THE PROPOSED CONSERVATION OF THE GENERIC
NAME VIBRIO PACINI 1854 AND DESIGNATION OF THE
NEOTYPE STRAIN OF VIBRIO CHOLERAE PACINI 1854

Request for an Opinion

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SUMMARY. Vibrio Müller has not been used in the literature of protozoology for over 100 years. Article 23 of the International Zoological Code of Nomenclature says "a name that has remained unused as a senior synonym in the primary zoological literature for more than 50 years is to be considered a forgotten name (nomen oblitum).". For a little more than 100 years Vibrio has become established as a commonly accepted generic name in bacteriology. It is recommended from the standpoint of bacteriology that Vibrio Müller 1773 be regarded as the name of a genus in zoology and in conformity with the International Code of Zoological Nomenclature be recognized as a nomen oblitum and without standing in bacteriological nomenclature. It is further recommended that Vibrio Pacini 1854 be placed in the list of conserved generic names (nomina generum conservanda) with the type species Vibrio cholerae Pacini 1854 and that the specific epithet cholerae in the binary combination Vibrio cholerae Pacini 1854 also be conserved. It is also proposed that the National Collection of Type Cultures (NCTC) strain 8021 (American Type Culture Collection (ATCC) strain 14035) be recognized as the neotype strain of Vibrio cholerae Pacini 1854. The morphological and physiological characteristics, including a photomicrograph

of a stained preparation showing monotrichous cells each with a polar flagellum, are presented of the proposed neotype strain.

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Müller (1773, 1786) used binomina in the nomenclature and classification of microscopic organisms following the publication by Linnaeus of the 1758 edition of Systema Naturae. Many of Müller's genera and species are recognized and in use today. Some of his organisms are known to be protozoa, some are now placed with nematodes, some of his genera and species are recognized as algae. The descriptions and illustrations of his species make it highly probable that some were bacteria. Müller's (1773, 39) genus Vibrio is of particular importance from the standpoint of bacteriology. In this genus, placed in his Infusoria crassiuscula¹ (Müller 1773, 23), he included 15 species with names and descriptions. Some of the species named by Müller (1773, 1786) were later placed in other genera, first of protozoa, then of bacteria. For example Vibrio rugula, V. serpens, V. undula, and V. spirillum were placed by Ehrenberg (1830, 38) and Winter (1884, 63) in the genus Spirillum.

Leidy (1849) examined the intestinal contents of the millipede (Julus marginatus), the toad (Bufo americanus), and the contents of the caecum of the rat, and appears to be the first to conclude that Vibrio lineola Müller 1773, 39 and Spirillum undula (Müller) Ehrenberg 1830, 38, found therein, are plants rather than animals. Buchanan (1925, 522) proposed that the species whose name and description came first in Müller's (1773, 39) listing, Vibrio lineola, be designated as the type species. Although described as an infusorian, Müller's description makes probable that this organism was a schizomycete.

The generic name Vibrio Müller 1773 has been arbitrarily used in several senses by different writers; Vuillemin (1913) reviewed the problem and suggested that the generic name should be suppressed to avoid further confusion. If Vibrio is regarded as a genus of protozoa, the fact that the

¹ Crassiuscula is the diminutive of the comparative of the Latin adjective crassus (thick, dense, not flowing, or amoeboid) meaning "infusoria that are somewhat more thick or solid."

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type species cannot be identified, and the further fact that present-day protozoologists do not accept or use the name Vibrio Müller 1773 would mean that the acceptance of the name in zoology is subject to the rules of the International Code of Zoological Nomenclature. Article 23 of the zoological code states that "a name that has remained unused as a senior synonym in the primary zoological literature for more than 50 years is to be considered a forgotten name (nomen oblitum).

A survey of "primary zoological literature" based upon usage during the past 50 years (1914-1964) has revealed no use of Vibrio as a generic name in zoology. While it was used as a generic name somewhat more than 100 years ago in naming certain organisms as protozoa now placed with the bacteria (Naegali 1857, Cohn 1872), it seems clear that as a generic name it has not been recognized in zoology for more than 100 years. In accordance with Article 23 of the zoological code there is no reason to regard the generic name Vibrio Müller 1773 as a senior synonym in primary zoological literature and there should be no obstacle under the bacteriological code to its use as a generic name in bacteriology. A recommendation made in conformity with Article 23 of the International Code of Zoological Nomenclature will be formulated and published.

The question to be considered next is the validity and legitimacy of the recognition of Vibrio as a generic name in bacteriology with the goal of fixing its status.

