A Taxonomic Study of Certain Bacteria Currently Classified as *Vibrio* Species

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SUMMARY

Twenty-five strains currently classified into 14 serological types or species of the genus *Vibrio* obtained from the NCIB and NCTC have been studied in detail for morphological, cultural and biochemical characters in two independent studies. The results indicate that these strains include members of three distinct taxonomic groups or genera, *Vibrio*, *Pseudomonas* and *Comamonas*. The new generic name *Comamonas*, type species *C. percolans*, replaces *Lophomonas* (Galarneault & Leifson) which is invalid.

INTRODUCTION

Thirty-four species are listed in the genus *Vibrio* of *Bergey's Manual* (1957). These species are so heterogeneous in character that it is impossible to define the genus in exclusive terms.

In recent years several new diagnostic tests have been developed for use in bacterial classification, especially of the Gram-negative, polarly flagellate bacteria. By applying a large range of tests to a small number of strains of so-called vibrios, we hoped to clarify the characters of the genus *Vibrio* and appraise the true taxonomic relationships of certain species at present included in the genus.

The work to be described was carried out as independent studies by the two authors. One of us (R.W.A.P.) examined a group of vibrios as part of a comparative study of the polarly flagellate bacteria commonly found in surface waters (see Park, 1961*a*). The other (G.H.G.D.) was asked by Dr R. Cain to determine the taxonomic relationships of a so-called vibrio (vibrio 01, Happold & Key, 1932) commonly used in biochemical studies, e.g. Cain (1961). In order to do this a number of other vibrio strains were obtained for comparative study. Although basically similar, the two studies differ in the tests, techniques and strains used and sometimes in the results obtained, but, as we hope to show, the conclusions which can be drawn from both studies are in good agreement. The simplest way of presenting our information is to divide each of the sections of this paper dealing with Methods and Results into two parts, distinguished by the initials of the author concerned.

METHODS. 1. (R.W.A.P.)

Strains examined

Vibrio comma (Inaba), NCTC 4693.

Vibrio sp. (Gardner & Venkatraman group III), NCTC 4711.

Vibrio sp. (Gardner & Venkatraman group V), NCTC 4715.

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- Vibrio* sp. (Gardner & Venkatraman group VI), NCTC 4716.
Vibrio sp. (Gardner & Venkatraman group II), NCTC 8042.
V. eltor NCTC 5895.
V. paracholerae (Gardner & Venkatraman group III), NCTC 30.
V. alcaligenes (Lehmann & Neumann, 1981), NCTC 9239.
V. percolans (Mudd & Warren, 1923), NCTC 1937.
V. proteus (Buchner, 1885), NCTC 8563.
V. cuneatus (Gray & Thornton, 1928), NCIB 8194.
V. cyclosites (Gray & Thornton, 1928), NCIB 2581.
V. neocistes (Gray & Thornton, 1928), NCIB 2582.
Vibrio 01 (Happold & Key, 1982), NCIB 8250.

Strain maintenance

Stock cultures were grown 24 hr. on nutrient agar slopes in loosely closed screw-capped bottles and stored at room temperature with the caps screwed down; fresh cultures were prepared monthly.

Incubation at 30° was used throughout this study, except in certain tests as indicated. Storage at room temperature was necessary because *Vibrio comma* does not survive well at low temperatures, e.g. 4°.

Characters examined

1. Cell-form and Gram-reaction of 24 hr. nutrient agar growth.
2. Occurrence and type of motility in hanging-drop preparations of 18 hr. peptone water culture.
3. Disposition and number of flagella using electron microscopy.
4. Metabolism of carbohydrates:
 - (i) Acid and gas production from the following carbohydrates: DL-arabinose, D-glucose, D-fructose, D-mannose, D-galactose, mannitol, dulcitol, sorbitol, sucrose, lactose, maltose, salicin; tested in 1% (w/v) concentration in peptone water (Mackie & McCartney, 1953) and also in a dilute meat extract medium (Simon, 1956), both media containing bromothymol blue as pH indicator. Arabinose was added as a Seitz-filtered solution to sterile base: gas production from this sugar was not tested for. All other carbohydrate media were steam sterilized. Control tests without carbohydrate were also inoculated. Initial pH of all tests was 7.4, and tests were read after 24 hr. and 7 days' incubation. As results were identical by both methods, only one set has been recorded.
 - (ii) Utilization of carbohydrates as sole carbon sources. Tested upon following medium (% w/v): NH_4Cl , 0.1; K_2HPO_4 , 0.1; MgSO_4 , 0.05; agar, 1.5. Same carbohydrates used as in (i) above, at same concentration, all added as Seitz-filtered solutions. Utilization was inferred from the growth of an organism within 7 days in two successive subcultures. Controls without carbohydrate were included.
 - (iii) Oxidation *versus* fermentation of glucose; test as described by Hugh & Leifson (1953), initial pH 7.4.
 - (iv) Production of reducing compounds from gluconate (Haynes, 1951). Medium (% w/v): Oxoid peptone, 0.2; Oxoid yeast extract, 0.1; K_2HPO_4 , 0.1; pH 7.0. Basal medium autoclaved and Seitz-filtered 40% (w/v) aqueous potassium gluconate

solution added to give final concentration of 4% (w/v). Dispensed aseptically, inoculated and shaken by hand twice daily during 5 days' incubation. Tested with Benedict's qualitative reagent.

(v) Acetylmethylcarbinol production from glucose, using Barritt's (1936) method, but incubated for 5 days.

(vi) Methyl-red test after 5 days' incubation (Mackie & McCartney, 1953).

(vii) Modified Eijkman test (*Topley & Wilson's Principles*, 1955).

(viii) Hydrolysis of starch within 5 days, tested upon nutrient agar containing 0.2% (w/v) soluble starch by flooding with iodine solution (Allen, 1918).

5. Nitrate reduction, tested in 5-day-old peptone water cultures containing 0.1% (w/v) KNO_3 , using the Griess-Ilosvay reagents and zinc powder test for false negatives (ZoBell, 1932).

6. Cholera-red reaction, tested by adding conc. H_2SO_4 (0.5 ml.) to a 5-day old culture in peptone water containing 0.001% (w/v) KNO_3 (Beam, 1959).

7. Indole production, tested in 5-day-old peptone (Oxoid) water cultures using Ehrlich reagent and ether extraction.

8. Utilization of tryptophan, phenylalanine, phenol, benzoic acid, and catechol as sole carbon sources. Compounds were supplied in 0.1% (w/v) concentration in the inorganic base described in 4 (ii) above. (Note: phenol was used in 0.05% (w/v) as 0.1% inhibited certain strains; autoxidation of catechol by light, heat or high pH was avoided by adjusting basal medium to pH 6.5 and sterilizing by Seitz-filtration.)

