week incubation at 36 C. The pH of all tube contents was determined electrometrically.

**Biochemical tests.** Glucose was estimated by means of a coupled enzyme system involving glucose oxidase and peroxidase (Keilin and Hartree, 1948; Keston, 1956; Teller, 1956). The "glucostat" reagents supplied by the Worthington Biochemical Corp. (Freehold, N.J.) were used, and the determinations were accurate to ±3%.

Tests for indole were performed in V17 medium by the Kovaes method described in the *Manual of Microbiological Methods* (Society of American Bacteriologists, 1957), and were repeated many times in 107 cultures during growth periods of 6 hr to 2 weeks. Positive reactions were detected by the immediate red-color development when drops of reagent were introduced at the surface of cultures. Delayed reactions in which brown or greenish-brown colors occur are not caused by the presence of indole but are the result of nonspecific reactions and decomposition of the reagent (Society of American Bacteriologists, 1957).

"Catalase" tests were done repeatedly on washed cells from V17 broth, from growth on anaerobic streak plates, and from colonies in pour-plates; 5% H2O2 freshly diluted from refrigerated 30% H2O2 was used, and special care was taken to avoid mixing peroxide and cells with any metal objects. These tests were done immediately after removing cultures or cells from an anaerobic environment, and also after at least 1-hr exposure in the air.

The benzidine test, using both the benzidine base and the dihydrochloride for the presumptive detection of iron porphyrin compounds, was generally done as described by Deibel and Evans (1960) on plate cultures and cell suspensions from broth cultures. Since the benzidine base is ten times more sensitive than the hydrochloride salt, cell suspensions were washed three times in distilled water to remove interfering components (e.g., excess Fe and Cu), and were resuspended in 0.5 ml of the usual 5% H2O2. H2S production was detected by the blackening of cultures grown in V17 and V23 broth media supplemented with 0.5 g per liter of ferric ammonium citrate as an internal indicator. This compound was nontoxic. Tests were performed under a variety of conditions and medium supplementations with 26 sulfur-containing compounds.

Nitrate reduction was determined in V17 broth containing 0.1% KNO3 and adjusted to pH 7.5. Cultures were grown in this medium in the presence and absence of thioglycolate, and nitrite was detected with the reagents and the spot-plate technique described by Rogosa (1961).

Tests for oxidase enzyme were conducted by flooding the growth on V15 agar plates with 1 ml of a 1% solution of dimethyl-p-phenylenediamine hydrochloride or the oxalate salt, and were observed for a period of 10 min for the usual color changes from pink to black.

The ability of resting cells to metabolize a number of compounds with the production of CO2, H2, or both, was tested by Warburg manometry at 37 C in an atmosphere of N2 or 95% N2 plus 5% CO2. Cultures were grown 1 day or less in V23 lactate broth. The cells were washed once in distilled water, and resuspended in distilled water to contain ca. 20 mg (dry weight) per ml. The cells were kept cold during centrifugation and washing procedures, gassed with the above mixture in all resuspending operations, quickly introduced into the flasks already containing all other components, and gassed immediately while shaking for 10 min. The main compartment of each flask contained 1.0 ml of 0.05 M potassium phosphate buffer (pH 6.5), 0.3 ml of water, and 1.0 ml of the cell suspension. From a side arm was tipped 0.2 ml (20 μM) of the Na or K salts of each of the following acids: pyruvic, oxaloacetic, l(-)-malic, fumaric,
succinic, isocitric, citric, malonic, L(+)-tartaric, and formic. Lactic acid was used as the Li, Na, or K salt.

Propionic and acetic acids were detected by Celite chromatography essentially as described by Swim and Utter (1957). Also, ether extracts of the fatty acids were analyzed by means of a Beckman GC-2A gas chromatograph and recorder. The copper column was packed with 15% polydiethylene glycol adipate (Hunter, Ortegren, and Pence, 1960) on Chromosorb W (60 to 80 mesh; Johns-Manville Co., New York, N.Y.). Helium at 30 psi was the carrier gas, and the temperature was 130 C. CO₂ and H₂ were determined by differential manometry, in which 0.1 ml of 50% KOH was injected at the end of the growth period through a rubber cap sealed on one of the side bulbs. The flasks were then shaken until equilibrated.