Ehrenberg (1838) used the infusorian generic name Vibrio for organisms which were later recognized as Schizomycetes. Vibrio synxanthus Ehrenberg (1840, 202) and Vibrio syncyanus Ehrenberg (1840, 202) have been transferred from this protozoon genus to the bacterial genus Pseudomonas Migula 1894 (conserved 1952, Opinion 5). Fuchs (1841, 190) named and described Vibrio cyanogenus and V. xanthogenus; according to Trevisan (1885, 104) and Schroeter (1886, 157) these names are junior synonyms of the organisms currently classified as Pseudomonas syncyanea (Ehrenberg) Migula 1895, 29 and P. synxantha (Ehrenberg) Breed 1948, 700 respectively and hence illegitimate. Most of the species of bacteria included in the genus Vibrio by writers before 1854 are now definitely placed in other genera or cannot be identified.

Pacini (1854) appears to be the first to describe an organ-

ism which is recognized today as a species of bacteria currently classified in the genus Vibrio. The etiologic agent of Asiatic cholera was first described and named vibrio cholera (sic) by Pacini 1854, 411. He was the first to use the generic name Vibrio with a legitimate specific epithet, cholerae, for the bacterium which is the causal agent of Asiatic cholera. Filippo Pacini, for many years Professor of Pathology at the Royal Institute of Superior Studies in Florence, studied Asiatic cholera after its 1854 appearance in Tuscany and remained interested in this disease for the remainder of his life. Bianchi (1885) published, after the death of Pacini, the unedited laboratory notes and illustrations which Pacini made while examining cadavers of cholera victims in Florence. Pacini saw the curved etiologic agent of Asiatic cholera in tremendous numbers in denuded intestinal epithelium and intestinal contents of victims. The pathological changes in the intestine and the organism itself were described in such a manner that they may be recognized. Pacini's contemporary Count Vittore Trevisan, a systematic bacteriologist, (1884, 373; 1884, 427) had no doubts that Pacini had described and named the etiologic agent of Asiatic cholera. The generic name Vibrio should therefore be ascribed to Pacini 1854 and should be conserved against Vibrio Müller 1773 and all other zoological names for the taxon to avoid further changes in the nomenclature of the genus. Vibrio cholerae Pacini 1854, the type species by monotypy, should also be conserved.

Koch (1884) was the first to isolate the etiologic agent of Asiatic cholera. He gave a more detailed description of the organism and used the vernacular name Kommabacillus instead of the earlier scientific name Vibrio cholerae Pacini 1854. Koch went to Egypt without previous knowledge of cholera or of its infectious nature. He did not know that Pacini had described and named Vibrio cholerae 30 years earlier. Trevisan was convinced of the identity of Vibrio cholerae Pacini and Koch's Kommabacillus which was recovered from Egyptians with Asiatic cholera. Trevisan was aware that Pacini (1854) had placed a bacterium in the zoological genus Vibrio Müller 1773 and he transferred Vibrio cholerae Pacini to the bacterial genus Bacillus as B. cholerae (Pacini) Trevisan 1884, 374. The generic name Pacinia was proposed by Trevisan (1885, 83) with the Asiatic cholera organism as the first species named.

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Zopf transferred Bacillus cholerae-asiaticae Trevisan 1884 to the genus Spirillum as S. cholerae-asiaticae (Trevisan) Zopf 1885, 69. Schroeter proposed the generic name Microspira and renamed the etiologic agent of Asiatic cholera, Microspira comma Schroeter 1886, 168. Blanchard (1906, 1) appears to be the first to use the specific epithet comma with the genus Vibrio.

Several authors (Blanchard 1906, 1; Castellani and Chalmers 1919, 962; Krasil'nikov 1949, 530) described the etiologic agent of Asiatic cholera as the first species in the genus Vibrio; others actually designated this schizomycete as the type species of the genus Vibrio (Buchanan 1918, 178; Bergey et al. 1923, 77; Enderlein 1925, 255; Pribram 1933, 49; Wilson and Miles 1946, 514; Hauduroy 1953, 648; Wilson and Miles 1955, 604; Breed et al. 1957, 229; Sneath and Cowan 1958, 554; Haupt 1964, 48).

The following names (synonyms) have been used for:

Vibrio cholerae Pacini 1854, 411.

Bacillus cholerae (Pacini) Trevisan 1884, 374.

Bacillus cholerae-asiaticae Trevisan 1884; according to Trevisan 1885, 84.

Bacillus virgula (sic) Trevisan 1884, 376.

Pacinia cholerae-asiaticae (Trevisan) Trevisan 1885, 84.

Spirillum cholerae-asiaticae (Trevisan) Zopf 1885, 69.