9. Tyrosine breakdown, tested by the ability to dissolve tyrosine (0.5%, w/v) suspended in nutrient agar within 7 days.

10. Phenylalanine and malonate breakdown, tested by the method of Shaw & Clarke (1955).

11. Gelatin hydrolysis, using 0.5% (w/v) gelatin in nutrient agar and flooding 5-day growth with acid mercuric chloride (Frazier, 1926).

12. Serum hydrolysis, observed after 24 hr. and 7 days' growth upon inspissated serum.

13. Casein hydrolysis, using Hastings' (1904) method with 10% (v/v) skimmed milk in nutrient agar; examined after 5 days.

14. Hydrogen sulphide production from peptone water, detected with lead acetate paper; examined after 24 hr. and 7 days.

15. Urea breakdown within 7 days, using Christensen (1946) medium in liquid form. Controls without urea were included.

16. Tributyrin hydrolysis, detected by clearing of 0.2% (v/v) tributyrin in nutrient agar after 24 hr. and 7 days.

17. Utilization of organic acids as sole carbon sources, inferred from growth within 7 days in two successive subcultures upon the inorganic medium described in 4 (ii) above containing 0.25% (w/v) of acid. Tested: succinic, malic, acetic, citric, pyruvic, lactic, malonic, and formic acids, all supplied as Seitz-filtered solutions of the sodium salts.

18. Utilization of ethanol and production of excess acid was tested by growth within 7 days in two successive subcultures upon inorganic medium (4 ii above) containing 1% (w/v) calcium carbonate and 1% (v/v) ethanol. Excess acid production was shown by clearing of CaCO_3 around growth. Controls without ethanol

were incubated separately. (Note: this is not the test of Shimwell, Carr & Rhodes, 1960, as used by G. H. G. D., Methods, 2.)

19. Catalase production, tested with 10 vol. H_2O_2 upon 24 hr. nutrient agar growth.

20. Kovacs (1956) oxidase test.

21. Cytochrome oxidase test of Gaby & Hadley (1957).

22. Growth in peptone water at 0° (within 14 days), 30° , 37° , 42° , 45° (within 7 days). Tested twice.

23. Sodium chloride tolerance, tested by growth within 7 days in peptone water containing 0.5, 1, 3, 6 or 9% (w/v) NaCl. Tested in triplicate.

24. Growth upon MacConkey agar (Oxoid) and Aronson (pH 9) medium (Mackie & McCartney, 1953) within 7 days.

25. Pyocyanin production. In addition to routine observation of all cultures, 7-day growth upon the glycerol-peptone medium of Gessard (1891) was examined for diffusible pigment in daylight and under a source of ultraviolet radiation fitted with a Wood's glass (u.v.).

26. Fluorescein production. In addition to routine observation, strains were tested for this character in the medium of Georgia & Poe (1931) and upon the inorganic base described in 4 (ii) above containing D-glucose (1%, w/v) and CaCO_3 (1%, w/v). Cultures were examined after 1, 3, and 7 days in daylight and under u.v.

27. Resistance to antibacterial agents. Tested: penicillin, 2.5 i.u.; Terramycin, 10 μg .; streptomycin, 80 μg .; chloramphenicol, 100 μg ., and 2:4-diamino 6:7-diisopropyl pteridine (vibriostatic agent O/129). Inhibition of growth at 24 hr. by these substances was tested upon nutrient agar seeded with organisms using Evans 'Sentest' disks (antibiotics) or, in the case of O/129, a few crystals placed upon the agar surface.

METHODS. 2. (G.H.G.D.)

Strains examined

Vibrio comma, NCTC 4693, NCTC 6585, NCTC 7270, NCTC 8021, NCTC 8367.

V. percolans, NCIB 8193.

V. cuneatus, NCIB 8194.

V. cyclosites, NCIB 2581.

V. neocistes NCIB 2582.

V. 01, NCIB 8250.

Strain maintenance

As R.W.A.P., but using cotton-wool-plugged tubes and subcultured every 2 weeks.

Characters examined

1. Cell-form and Gram-reaction, as R.W.A.P.

2. Motility in nutrient broth at 25° , observed by phase-contrast microscopy after 1-8 days.

3. Flagellation, as R.W.A.P., and various staining methods.

4. Carbohydrate metabolism:

(i) Acid production. Basal medium: Koser salt solution (Mackie & McCartney, 1953, p. 429) plus Oxoid yeast extract 0.3% (w/v). Initial pH adjusted to 7.0, 2 ml. of 1.5% (w/v) solution of bromocresol purple added per litre and medium

autoclaved. Carbohydrates were added as 20 % (w/v) autoclaved solutions to give 1 % (w/v) final concentration. The complete media were aseptically dispensed and tested for sterility by incubating for 48 hr. at 30°. Carbohydrates used: arabinose (L+), xylose, glucose, fructose, mannose, galactose, rhamnose, mannitol, sorbitol, dulcitol, inositol, sucrose, maltose, lactose melibiose, raffinose, dextrin, inulin, glycogen, salicin. Tests read daily over 14 days.

(ii) Oxidation *versus* fermentation of glucose. Modified from Hugh & Leifson (1953). Basal medium as in (i) above, plus 1 % (w/v) glucose and 0.3 % (w/v) agar. Initial pH 7.0 and 2 ml. of 1.5 % (w/v) bromocresol purple added per litre. (Note: these modifications were recommended by Dr A. C. Hayward, Commonwealth Mycological Institute, Kew, and Dr A. C. Baird-Parker, Unilever, Bedford, and do appear to clarify the results of this test.)

(iii) Gas from glucose, in addition to observations of (ii) above, routine peptone water fermentation tests containing Durham tubes were observed over 14 days.

(iv) Voges-Proskauer and methyl-red tests as Mackie & McCartney (1953).

(v) Acetylmethylcarbinol (acetoin) production and final pH from 1 % (w/v) glucose in the basal medium of (i) above, initial pH 7.0. Tested after 4 days using pH test papers and Barritt's (1936) method for acetylmethylcarbinol.

(vi) Starch hydrolysis, as R.W.A.P.

(vii) Aesculin hydrolysis within 7 days in the basal medium of (i) above, plus 0.5 % (w/v) aesculin and 0.005 % (w/v) ferric ammonium citrate.

(viii) Dihydroxyacetone production from glycerol, test of Shimwell, Carr & Rhodes (1960).

5. Nitrate reduction: Basal medium 4 (i) above plus 0.1 % (w/v) KNO_3 and 0.1 % (w/v) agar. Procedure as R.W.A.P.

6. Cholera-red test, in Oxoid peptone water (Mackie & McCartney, 1953).

7. Indole production, in 1 % (w/v) Oxoid tryptone water, tested after 3 days by ether extraction and Ehrlich reagent.

8. Tyrosine breakdown, as R.W.A.P.

9. Gelatin hydrolysis, as R.W.A.P., but using 0.2 % (w/v) gelatin.

10. Serum hydrolysis, as R.W.A.P.

11. Casein hydrolysis, as R.W.A.P.

12. Lysine decarboxylase activity. Method of Carlquist (1956). Medium: Basal medium of 4(i) above plus 0.5 % (w/v) lysine hydrochloride. Tested after 5 days.