**Results**

**Occurrence and frequency distribution.** In hundreds of human salivary samplings, and in equally large numbers of oral specimens from the rat, the hamster, the rabbit, and the guinea pig, there has never been an instance in which Veillonella organisms were not recoverable in the selective media of Rogosa (1956) and Rogosa et al. (1958). However, only one strain from 1 of 78 mice was isolated, despite the use of enrichment cultures of combined fecal and oral material in V16 medium with and without vancomycin, streptomycin, azide, or cyanide. In the present study, confirming Douglas’ (1950) observation, the Veillonella organisms were present in human saliva in nearly the same order of magnitude as the streptococci, the single largest group of organisms. In the rat, also, the veillonelae constituted 9% of the measurable total organisms and were half a numerous as the streptococci.

**Morphology.** The organisms were small spherical cocci, generally 0.3 to 0.4 μ in diameter, but occasionally smaller. Although Langford et al. (1950) contended that the basic cellular arrangement was the diplococcus, in wet mounts or gram-stained preparations from liquid media, the cells occurred as diplococci, irregular masses, short chains, or combinations of these forms. This agrees with descriptions of Prévot (1933). Some strains occasionally did not counter-stain well, and were barely visible at a magnification of 1,350 ×. Cells from fluid media were gram-negative in thin smears by a variety of gram-stain modifications. Motility was never detected.

Colonies in uncrowded poured-agar plates were generally 1 to 3 mm in their greatest dimension, and were smooth; entire; lens-, diamond-, or heart-shaped; opaque; grayish white; and butyrous or soft to the touch.

**Temperature relations.** Growth was good in the range of 30 to 37 C. At 40 C, all 107 strains grew through one transfer, but 42% of them failed to grow subsequently. There was no growth of any strain in the second serial passage at 45 C, and only infrequently (16%) did growth occur in the first transfer. At 24 C, however, all cultures grew well through serial transfers, although sometimes marked turbidity or other evidence of growth developed only after 7 days of incubation. There was no growth at 18 C, and no organism survived heating at 60 C for 30 min.

**General cultural and physiological characteristics.** Continued aerobic growth occurred only occasionally when such massive inocula as 5 to 10% of the total culture were employed. In media containing reducing agents such as ascorbic acid (0.05%), thioglycolate (0.1%), or cyanide (0.01%), growth often occurred in freshly made or boiled medium without further anaerobic precautions, particularly if the surface area-volume ratio was small, and if the medium was dispensed in air-tight containers. There was no growth on the surface of Trypticase Soy Agar (BBL) slants inoculated with either a loop or a drop of broth culture and incubated in air.

All strains were cytochrome oxidase- and benzidine-negative. Indole was not produced, and gelatin was not liquefied. Nitrate was reduced to nitrite or further-reduced products. H₂S was produced in V17, V23, or in a defined medium to be described elsewhere, from reduced glutathione (1.65 × 10⁻⁵ M), cysteine (8.25 × 10⁻⁴ M), or both, and from KSCN (1.65 × 10⁻³ M).

Carbohydrates were not fermented by any of the 107 strains of Veillonella. Litmus and a number of sulfonphthalein indicators, particularly phenol red, bromothymol blue, bromoresol purple, and chlorophenol red, were reduced to their leuco state. Therefore, electrometric pH determinations were made, with results from inoculated tubes without added carbohydrate always included. Such media initially adjusted to pH 7.5 and incubated anaerobically in 95% N₂ plus 5% CO₂ or 95% H₂ plus 5% CO₂ gen-

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eraly equilibrated at pH 7.2. If inoculated media without added carbohydrates contained sufficient lactate, hypoxanthine, and other fermentable substrates derived from enzymatic digests of casein and yeast extract, the pH value fell to 6.8 as the medium became saturated with the CO₂ produced. It is only at initial acidic pH values, as demonstrated by Douglas (1950), that a rise in pH takes place.