Microspora comma Schroeter 1886, 168.

Spirillum cholerae (Pacini) Macé 1889, 606.

Vibrio cholerae-asiaticae (Trevisan) Pfeiffer 1896, 527.

Liquidovibrio cholerae (Pacini) Orla-Jensen 1909, 333.

Vibrio comma (Schroeter) Blanchard 1906, 1.

Bacillus comma (Schroeter) Holland 1920, 218.

The National Collection of Type Cultures strain 8021 (ATCC 14035, RH 1094) is herein proposed as the neotype strain of Vibrio cholerae Pacini 1854 (vide Sneath and Cowan 1958; Hugh and Ryschenkow 1961). This strain conforms to the description of the etiologic agent of Asiatic cholera as given by Pacini (1854) and Koch (1884).

MATERIALS AND METHODS

The proposed neotype strain was deposited in the NCTC by the Standards Laboratory, Central Public Health Labora-

tory, Colindale, London, N.W.9, in September 1949 as Vibrio choleraeasiaticae, Gardner and Venkatraman O group I, Ogawa O form (Kauffmann 1950). A descendant of this strain was deposited in ATCC in 1960. The date and source of isolation of this strain was not obtainable from the NCTC. Leifson's (1951, 1960) technique was employed to stain flagella.

The effects of the organism on sugars and alcohols was determined in purple broth base (Difco 0227) containing 1% (weight per volume, w/v) of each of the 22 carbon compounds. Aesculin was added to purple broth base in a 0.5% (w/v) concentration. Modified Kovacs' reagent (Gadebusch and Gabriel 1956) was used to detect indole in 48-hour 1% (w/v) tryptone broth culture. Buffered peptone glucose broth in 3 ml and 1 ml quantities was inoculated for the methyl red (MR) and Voges-Proskauer (VP) tests respectively. The MR broth was incubated for 5 days, and the VP broth for 2 days before the tests were performed as described by Ewing (1960). Citrate utilization was determined in Simmons' (1926) and Christensen's (1949) medium and final observations were recorded after 2 days incubation. Hydrogen peroxide (3%, w/v) was dropped on 24-hour nutrient agar slant growth to detect catalase production. The indophenol oxidase test, as described by Ewing and Johnson (1960), was performed on discrete 24-hour colonies on infusion agar. Kohn's (1953) charcoal gelatin was used to detect gelatinolytic activity. Kligler's iron agar was used to detect hydrogen sulfide production, and final observations were made after 2 days of incubation. The ninhydrin test was performed according to the method of Carlquist (1956). Malonate utilization was determined by the use of Leifson's (1933) malonate broth modified by the addition of 0.1% (w/v) yeast extract as described by Ewing, Davis and Reavis (1957). The final observation was made after 7 days incubation.

Nitrate reduction was determined in broth at pH 7.1 containing 1% (w/v) caseitone, 0.3% (w/v) yeast extract, and 0.2% (w/v) potassium nitrate. The broth was dispensed in 4 ml quantities in 13 x 100 mm test tubes containing Durham vials for the collection of gas and autoclaved at 121°C for 15 min. Cultures were examined for nitrate reduction to nitrite after 2 days of incubation by adding the reagents described by Wallace and Neave (1927) and Tittsler (1930). The phenylalanine deaminase test was performed after 24 hours of

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incubation as described by Ewing, Davis and Reavis (1957). Ability to grow in the presence of potassium cyanide was determined by using Møller's (1954) medium and final observations were made after 2 days of incubation. Christensen's (1946) agar was used to detect urea hydrolysis and final observations were recorded after 2 days incubation. Falkow's (1958) decarboxylase medium containing lysine, arginine and ornithine was used. A control and the 3 amino acid-fortified media were inoculated and layered with sterile, melted petrolatum to a depth of 10-20 mm. Final observations were made after 7 days of incubation. Hemolysis of washed sheep erythrocytes was estimated by the test tube technique using a 24-hour culture grown in physiological pancreatic digest of casein. The results were read after incubation at 37°C for two hours followed by 18 hours of refrigeration at 6°C.

All media were incubated at 37°C. The criteria for interpretation of positive and negative tests were described in the reference cited and amplified by Ewing (1960) and Edwards and Ewing (1962).

RESULTS

Morphology: Gram-negative, asporogenous, rod-shaped; cells straight or slightly curved with soma size 0.3 to 0.6 x 1 to 5 μ . Motile with predominantly one polar flagellum having a mean wavelength of 2.4 μ (see Figs. 1, 2). Cells with two flagella at one pole or one flagellum at both poles are rarely encountered. Formalin treatment does not significantly change the flagellar shape. Capsules are not evident on the media employed in this study.