13. Hide-powder lysis. Method of Evans (1947) in double-layer plates of basal medium 4(i) above with hide-powder (Baird & Tatlock) incorporated in top layer. Examined daily.

14. Hydrogen sulphide production. Basal medium of 4(i) above, plus (i) 0.05 % (w/v) L-cysteine-HCl, or (ii) 0.1 % (w/v) sodium thiosulphate. H_2S detected by lead acetate paper, over 7 days.

15. Ammonia production from arginine. Medium: (%, w/v), Oxoid tryptone, 0.2; NaCl, 0.5; K_2HPO_4 , 0.08; L-arginine-HCl, 0.5; glucose, 0.1; pH 7.1. Tested with Nessler reagent after 5 days.

16. Urea breakdown by Christensen's (1946) method, in cotton-wool-plugged tubes, read over 7 days.

17. Egg-yolk reaction, tested as recommended by Willis (1960) after 6 days.

18. Tributyrin hydrolysis, tested by clearing within 7 days of Oxoid glycerol tributyrinate (1%, w/v) agar.

19. Utilization of organic acids as sole carbon sources. Medium: Koser salt solution plus 1.5% (w/v) agar and 0.008% bromothymol blue, pH 6.8. Acids added as sodium salts to give 0.25% (w/v) final concentration. Succinic, malic, acetic, citric, lactic and oxalic acids tested. Tested on slopes, read up to 7 days.

20. Acid production from ethanol, test of Shimwell *et al.* (1960).

21. Oxidation of calcium lactate to carbonate, test of Shimwell *et al.* (1960).

22. Catalase production, as R.W.A.P.

23. Oxidase production, as R.W.A.P.

24. Cytochrome oxidase production, as R.W.A.P.

25. Phosphatase production, tested upon nutrient agar containing 0.006% (w/v) phenolphthalein phosphate, exposed after 5 days to vapour from 0.880 ammonia solution.

26. Growth within 7 days on nutrient agar containing 5% (w/v) NaCl, 5 or 10% (w/v) sodium taurocholate; growth and reduction with 0.2 or 1% (w/v) triphenyl tetrazolium chloride (T.T.C.) (Meitert, Meitert & Horodniceanu, 1960).

27. Growth and characteristics upon MacConkey, Wilson & Blair, Dieudonné and deoxycholate-citrate agars (Mackie & McCartney, 1953), over 7 days.

28. Growth on nutrient agar under strict anaerobiosis, 7 days.

29. Growth in nutrient broth at 4° (14 days), 14°, 25°, 30°, 44° (7 days).

30. Growth from initial pH 4.5, test of Shimwell *et al.* (1960).

31. Pigment production. Routine observation of all cultures in daylight; fluorescence under u.v. of growth upon the glucose-calcium carbonate medium of R.W.A.P.

32. Resistance to antibacterial agents. Tested using Evans 'Sentest' or Mast disks on surface seeded nutrient agar. Antibiotics: penicillin, 1 and 2.5 i.u.; streptomycin, 20 and 80 µg.; chloramphenicol, 40 and 100 µg.; Aureomycin, 10 and 100 µg.; Terramycin, 10, 25 and 100 µg.; erythromycin, 1 and 10 µg.; tetracycline, 100 µg.; neomycin, 100 µg.; bacitracin, 5.5 units; novobiocin, 5 and 10 µg.; oleandomycin, 5 and 20 µg. Also tested O/129 (see R.W.A.P.) using an aqueous suspension (c. 50 mg. in 20 ml.) and agar well technique. (Note: a saturated aqueous solution of O/129 proved non-inhibitory against *Vibrio comma* in preliminary tests.)

RESULTS

Tables 1 (R.W.A.P.) and 2 (G.H.G.D.) show the results obtained by the two authors and the classification of strains into three groups on the evidence of these results. The overall characters of these three groups are compared in Table 3.

Morphological details are not readily presentable in tables and we therefore include here an account of our observations. The six strains of *Vibrio comma* used by G.H.G.D., and strains 4698 (*V. comma*), 5895 (*V. eltor*), 8568 (*V. proteus*), 8042, 4711, 4715 and 4716 studied by R.W.A.P. all exhibited pleomorphism. Straight and curved short rods, S-forms, spirals, undulating filaments and spheroplasts (Pitzurra & Szybalski, 1959) were regularly seen. This variety of forms has been reported before in studies upon *V. comma* (Koch, 1886; Henrici, 1925). Plate 1, figs. 1 and 7, illustrates some of these forms. *V. paracholerae* (NCTC 30) did not

Table 1. *Results of R.W.A.P.*

Strain	<i>Vibrio</i> spp.								<i>Pseudo-</i> <i>monas</i> spp.	<i>Comamonas</i> spp.				
	4693 1L	4711 1L	4715 1L	4716 1L	8042 1L	5395 1L	8563 1L	80 1L	8194 M	2581 ML	2582 ML	1937 M	9239 1	8250 1
Flagellation														
Acid production and utilization of:														
Arabinose	-	-	-	-	-	-	-	-	+U	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+U	-	-	-	-	-
Fructose	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Mannose	+	-	+	+	+	+	+	+	+U	-	-	-	-	-
Galactose	±	+	+	±	+	+	±	+	+U	-	-	-	-	-
Mannitol	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	±	-	-	-	-	-	-	-
Lactose	±	±	±	±	+	±	±	±	-	-	-	-	-	-
Maltose	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Salicin	-	-	-	-	+	-	±	-	-	-	-	-	-	-
Hugh & Leifson test	F	F	F	F	F	F	F	F	O	Al.	Al.	Al.	Al.	Al.
Gluconate test (Haynes)	-	+	+	+	-	-	-	+	-	-	-	-	-	-
Acetoin production	-	+	+	+	+	+	-	-	-	-	-	-	-	-
Methyl-red test	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	+	-	+	-	-	-	+	+	-
Cholera-red test	+	+	+	-	+	-	-	+	-	-	-	-	-	-
Indole production	+	+	+	+	+	-	+	+	-	-	-	-	-	-
Utilization of:														
Tryptophan	-	-	-	-	-	-	-	-	+B	-	-	-	-	+
Phenylalanine	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Phenol	-	-	-	-	-	-	-	-	+	-	-	-	-	+
Benzoic acid	-	-	-	-	-	-	-	-	+	-	-	-	-	+
Catechol	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Tyrosine dissolution	+	+	-	-	-	-	-	-	+	-	+	+	+B	+
Phenylalanine (Shaw & Clarke)	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	-	-	-	+	-
Serum hydrolysis	±	+	+	-	±	+	±	±	-	-	-	-	-	-
Casein hydrolysis	+	+	+	+	+	+	+	+	-	-	-	-	-	-
H ₂ S from peptone	±	±	-	±	±	-	-	±	-	-	-	±	-	-
Urease	-	-	-	-	-	-	-	-	±	-	+	+	-	-
Utilization of:														
Succinic	+	+	+	+	+	+	+	-	+	-	-	-	-	+
Malic	+	+	+	+	+	+	+	-	+	-	-	-	-	+
Acetic	+	-	-	+	-	+	+	-	+	-	-	-	-	+
Citric	-	+	+	+	+	+	+	-	+	-	-	-	-	+
Pyruvic	+	-	+	+	+	+	+	-	+	-	-	-	-	-
Lactic	+	+	+	+	+	+	-	-	+	-	-	-	-	-
Malonic	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Formic	-	-	-	-	-	-	-	-	+	-	-	-	-	+
Utilization and acid from ethanol	-	-	-	-	-	-	-	-	U	-	-	-	-	UA
Catalase	±	±	±	±	±	±	±	±	+	+	±	±	+	+
Kovacs oxidase	+	+	+	+	+	+	-	-	+	-	-	-	+	+
Cytochrome oxidase	±	±	±	-	-	-	-	-	±	±	±	-	-	-
Growth at 0°	-	-	-	-	-	-	+	-	+	-	-	-	-	-
Growth at 37°	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Growth at 42°	+	+	+	+	-	+	-	+	-	+	+	-	-	-
Growth in 3% NaCl	+	+	+	+	+	+	+	+	+	-	-	-	+	-
Growth in 6% NaCl	-	-	+	-	-	-	+	-	-	-	-	-	-	-
MacConkey	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Aronson	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Fluorescein	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Fluorescence u.v.	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Susceptible to:														
Penicillin 2-5	+	-	-	±	-	±	±	-	-	-	-	-	-	-
Streptomycin 80	+	+	+	+	+	+	+	+	+	+	+	+	-	+
O/129	+	+	+	+	+	+	+	-	-	-	-	-	-	-