Because V. parvula is described as fermenting glucose by Prévot (1933) and in the 5th, 6th, and 7th editions of Bergey's Manual, 107 strains of Veillonella, including a strain of V. parvula strain Te3 originally from Prévot, were tested for this property by means of the glucose oxidase technique described earlier. Glucose was not utilized by any of the strains. The mean residual glucose concentration after 96 hr at 36 C was 99.2 ± 3% of the initial 0.0096 M concentration.

The effect of CO₂ in the initiation of growth of 50 representative strains was tested by inoculating a drop of culture from V23 broth into two tubes of the same medium. To one duplicate set of plugs were added 3 drops of 50% pyrogallic acid and 3 drops of 10% KOH, and to the other set were added 3 drops of pyrogallic acid and 3 drops of freshly prepared 10% K₂CO₃. Rubber stoppers were quickly inserted. Only six strains were not limited in growth in a CO₂-free atmosphere after 16 hr of incubation. In the absence of CO₂, 83% of the cultures had a mean optical density (OD) of 0.08. This contrasts with the cultures grown in CO₂, 77% of which had an OD > 1.0 after 1 day, and none of which had an OD < 0.82. Although most strains eventually grew to some extent in the absence of CO₂, 20% of the cultures did not grow, or grew very sparsely (OD < 0.10), even after long incubation.

After 16 hr at 36 C in an atmosphere of 95% N₂ plus 5% CO₂, growth was absent at pH values < 5.5, barely observable at pH 6.0, good from pH > 6.0 to 6.5, maximal from pH 7.0 to 8.5, and suddenly ceased between pH 8.5 and 9.0. The initial pH values supporting growth in similar media were generally higher than Douglas (1950) reported for one strain (>5.5 as compared with 4.8). When the fermentation of lactate took place in an initially acidic environment (pH 6.0), the pH value increased to 7.5. At slightly alkaline initial pH values, the resultant pH always decreased (from pH 8.0 to pH 6.9 to 7.6).

The Veillonella strains were markedly resistant to Tween 80. With 10% Tween 80, the medium tended to separate into distinct layers, indicating obvious physical changes; yet, most strains were able to grow. Further experiments with six detergents were performed with V23 medium without Tween 80. Heavy precipitates and cloudiness were characteristic of media containing the higher concentrations of Triton Gr-5 (Rohm & Haas, Inc., Philadelphia, Pa.), Glim (B. T. Babbitt, Inc., New York, N.Y.), or sodium dodecylsulfate. Nevertheless, growth was evident in such nonionic substances as Triton X-100 and Glim at the surprisingly low dilution of 1:250; in trithiosulfonate at 1:1,000; and even in the more highly ionized Aerosol OT (Fisher Scientific Co., Pittsburgh, Pa.) and sodium dodecylsulfate, where the antibacterial effect may be due only partially to decreased surface tension, at dilutions of 1:26,666 or less.

The Veillonella were inhibited by 1% NaCl in V23 medium, and failed to grow in 4% NaCl media. The resistance of these organisms to bile was often not as great as was anticipated, even in the case of those strains isolated from the intestinal tract. Although all strains grew in 5% bile, growth was often markedly delayed. Of 18 representative strains tested, 7 did not grow in 10% bile media, 11 in 20%, and 16 in 30%; only 3 strains grew weakly in 40% bile after extended incubation. Also, no growth occurred in the presence of potassium tellurite (10 mg per liter), 2,3,5-triphenyl tetrazolium chloride was not reduced, and certain dyes (0.0002%) were variably inhibiting. Crystal violet in V23 medium prevented the growth of 9 of the 18 strains after 1 day of incubation; after 96 hr, 1 strain still did not grow. Although growth generally occurred with relatively long incubation, at 96 hr cell crops were usually only equal to, or less than, control growth after 24 hr or less. Brilliant green, although in most cases considerably less inhibiting than crystal violet, prevented the growth of 3 of the 18 strains in Sims and Snyder's (1958) selective medium, and 8 of 18 in V23 medium after 24 hr at 36 C. Because the dye was reduced to the leuco compound in V23 medium, it appears that the leuco compound was more toxic than its colored counterpart. However, when growth without lag occurred in brilliant green media, it was as good as in control media without dye. But even with extended incubation, 3 of 18 and 2 of 18 strains failed to grow in Sims and Snyder's and V23 medium, respectively.