Physiology: This organism grows aerobically in peptone broth free of fermentable carbohydrate and facultatively in peptone containing dextrose. It fails to grow on desoxycholate agar.

Dextrose is fermented without gas production. Acid is produced from dextrose under anaerobic and aerobic conditions. Acid is produced from fructose, galactose, lactose, maltose, mannitol (without gas), mannose, sucrose, and trehalose. A transient weak acidity is produced in sorbitol broth. The lactose and trehalose reactions are delayed from 2 to 4 days. Indole, citrate, catalase, indophenol oxidase, gelatin, lysine decarboxylase (ninhydrin), nitrate reduction

Figures 1 and 2. Vibrio cholerae (NCTC 8021) showing the polar monotrichous flagellum characteristic of the organism in broth culture with formalin fixation. Leifson flagella strain, X 2,000.

to nitrite, lysine and ornithine (Falkow) tests positive. The methyl red indicator in the MR test becomes orange rather than red or yellow. The VP test is weakly positive.

Acid is not produced from adonitol, aesculin, arabinose, cellobiose, dulcitol, inositol, inulin, melezitose, melibiose, raffinose, rhamnose, salicin, and xylose. Hydrogen sulfide, malonate, nitrate reduction to nitrogen gas, phenylalanine deaminase, urea, arginine tests are negative. The organism does not grow in potassium cyanide broth. Sheep erythrocytes are not hemolysed and the cells are agglutinated by O-group I antibodies.

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DISCUSSION

Bacteriophage studies (Mukerjee 1961, 1963) have indicated that the etiologic agent of classical Asiatic cholera is generally susceptible to a specific bacteriophage (Group IV) whereas El Tor vibrios are lyso-resistant to this same bacteriophage. Stable nonhemolytic Vibrio cholerae strains which are insensitive to Group IV bacteriophage were isolated during the 1962 New Guinea epidemic. Hugh (1962) reported that O-group I hemolytic El Tor vibrios (Vibrio eltor Eisher 1909, 549) were a biotype (physiological type) of Vibrio cholerae Pacini 1854. The rules of nomenclature clearly recommend that biotypes and phagotypes (phage types) be recognized as infrasubspecific subdivisions of a species.

Comparative taxonomic studies appear to agree that organisms which fail to produce anaerobic acidity from dextrose should be excluded from the genus Vibrio and that the following provisional definition of the genus Vibrio is warranted:

Gram-negative; asporogenous straight rods or rods with a single rigid curve; polar flagella; produce anaerobic acidity from dextrose without gas production. It is recognized that this incomplete definition may include a few species of other genera but further studies appear to be necessary before agreement may be reached on how this definition may be emended to include or exclude organisms (e.g. Aeromonas formicans (Crawford) Pivnick and Sabina 1957, Aeromonas caviae (Scherago) Liu 1962) morphologically and physiologically related to Vibrio cholerae Pacini. It should be apparent that some Gram-negative rods with a polar flagellum and a single somatic curve have been classified in Pseudomonas Schroeter and Comamonas Park.

CONCLUSIONS

Five recommendations, based on the observations presented above, have been prepared in the interest of nomenclatural stabilization.

1. Careful research indicates that the infusorian generic name Vibrio Müller 1773, 39 has not been used in the primary zoological literature for more than 100 years. It is

recommended that Vibrio Müller 1773 be placed in the Official Index of Rejected Zoological Names.

2. Pacini (1854) was the first to describe a microbe which is recognized today as a species of bacteria currently classified in the genus Vibrio. It is recommended that the generic name Vibrio Pacini 1854 be conserved against Vibrio Müller 1773 and all other zoological names for the taxon to avoid further changes in the nomenclature of the genus, and that it be placed in the list of conserved bacterial generic names (Nomina generum bacteriorum conservanda).

3. Pacini (1854, 411) described and named vibrio cholera (sic). He was the first to use the generic name Vibrio with a validly published and legitimate specific epithet, cholerae, for the bacterium which is recognized as the causal agent of Asiatic cholera. It is recommended that Vibrio cholerae Pacini 1854, the type species by monotypy, be placed in the list of names of type species of genera (Nomina generum bacteriorum conservanda).

4. It is recommended that the specific epithet cholerae in the binary combination Vibrio cholerae Pacini 1854 be placed in the list of conserved specific epithets (Epitheta specifica conservanda).

5. It is recommended that the National Collection of Type Cultures strain 8021 (American Type Culture Collection strain 14035) be recognized as the neotype strain of Vibrio cholerae Pacini 1854.

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