Key, see p. 119.

Table 2. *Results of G.H.G.D.*

Strain	<i>Vibrio</i> spp.						<i>Pseudo-</i> <i>monas</i> spp.	<i>Comamonas</i> spp.			
	4698	6585	7270	7254	8021	8367	8194	2581	2582	8198	8250
Flagellation	1L	1L	1L	1L	1L	1L	M	M	M	M	—
Acid from:											
Arabinose	—	—	—	—	—	—	7	10	—	—	—
Xylose	—	—	—	—	—	—	8	—	—	—	—
Glucose	8	8	8	8	8	8	8	12	—	—	—
Fructose	8	8	8	8	8	8	10	10	—	—	—
Mannose	8	8	8	8	8	8	8	14	—	—	—
Galactose	—	8	8	8	8	8	8	14	—	—	—
Rhamnose	—	—	—	—	—	—	—	7	—	—	—
Mannitol	7	8	8	8	8	8	7	10	—	—	—
Inositol	—	—	—	—	—	—	7	—	—	—	—
Sucrose	8	8	8	8	8	8	5	7	—	—	—
Maltose	8	8	8	8	8	8	—	14	—	—	—
Lactose	—	7	12	7	12	—	—	12	—	—	—
Melibiose	—	—	—	—	—	—	8	12	—	—	—
Dextrin	8	8	8	8	8	8	—	10	—	—	—
Glycogen	8	8	8	8	8	8	—	—	—	—	—
Salicin	—	—	—	—	—	—	—	12	—	—	—
Hugh & Leifson test	F	F	F	F	F	F	O	O±	—	—	—
Methyl-red test	+	+	+	+	+	+	—	—	—	—	—
Acetoin	—	—	±	—	—	—	—	—	—	—	—
pH 1 % glucose	5.1	5.1	5.1	6.1	5.1	4.5	6.1	6.7	6.7	7.1	7.1
Starch hydrolysis	+	+	+	+	+	+	—	±	—	—	—
Aesculin hydrolysis	—	—	—	—	—	—	—	+	—	—	—
Nitrate reduction	+	+	+	+	+	+	+	+	+	—	—
Cholera-red test	+	+	+	+	+	+	—	—	—	—	—
Indole	+	+	+	+	+	+	—	—	—	—	—
Tyrosine dissolution	±	—	±	—	—	—	+	—	+	+	+
Gelatin hydrolysis	+	+	+	+	+	+	+	—	—	—	—
Serum hydrolysis	—	+	+	+	+	+	+	—	—	—	—
Casein hydrolysis	+	+	+	+	+	+	—	±	—	—	—
Lysine decarboxylase	+	+	+	+	+	+	—	—	—	—	—
Hide-powder lysis	+	+	+	+	+	+	—	—	—	—	—
H ₂ S cysteine	+	+	+	+	+	+	±	±	±	±	±
H ₂ S thiosulphate	+	+	+	+	+	+	—	±	±	±	—
NH ₃ from arginine	—	—	—	—	—	—	+	—	—	—	—
Urease	—	—	—	—	—	—	+	—	+	+	—
Egg yolk reaction	Li	Li	Li	Li	Li	Li	Li	—	—	—	Li±
Tributyrin	NG	NG	NG	NG	NG	NG	+	+	+	+	+
Utilization of											
Malic	+	+	+	+	+	+	+	—	+	+	+
Acetic	+	+	+	+	+	+	+	—	+	+	+
Lactic	+	+	+	+	+	+	+	—	+	+	+
Oxalic	—	—	—	—	—	—	—	—	—	+	—
Acid from ethanol	—	—	—	—	—	—	—	—	—	—	±
Lactate oxidation	—	—	—	—	—	—	—	—	—	—	+
Catalase	±	±	±	±	±	±	+	+	+	+	+
Kovacs oxidase	+	+	+	+	+	+	+	—	+	+	—
Cytochrome oxidase	+	+	+	+	+	+	—	—	±	—	—
Phosphatase	—	—	—	—	—	—	+	—	—	—	±
5 % NaCl tolerance	+	±	—	+	—	+	±	+	—	—	±
5 % Bile tolerance	±	+	+	+	+	+	+	—	+	±	+
10 % Bile tolerance	±	—	+	+	+	+	+	—	—	—	+

Key, see p. 119.

Table 2 (cont.)