Biochemical characteristics. In the present work,
the only significant fermentation products detectable in the fermentation of lactate by growing cultures were propionic acid, acetic acid, CO₃⁻, and H₂. This agrees with previous observations by Johns (1951b) and Foubert and Douglas (1948b).

We wished to determine whether resting cells of our strains would utilize pyruvate, oxaloacetate, malate, fumarate, and succinate, as reported by Johns (1951a, b) for a single strain isolated from the rumen of the sheep and designated by him as V. gazogenes (V. alcalescens). It was indeed found that all strains, regardless of animal source, serological group, nutritional behavior, and lack or possession of a peroxide-decomposing enzyme, would metabolize these compounds in an atmosphere of N₂. Compounds not attacked were L(+)−tartaric acid (by unadapted cells) and formic acid, as Johns (1951b) showed. In addition, citric, isocitric, and malonic acids were not attacked. Although succinic acid was dissimilated by resting cells with the production of propionic acid and CO₃⁻, all strains were incapable of growth in succinate media. This phenomenon will be discussed more fully elsewhere.

Lactic acid was fermented poorly or not at all by resting cells, and Johns' (1951b) finding that bicarbonate and CO₂ stimulated the attack on lactate was confirmed with all our strains. However, in Johns' (1951b) experiments, and also in ours, the rate and extent of lactate fermenta-

![FIG. 1. Aerobic respiration of Veillonella on lactate typified by the data obtained with V. alcalescens BL-78.](http://jb.asm.org/)

![FIG. 2. Typical aerobic dissimilation of oxaloacetate by Veillonella sp. Z24B.](http://jb.asm.org/)
obtained. We have seen that pure cultures of Veillonella do not utilize glucose or any other carbohydrates. However, in some fresh isolates of apparently pure cultures of Veillonella, glucose rapidly disappeared; but, on careful examination, it was discovered that these were always contaminated with glucose-utilizing organisms such as streptococci, diphtheroids, Bacteroides, and Proteus. After purification of the original isolation, however, carbohydrates were not fermented.

Berger (1960) was not sure of the significance of weak, delayed, benzidine reactions. Such delayed results are highly doubtful because the peroxidase-H$_2$O$_2$ complex has an extremely rapid reaction rate (Chance, 1949a, b), and Deibel and Evans (1960) showed that the benzidine base, as contrasted with the chloride salt, gave rise to false-positive weak reactions. The Veillonella species gave us a benzidine-negative reaction. Berger (1961) described the Veillonella genus as the “anaerobic, oxidase-negative species.” We agree with this description, and this plus the negative benzidine reaction serve, among other things, to differentiate the genus Veillonella from the genus Neisseria.

Deibel and Evans (1960) showed that an organism may decompose peroxide to water and O$_2$ (a catalase?), and may not have detectable iron porphyrin compounds; Delwiche and Johnston (1962) confirmed the existence of a nonheme, peroxide-decomposing enzyme. V. alcalescens and certain animal strains appear to be in this category.

Of the species described in the 7th edition of Bergey’s Manual, V. disoideus, V. reinfornis, and V. orbiculcus have disc- or kidney-shaped cells two to five times greater in diameter than the spherical cells of V. alcalescens and V. parvula, the type species. The metabolism of V. disoideus, V. reinfornis, and V. orbiculcus seems distinctively different from that of the serogenic organisms, V. parvula and V. alcalescens, because the other three species are described as not producing gas. Another organism, V. vulnovaginitidis (Breed et al., 1957), although described as having spherical cells three times larger than those of the type species, liquefies gelatin, and does not produce gas. V. vulnovaginitidis was originally designated as N. vulnovaginitidis by Reynes (1947) on the basis of a single reported isolation. The observation by Langford et al. (1950) that this single strain is a long-chained, gram-negative streptococcus, occurring often in chains of ten or more cells, was confirmed by us from studies of the only available strain (247-D) received from Prévot. A rereading of Reynes’ (1947) short note leaves an impression consistent with the judgment that this organism is a streptococcus. Thus, there are basic differences in morphology and metabolism between these four organisms and V. parvula and V. alcalescens. It is therefore suggested that V. disoideus, V. reinfornis, V. orbiculcus, and V. vulnovaginitidis be excluded from the genus Veillonella.