Strain	<i>Vibrio</i> spp.						<i>Pseudo-</i> <i>monas</i> spp.	<i>Comamonas</i> spp.			
	4693	6585	7270	7254	8021	8367	8194	2581	2582	8193	8250
0.2% T.T.C. tolerance	—	—	+	—	+	—	+	—	+	+	+
1% T.T.C. tolerance	—	—	—	—	—	—	+	—	—	—	—
MacConkey	+	+	+	+	+	+	+	—	+	+	+
Wilson and Blair	—	+	+	+	+	+	+	—	+B	+	+B
Dieudonné	+	+	+	+	+	+	±	—	—	—	—
Deoxycholate-citrate	—	+	+	+	+	+	+	—	—	—	—
Anaerobic growth	±	±	±	±	±	±	—	—	—	—	—
Growth at 4°	—	—	—	—	—	—	—	±	—	—	±
Growth at 14°	+	+	+	+	+	+	+	±	+	±	+
Growth at pH 4.5	—	—	—	—	—	—	+	—	+	+	+
Pigment daylight	—	—	—	—	—	—	YG	Y	—	—	—
Fluorescence, u.v.	—	—	—	—	—	—	+	—	—	—	—
Susceptible to											
Chloramphenicol 100	+	+	+	+	+	+	±	+	+	±	+
Chloramphenicol 40	+	+	+	+	+	+	±	—	+	—	+
Terramycin 10	+	+	+	+	+	+	+	+	+	±	+
Tetracycline 100	+	+	+	±	+	+	+	+	+	+	±
Aureomycin 100	+	+	+	+	+	+	±	+	+	+	±
Aureomycin 10	+	+	+	+	+	+	—	+	+	+	—
Streptomycin 80	+	+	+	+	+	+	+	+	+	—	+
Streptomycin 20	+	+	+	+	+	+	+	+	+	—	+
Neomycin 100	+	+	+	+	+	+	+	+	+	—	—
Erythromycin 10	+	+	+	+	+	+	—	+	—	—	—
Erythromycin 1	+	+	+	—	+	±	—	±	—	—	—
Novobiocin 10	+	+	+	+	+	+	—	+	—	—	—
Novobiocin 5	+	+	+	+	+	+	—	—	—	—	—
Oleandomycin 20	+	+	+	—	+	±	—	+	—	—	—
O/129	±	±	+	—	±	±	—	—	—	—	—

Key, see p. 119.

show the same degree of pleomorphism, only straight and curved rods and spheroplasts were seen (Pl. 1, fig. 2). All these strains exhibited single polar flagella upon the short cell forms and often had a flagellum at both poles of the longer forms, some of which appeared to be dividing (Pl. 1, fig. 1). Spheroplasts commonly retained flagella but motility of spheroplasts was never observed in living material (cf. Jeynes, 1961).

Strain 8194 (*Vibrio cuneatus*) consisted of robust, round-ended rods which were usually curved (Pl. 1, fig. 4). One or more polar flagella were seen (1–10, R.W.A.P.). No pleomorphism other than variation in cell length was observed and this may correlate with observed colonial variations between smooth soft and rough hard forms. The tapering cell forms described by Gray & Thornton (1928) were not seen.

Strains 2581 (*Vibrio cyclosites*) and 2582 (*V. neocistes*) were observed by R.W.A.P. as fairly robust, straight rods with round ends, usually bearing 2–6 flagella at one pole and one at the other (Pl. 1, fig. 3). Leifson (1960) has illustrated a very similar form (Leifson, fig. 16*d*) showing division of the organism. Our figure does not show division in progress. G.H.G.D. observed lophotrichous but not bipolar, flagellation in strain 2518. Loss of motility appeared to occur readily in this strain and

coccoid and swollen forms were sometimes seen. Strain 2582 was similar in flagellation to 2581, and regularly motile. Filamentous forms that divided by constriction (Bisset, 1952), and appeared as chains of fusiform cells were observed in strain 2582.

Strains 8193 (G.H.G.D.) and 1937 (R.W.A.P.) (*Vibrio percolans*) were observed by both authors to consist of robust, straight rods with round ends and 1-5 flagella at one pole (Pl. 1, fig. 5). Filamentous forms showing constrictions were common, cf. Leifson (1960, fig. 16c), and strain 2582 above. Similar tendencies to filamentous

Table 3. Comparison of certain results from R.W.A.P. and G.H.G.D. for the groups indicated in Tables 1 and 2

Group Author and number of strains	<i>Vibrio</i>		<i>P. fluorescens</i> (8194)		<i>Comamonas</i>	
	R.W.A.P. (8)	G.H.G.D. (8)	R.W.A.P. (1)	G.H.G.D. (1)	R.W.A.P. (5)	G.H.G.D. (4)
Flagellation	1L	1L	M	M	1, M or ML	3/4 M
Acid from						
Arabinose	—	—	+U	+	—	1/4 ±
Xylose	NT	—	NT	+	NT	—
Glucose	+	+	+U	+	—	1/4 ±
Fructose	+	+	—	±	—	1/4 ±
Mannose	7/8	+	+U	+	—	1/4 ±
Galactose	+ or ±	5/6	+U	+	—	1/4 ±
Rhamnose	NT	—	NT	—	NT	1/4 ±
Mannitol	+	+	—	±	—	1/4 ±
Inositol	NT	—	NT	±	NT	—
Sorbitol	1/8	—	—	—	—	—
Sucrose	7/8	+	—	+	—	1/4 ±
Lactose	±	4/6 ±	—	—	—	1/4 ±
Maltose	+	+	—	—	—	1/4 ±
Melibiose	NT	—	NT	+	NT	1/4 ±
Dextrin	NT	+	NT	—	NT	1/4 ±
Glycogen	NT	+	NT	—	NT	—
Salicin	2/8	—	—	—	—	1/4 ±
Hugh & Leifson test	F	F	O	O	Al	1/4 ± O
Methyl-red test	2/8	+	—	—	—	—
Acetoin	5/8	1/6	—	—	—	—
pH 1 % glucose	NT	below 6	NT	7.1	NT	c. 7
Starch hydrolysis	+	+	—	—	—	1/4 ±
Aesculin hydrolysis	NT	—	NT	—	NT	1/4 ±
Nitrate reduction	7/8	+	—	+	2/5	2/4
Cholera-red test	5/8	+	—	—	—	—
Indole	7/8	+	—	—	—	—
Tyrosine dissoluton	2/8	2/6 ±	+	+	4/5	3/4
Gelatin hydrolysis	+	+	+	+	1/5	—
Serum hydrolysis	7/8 ± or +	5/6	+	+	1/5	—
Casein hydrolysis	+	+	—	—	—	1/4 ±
Lysine decarboxylase	NT	+	NT	—	NT	—
Hide-powder lysis	NT	+	NT	—	NT	—
H ₂ S cysteine	NT	+	NT	±	NT	±
H ₂ S thiosulphate	NT	+	NT	—	NT	3/4 ±
H ₂ S peptone	5/8 ±	NT	—	NT	1/5 ±	NT
NH ₃ from arginine	NT	—	NT	+	NT	—
Urease	—	—	±	+	2/5	2/4
Tributyryn 0.2 %	+	NT	+	NT	+	NT
Tributyryn 1 %	NT	NG	NT	+	NT	+
Egg-yolk reaction	NT	Li	NT	Li	NT	1/4 Li ±

Key, see p. 119.

Table 3 (cont.)

Group Author and number of strains	<i>Vibrio</i>		<i>P. fluorescens</i> (8194)		<i>Comamonas</i>	
	R.W.A.P. (8)	G.H.G.D. (6)	R.W.A.P. (1)	G.H.G.D. (1)	R.W.A.P. (5)	G.H.G.D. (4)
Utilization of:						
Tryptophan	—	NT	+	NT	1/5	NT
Phenylalanine	—	NT	+	NT	—	NT
Phenol	—	NT	—	NT	1/5	NT
Benzoic acid	—	NT	+	NT	1/5	NT
Catechol	—	NT	—	NT	1/5	NT
Phenylalanine (Shaw & Clarke)	—	NT	—	NT	1/5	NT
Malonate (Shaw & Clarke)	+	NT	+	NT	+	NT
Succinic	7/8	+	+	+	1/5	+
Malic	7/8	+	+	+	1/5	3/4
Acetic	4/8	+	+	+	1/5	3/4
Citric	6/8	+	+	+	1/5	+
Pyruvic	6/8	NT	+	NT	—	NT
Lactic	6/8	+	—	—	—	3/4
Oxalic	NT	—	NT	—	NT	1/4
Malonic	—	NT	+	NT	—	NT
Formic	—	NT	+	NT	1/5	NT
Utilization of ethanol	—	NT	+	NT	1/5	NT
Acid from ethanol	—	—	—	—	1/5	1/4
Lactate oxidation	NT	—	NT	—	NT	1/4
Catalase	±	±	+	+	+ or ±	+
Kovacs oxidase	6/8	+	+	+	2/5	2/4
Cytochrome oxidase	3/8 ±	+	±	—	2/5 ±	1/4 ±
Phosphatase	NT	—	NT	+	NT	1/4 ±
NaCl tolerance:						
3 %	+	NT	+	NT	1/5	NT
5 %	NT	4/6	NT	±	NT	2/4
10 % bile tolerance	NT	5/6	NT	+	NT	1/4
1 % T.T.C. tolerance	NT	—	NT	+	NT	—
Growth at:						
0°	1/8	NT	+	NT	—	NT
4°	NT	—	NT	+	NT	2/4 ±
42°	6/8	NT	—	NT	2/5	NT
MacConkey	+	+	+	+	4/5	3/4
Aronson	+	NT	—	NT	—	NT
Dieudonné	NT	+	NT	±	NT	—
Wilson & Blair	NT	5/6	NT	+	NT	3/4
Deoxycholate citrate	NT	5/6	NT	+	NT	—
Anaerobic growth	NT	±	NT	—	NT	—
Growth at pH 4.5	NT	—	NT	+	NT	3/4
Fluorescence u.v.	—	—	+	+	—	—
Pigment, daylight	—	—	+	+	—	1/4
Susceptible to O/129	7/8	5/6	—	—	—	—

Key, see p. 119.

growth were observed by R.W.A.P. in certain freshly isolated water bacteria classified as *Comamonas* sp. (Park, 1961*a*). Variation from polar to peritrichous flagellation as described by Leifson & Hugh (1958) for this organism was not observed, although in a preparation stained by Leifson's (1960) method, many cells

exhibited flagellation similar to that described as 'degenerate peritrichous' by Conn & Wolfe (1938), or 'subpolar' by Leifson (1960). When originally isolated by Mudd & Warren (1923) this organism was found to display a range of morphological forms including what may have been spheroplasts.

Strain 8250 (*Vibrio* 01) had a regular cell form of single and paired, short, thick, straight rods. They appeared to be square-ended in cell-wall stained preparations. R.W.A.P. observed a few motile organisms in peptone water cultures and found occasional organisms bearing one or two flagella at one pole by electron microscopy. Motility was presumably a character of this strain when originally isolated (Happold & Key, 1932) but it appears to be easily lost. No motility was detected by G.H.G.D. and no flagella demonstrated.

Strain 9239 (R.W.A.P.) (*Vibrio alcaligenes*) consisted of round-ended straight rods having one or two flagella at one pole. No other forms were seen (Pl. 1, fig. 6).

Table 4. *Comparison of Vibrio, Aeromonas, Pseudomonas and Comamonas reactions in possible diagnostic tests*

	<i>Vibrio</i>	<i>Aeromonas</i>	<i>Pseudomonas</i>	<i>Comamonas</i>
Spheroplasts regularly formed	+	—	—	—
Pleomorphic	+	—	—	—
Single polar flagellum	+	V	V	V
Fluoresce under u.v.	—	—	+	—
Growth at pH 9	+	V	V	V
Susceptible to O/129	+(V)	—?	—	—
Hugh & Leifson test	Ferment	Ferment	Oxidize	Alkaline or Nil
Gas from glucose	—	+(V)	—	—
Cholera-red test	V	—	—	—

Key: + = positive; — = negative; V = some strains positive; +(V) = most strains positive; ? = see Rhodes (1959) and text.

Note. This table is based on our own results, see Park (1961*a*), and those of previous workers.

Detailed comparisons of our other results can readily be made from the tables we have given. One or two features of these results will serve as examples.

Differences in the results of Kovacs oxidase test and the cytochrome oxidase test in the two studies probably reflect a lack of definition in the execution and reading of these tests. One of us (G.H.G.D.) found some variation in results of the cytochrome oxidase test when the strains were tested in various ways, e.g. young and old, broth and agar cultures. Agar cultures tested by the filter-paper technique gave the clearest results. On the other hand, differences in the results for tributyrin attack are explained by the use of different tributyrin concentrations by the two authors (see Methods). It is interesting to note that all six strains of *Vibrio comma* tested by G.H.G.D. failed to grow in 7 days on 1% (w/v) tributyrin agar (cf. Collins & Hammer, 1934).

Good general agreement in carbohydrate metabolism tests is noticeable. The detection of weak oxidation of glucose by strain 2581 (G.H.G.D.) and late attack of various sugars by the same strain may be explained by the absence of peptone from the basal medium used (cf. Liu, 1952). Ammonia production from peptone or arginine was not detected for this strain (G.H.G.D.), but an alkaline reaction was recorded in the standard Hugh & Leifson test (R.W.A.P.). The strain grew in peptone

water which indicates that peptone was attacked, and breakdown products could have masked weak acid production in the tests of R.W.A.P. This does not, however, explain the fact that R.W.A.P. obtained exactly similar results in Simon medium (see Methods). It is possible that the yeast extract used in the medium of G.H.G.D. may have given rise to false late positive results, and it is also possible that differences in the initial pH value and pH indicators employed may have contributed to the different results obtained. Strains 2581 and 2582 were originally described as producing acid from glucose (Gray & Thornton, 1928). R.W.A.P. found that strain 9239 produced an acid reaction in peptone water and also in all the carbohydrate-peptone water media except those containing sucrose or maltose, which became very alkaline. These findings emphasize the need for controls when peptone water is used as a base for carbohydrate breakdown tests (cf. also Orcutt & Nutting, 1942).

The attack on hide powder by *Vibrio comma* confirmed to some extent the report by Narayanan & Menon (1952) of collagenase activity in this organism. This character is fairly common in other Gram-negative polarly flagellate bacteria.