The Veillonella strains responded to polymyxin B, an agent considered to be specific against gram-negative species, as would be expected of typical gram-negative organisms; in this respect as well as in the responses to vancomycin and novobiocin, the organisms resembled the seven nonpathogenic strains of Neisseria tested (Fitzgerald, Parramore, and Mackintosh, 1959). Colonies were not formed in media containing 0.25% phenethyl alcohol; this is also generally characteristic of gram-negative bacteria (Lilly and Brewer, 1953; Berrah and Konetzka, 1962). In addition, lipo-polysaccharide fractions with properties characteristic of endotoxins from gram-negative bacteria were extracted from Veillonella cultures (Mergenhagen, Hampp, and Scherp, 1961). This, and direct results from gram-stained smears, reinforce the view that the genus Veillonella is gram-negative. M. lactilyticus strains T9 and T13 (Foubert and Douglas, 1948a, b) have behaved like Veillonella organisms in these and other respects, and it appears, therefore, that the name M. lactilyticus is not justified.

The results presented here are not in complete agreement with Prévot (1933) or with Bergey’s Manual, because V. parvula is described as fermenting glucose and certain other sugars and as producing indole, whereas V. alcalescens is described as not producing H$_2$S and as not reducing nitrate. On the other hand, the present results generally agree with those of Langford et al. (1950), Foubert and Douglas (1948a, b), Hare et al. (1952), Berger (1960), and Sims (1960). In addition, the present negative sugar reactions agree with those obtained by Hall and Howitt (1925) and the description for M. gasogenes alcalescens anaerobius by Lewkowicz (1901). Prévot (1933) and Bergey’s Manual state that V. parvula is identical with Staphylococcus parvulus (Veillon and Zuber, 1898). The evidence for this is a statement by Prévot (1933) that one
strain was authenticated by Veillon. However, it appears that Veillon and Zuber's (1895) strains were not extant, and the only differentiating information then available was that the original organism was a small gram-negative coccus, appearing as masses and diplococci, and producing gas. These limited criteria apply to either V. parvula (S. parvula; Veillon and Zuber, 1895), or V. alcalescens (M. gazogenes alcalescens anaerobius; Lewkowicz, 1901).

From the foregoing evidence, it is not possible to differentiate between V. parvula and V. alcalescens. Furthermore, since the described negative sugar reactions of V. alcalescens (Prévot, 1933; Bergey et al., 1939; Breed et al., 1948, 1957) may be considered correct, investigators generally designated any Veillonella isolate as V. alcalescens because their organisms possessed at least the important property of not fermenting carbohydrates, even if their strains did not otherwise conform to previously published descriptions. This situation seems discouraging, but, if we accept the present behavior of Prévot's original strains of V. parvula Te3, V. alcalescens 259, and many others identical with them, the description of the genus should be presently amended to include only the two species, V. parvula and V. alcalescens, as was done in the 6th edition of Bergey's Manual. In addition, the species descriptions (Breed et al., 1957) should be amended in the following specific respects: V. parvula, (i) carbohydrates are not fermented, (ii) indole is not produced; V. alcalescens, (i) H₂S is produced, (ii) nitrate is reduced.

Such amended descriptions of the genus and of the species would still not permit the differentiation of V. parvula and V. alcalescens. However, unpublished but completed experiments, too extensive to report here, indicate that the species of human origin are distinguishable in the following respects: (i) each species has distinctive noncrossing agglutinogens; (ii) V. alcalescens possesses a peroxide-decomposing enzyme not present in V. parvula; and (iii) V. alcalescens has an absolute growth requirement for putrescine or cadaverine in a nutritionally defined medium.

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