In our results the egg-yolk reaction of *Vibrio comma* was interpreted as due to lipolysis. The 'pearly layer' effect of Willis (1960) was evident, and zones of what appeared to be a crystalline precipitate which reacted with copper sulphate were produced. Felsenfeld (1944) reported lecithinase activity in *V. comma*.

The test for lysine-decarboxylase activity yielded clear results and may be of taxonomic value (Szturm-Rubinsten, Piéchaud & Piéchaud, 1960). Thibaut & Le Minor (1957) reported the use of this test for the differentiation of enterobacteria.

Preliminary analyses of the cell-wall amino-acid components of strains 4693, 8250 and 8194 by the technique of Davis & Freer (1960) indicated gross similarity between these strains and various other Gram-negative bacteria (e.g. *Neisseria* sp., coliforms, etc.). Qualitative differentiation was not possible and all strains so far examined yielded typical Gram-negative, i.e. complex, amino acid patterns.

DISCUSSION

In this study we have applied a large number and variety of simple tests to a small number of bacterial strains representing all but two (*Vibrio fetus*, see Park, 1961*b*, and *V. metschnikovii*) of the so-called vibrios available in the National Collection of Industrial Bacteria and National Collection of Type Cultures. Although the results obtained by the two authors differ in details, the classification of strains derived from these two independent studies is the same. Tables 1 and 2 show how the strains were analysed into groups; the first group in each table represents what we consider to be true members of the genus *Vibrio*, the second (strain 8194) exhibits characters typical of *Pseudomonas fluorescens*, while the third group is comprised of strains that we regard as representatives of the genus named *Lophomonas* by Galarneault & Leifson (1956). Before proceeding to discuss our results it is important to explain that a change of nomenclature is necessary regarding the genus *Lophomonas* (Galarneault & Leifson). In 1860 Stein gave the name *Lophomonas* to a genus of protozoa found in the cockroach colon; see Wenyon (1926) and Kudo (1954) concerning current usage of the name. Under Rule 24 (see also Principle 8 and Opinion 14) of the *International Code of Nomenclature of Bacteria and Viruses* (1958) such a name must not be used in bacteriology because it is a 'later homonym of the name

of a protozoa taxon'. We propose the name *Comamonas* (Latinized Greek: *Coma*—a lock of hair, and *monas*—a unit) to replace *Lophomonas* in bacteriology. The type species of *Lophomonas*, *L. alcaligenes* proposed by Galarneau & Leifson was presumably a new combination of the name *Vibrio alcaligenes* (Lehmann & Neumann, 1931) but this is not only uncertain but also invalid in view of certain differences in definition between *Lophomonas* and *Vibrio alcaligenes* (see Park, 1961*a*). We therefore designate the type species of *Comamonas* as *C. percolans* (Mudd & Warren, 1923) *nov. comb.* Cultures of the type strain of this species are maintained as ATCC 8461, NCTC 1987 and NCIB 8198. It is interesting to note here that *Bergey's Manual* (1957) suggests that *Vibrio percolans* and *V. alcaligenes* may be synonymous but no definite information on this point is given.

Strain 8194 is clearly distinct from *Vibrio comma* and as Hugh & Leifson (1958) have pointed out, there seems no justification for retaining this strain in the genus *Vibrio*. We see no reason why this organism should not be reclassified as a strain of *Pseudomonas fluorescens*. See Rhodes (1959) & Park (1961*a*) for comparable studies upon other strains of *P. fluorescens*.

The organisms of our third group gave either negative or weak positive results in most of the tests tried, and because they resembled each other in this respect we consider them sufficiently alike to be included in the genus *Comamonas*. Upon the available evidence we cannot decide whether only one species, i.e. *Comamonas percolans*, is represented but this seems probable. Certain variations between strains and even within single strains were noticeable in this group and these suggest a gradation toward the typical *Pseudomonas*. Strain 8250 displayed an ability to utilize various aromatic compounds, while strain 2581 showed some evidence of carbohydrate attack. Similar but more marked gradations towards *Pseudomonas* have been noted independently in other strains of *Comamonas* by both authors, e.g. Park (1961*a*). We thus conclude that we can detect in *Comamonas* a continuation of the trend towards inactivity, as judged by the biochemical tests currently used, that was observed by Rhodes (1959) in the phytopathogenic strains of *Pseudomonas*. None of the *Comamonas* strains examined in this work had any history of pathogenicity, but the production of yellow pigment by 2581 (notably on milk agar) is of interest in this connexion. In spite of these considerations we feel that it is taxonomically useful to place in a separate genus, *Comamonas*, those polarly flagellate bacteria that neither produce a compound which fluoresces under u.v. nor show any clearly defined attack on carbohydrates.

The three so-called vibrios of Gray & Thornton (1928), strains 8194, 2581, and 2582, gave different results in some cases from those originally reported for these strains. Of chief interest is probably the failure of the phenol-utilizing *Vibrio cyclosites* (2581) to utilize phenol in our test. It is to be expected, however, that after 80 years of laboratory maintenance these strains might show some changes in their reactions. We consider that although such changes may have occurred, the strains currently labelled as *V. cyclosites*, *V. neocistes* and *V. cuneatus* represent Gray & Thornton's original isolates. Gray & Thornton's classification was acknowledged to be 'one of convenience only and its temporary nature recognized'; over reliance upon their results from diagnostic tests is probably best avoided.

The utilization of oxalate by strain 8198 recorded above indicates a possible relationship with those oxalate decomposers of the *Comamonas* type reported

by Bhat & Barker (1948) and Jayasuriya (1955). In view of our recommendation to rename this organism *Comamonas percolans* as type species of the genus, it is important also to note here that Leifson & Hugh (1953) and R.W.A.P., using cultures ATCC 8461 and NCTC 1987, respectively, found that it reduced nitrate to nitrite, whereas Mudd & Warren (1928) and G.H.G.D., using the original isolate and NCIB 8198, respectively, found no nitrate reduction. The isolation of a peritrichous variant by Leifson & Hugh (1953) has already been mentioned.

Strain 8250 exemplifies some of the taxonomic difficulties which organisms of this type may create. If polar flagellation had not been detected the strain could have been classified as an *Alcaligenes* or *Protaminobacter* species or as *Achromobacter parvulus*, to quote only a few of the possibilities available in *Bergey's Manual* (1957). Possible relationship to the genus *Acetomonas* (Shimwell *et al.* 1960) was also suspected, but Dr J. G. Carr kindly examined the organism and confirmed that classification in *Acetomonas* or *Acetobacter* was unjustified. (Note: Dr Carr also confirmed the lack of motility in the strain supplied to him by G.H.G.D., and did not detect any acid production from ethanol although the strain did produce irisation on ethanol-chalk agar and grew with ethanol as sole carbon source.)

The main object of this study was to define more accurately the genus *Vibrio*. Our results show that the strains we studied include a clear-cut and compact group, all basically the same as *V. comma* in morphological, cultural and biochemical characters. The overall similarity of the members of this group leads us to conclude that they are members of a single species; the conclusion drawn by Sneath & Cowan (1958) that the genus *Vibrio* might reasonably be downgraded to species rank is of interest in this context.

In Table 4 we have listed certain characters which may have, or are commonly considered to have, diagnostic value in differentiating *Vibrio* from other polar flagellate bacteria. The closest similarity occurs between *Vibrio* and *Aeromonas* (see Park, 1961*a*) and the existence of anaerogenic strains of *Aeromonas* (Crawford, 1954; Pivnick & Sabina, 1957; Park, 1961*a*) further complicates their differentiation. Two characters, pleomorphism and sensitivity to O/129, usually found in *Vibrio* but not in *Aeromonas* strains appear to be of possible diagnostic value and require further assessment.

The distinctive morphology observed in all but one of our *Vibrio* strains appears to be of diagnostic value when present. Its absence, however, does not allow one to exclude an organism from *Vibrio* as it is well known that strains of *V. comma* may exist as almost straight rods (see Pollitzer, 1955). Some bacteria that are biochemically very different from *V. comma* also show this distinctive morphology (Smith & Taylor, 1919; Spencer, 1955; Bryant & Small, 1956; Deas, 1960; Park, 1961*b*). It is difficult to decide whether these organisms should be regarded as close relatives of *V. comma* on morphological grounds, or as not related to it because of their distinct biochemical characters. The presence merely of somatic curvature is of no diagnostic value. It is known to occur in strains of many genera, e.g. *Pseudomonas*, *Aeromonas*, *Comamonas*, *Selenomonas*, etc.

Susceptibility to the vibriostatic agent O/129 first used in systematic work by Shewan, Hodgkiss & Liston (1954) seems, like the distinctive morphology, to be of positive value when present, but of no value when absent. The report by Rhodes (1959) that two NCTC strains of *Aeromonas* were sensitive to a soluble phosphate

derivative of O/129 complicates the issue. It may be that results obtained with the phosphate derivative are not strictly comparable with ours. Whatever the explanation, the fact remains that the distinction between these two genera is not clear. (Note: the authors recognize the value of simple diagnostic procedures in routine identification of bacteria, but suggest that the above comments show how important it is to base such procedures upon sound taxonomic knowledge of the organisms concerned. It is certain that reliance upon single characters, however well authenticated, can lead to errors being made. The literature of *Vibrio* is largely concerned with diagnosis rather than with taxonomy.)

Finally, we should like to draw attention to an important aspect of the work carried out in Asia upon the identification and classification of *Vibrio*. The term 'cholera and cholera-like vibrios' frequently occurs in this literature and there is good reason to doubt the true *Vibrio* nature of some of the bacteria studied. The definition of *Vibrio* commonly applied (Taylor, Pandit & Reed, 1937) and the isolation techniques used, would not guarantee exclusion of all organisms which we now recognize as *Comamonas*, *Pseudomonas* or *Aeromonas* strains. The study of Pandit & Maitra (1938) is a good example. The 'water-vibrios' of Heiberg's (1935) Group VI (non-fermenting strains), the 'non-fermenters' of Gardner & Venkatraman (1935), and Linton's chemical group III (see Linton, 1940) are also interesting in this context. It is difficult in most cases to tell whether the organisms which showed no reaction with the cholera somatic O antigen (e.g. Taylor & Ahuja, 1938) were in fact non-vibrios or just non-cholera vibrios. It is similarly difficult to analyse the results of Jackson (1948) who isolated 49 strains of 'vibrio' from 82 of 48 English surface-water samples (cf. Park, 1961*a*). The serological heterogeneity of most of these strains does not necessarily exclude them from *Vibrio*, but neither do the isolation methods nor biochemical results reported ensure that all the strains examined were vibrios. In the circumstances it is easy to see why such workers as Gray & Thornton (1928) allotted dubious organisms to *Vibrio*.

Only by making closer and more detailed taxonomic studies of organisms that workers at present consider as *Vibrio* spp., and by unifying the diverse interests in vibrio-like bacteria from various habitats, e.g., marine, soil, animal, human, can we hope to rectify the situation that exists and has been responsible for the inclusion in the genus *Vibrio* of *Bergey's Manual* (1957) of such a heterogeneous collection of bacteria.

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EXPLANATION OF PLATE

Electronmicrographs; shadowed gold-palladium (60:40) mixture. From 24 hr. growth on nutrient agar.

Figs. 1, 7. Strain 8563 (*Vibrio proteus*) showing straight and curved rods, spheroplasts and long dividing forms, single and bipolar monotrichous flagellation. Fig. 1, $\times c. 3000$; Fig. 7, $\times c. 4500$.

Fig. 2. Strain 30 (*Vibrio paracholerae*) showing rod forms and a spheroplast. $\times c. 4000$.

Fig. 3. Strain 2582 (*Vibrio neocistes*, *Comamonas* sp.) showing bipolar flagellation as exhibited by this strain and strain 2581. $\times c. 4500$.

Fig. 4. Strain 8194 (*Vibrio cuneatus*, *Pseudomonas fluorescens*). $\times c. 6000$.

Fig. 5. Strain 1987 (*Vibrio percolans*, *Comamonas* sp.). $\times c. 15,000$.

Fig. 6. Strain 9239 (*Vibrio alcaligenes*, *Comamonas* sp.). $\times c. 5000$.

Key to Tables 1-3

Flagellation:

1 = 1 or rarely 2, polar

M = 1 or several, polar

L = at one or both poles

+ = positive

$\pm$ = weak, slow or late reaction or growth

Fractional figures = number of strains positive/number tested (in some cases $\pm$ /tested).

— = no reaction, growth, etc.

A = excess acid

F = fermentation

Al. = alkaline reaction in open tube

NG = no growth

YG = yellow-green

Y = yellow

U = utilized

Numbers = day reaction detected

O = oxidation

Li = lipolysis

NT = not tested

B = black-brown

Notes.

(a) All strains examined were Gram-negative.

(b) In R.W.A.P. results, all strains cleared tributyrin, attacked malonate (Shaw & Clarke), grew at 30° and in 0.5% and 1% NaCl, and were susceptible to Terramycin 10 units and Chloramphenicol 100 units. None gave positive Eijkman reaction, utilized or produced acid from dulcitol, grew at 45° or in 9% NaCl, produced pyocyanin, or gas.

(c) In G.H.G.D. results, all strains grew at 25° and 30°; all utilized succinate and citrate. None produced acid from dulcitol, sorbitol, raffinose or inulin. None produced gas from glucose, gave V.P. + reaction, grew at 44°, produced dihydroxy-acetone from glycerol, were susceptible to bacitracin 5.5 units, oleandomycin 5 μ g., penicillin 2.5 and 1 units. All were susceptible to Terramycin 100 and 25 units.

(d) In Table 1, under 'Acid production (and utilization of) : ' +, $\pm$ and — refer to acid production only.