A GUIDE TO THE IDENTIFICATION OF THE GENERA OF BACTERIA

BY V. B. D. SKERMAN

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TO THE IDENTIFICATION OF THE
GENERA
OF BACTERIA

WITH METHODS AND DIGESTS OF GENERIC CHARACTERISTICS

BASED ON DATA GIVEN IN THE SEVENTH EDITION OF BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY AND ON ORIGINAL PAPERS

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THE WILLIAMS & WILKINS COMPANY

BALTIMORE 2, MARYLAND 1959

PREFACE

The Key to the Genera of Bacteria has been compiled with the aim of placing in the hands of research workers, teachers, and students, a volume in which general directives for the identification of bacteria are supported by a complete list of the techniques needed for the purpose. In addition, a Digest of data published for the various species in the seventh edition of *Bergey's Manual of Determinative Bacteriology* and in several original papers has been included. The publication of the Key and Methods in the one book should encourage a more general application of common procedures for the description of bacteria. The main purpose of the digests is to draw attention in a more definite way to the deficiencies in descriptions within the various genera in the hope that steps may be taken to rectify them. A Guide to Study has been provided to ease the burden associated with the assimilation of knowledge over this varied field of science.

The volume is intended as a supplement to the *Manual* itself, and it is hoped that its use will contribute materially to the development of future editions of the *Manual*.

I am indebted to the late Professor R. S. Breed, at whose invitation the present keys were written for inclusion in the seventh edition of *Bergey's Manual*, to the Board of Trustees of the *Manual* for permission to draw so freely on the contents of the *Manual*, and particularly to Dr. R. E. Buchanan for a very critical perusal of the manuscript.

I am also indebted to the numerous authors, organizations, and publishers who have so readily given their permission to reproduce the material which appears in the Methods and illustrations, and whose names appear in the references to the respective items.

In drawing up the Methods, I have made use of some which have been in use for a long period in this laboratory and for which the original references can not be traced. These methods bear no reference and acknowledgement is due to the original authors and publishers whoever they may be.

I should like to express my appreciation to the Carnegie Corporation of New York through whose financial assistance I was able to visit the late Professor Breed and many other people whose assistance to me in the compilation of the keys has proved invaluable; to Mrs. Anne Pope, Miss Barbara Carey, and Mr. Ian MacRae for the very great assistance which has made the publication of the volume possible; and to Miss Barbara Steele who typed the manuscript and whose unusual interest in the work has resulted in the elimination of many errors and omissions. I am also indebted to the Department of Photography, University of Queensland, for the preparation of line drawings and the development and printing of the photographic illustrations; and finally to those colleagues whose comments, acid and otherwise, have contributed to formulation of the Key.

V. B. D. Skerman
October, 1958

INTRODUCTION

The Key is essentially the same as that which appeared in the *Manual*. Certain additions have been made of genera which were omitted from the *Manual* or appeared in the literature after the manuscript had gone to press, and the material has been annotated more freely than in the previous key, although perhaps not as extensively as some may wish. A major alteration which has been made is the insertion at Section **H93** of the statement "Not as above . . . **94.**" This became necessary following the application of this general comparative approach in our laboratory when it became obvious that numerous organisms which are not listed as doing so have the power of liquefying Loeffler's inspissated serum. The insertion provides a shunt for these organisms. A second major alteration is the inclusion of the keys for the Order *Myxobacterales* and the organisms in the Class *Microtatobiotes*. In both cases the keys are merely reconstructions of those provided in the *Manual* and are reproduced in this form with the permission of the authors and publishers.

The Key departs in several places from a simple dichotomy. Such departures occur where (*a*) there has been some doubt as to the validity of the insertion, and the use of other than a dichotomy permits subsequent removal without reconstruction and numbering of the key; (*b*) there has been a late insertion which would have necessitated a complete renumbering of the key; and (*c*) it appeared more convenient to depart from the dichotomy as in Section B.

Shunts have been used where there is a divided opinion on interpretation or a test is of limited usefulness. By this device, organisms may be separated into two groups by a dichotomy. Certain genera are then separated from one group on specified tests and the remaining organisms rechanneled into the other group for subsequent treatment. One such shunt has been used in the treatment of the multicellular organisms and another, in the separation of the cocci.

An alteration has been made of the definition of terms given as a footnote to Section A. The author has received some comments on the use of the term, trichome —all indicating that its use is undesirable. In this the author agrees but the term is still used in an attempt to define the different ways in which it has been employed in the *Manual*. Its elimination from subsequent editions of the *Manual* and from current literature is desirable.

The use of the term multicellular also warrants comment. Bisset regards organisms which, in their more stable rod form, are found to be composed of a small number of cells, as multicellular, *e.g.*, *Nocardia* and *Corynebacterium*. He does not regard an elongated rod similarly divided but into much longer cells as multicellular, *e.g.*, *Streptomyces*. This seems a little inconsistent and if applied to such genera as *Oscillatoria* or *Vitreoscilla* would give rise to the undesirable division of genera into multi- and unicellular species. In the Key those organisms which show a clear subdivision in the rods without resort to special staining methods are regarded as multicellular, *e.g.*, *Caryophanon*. Provision has been made, however, for those smaller cells such as *Nocardia* by means of a shunt in the Key which admits them as multicellular although special methods are needed to demonstrate this.

From cytological examination there is no suggestion that these cells are physiologically interdependent. That they may be so in some cases is evident from the great sensitivity of *Beggiatoa* to mechanical transfer in attempts to isolate it.

Page references following genera in the Key and in the Digest of Genera are in two forms. Those preceded by the letter *M* refer to the pages in the seventh edition of

Bergey's Manual of Determinative Bacteriology; the others refer to pages in this volume.

As with the previous Key, the alternative terminology given by Topley and Wilson (indicated by (T and W, 4th ed.)) is used in the Key wherever the correlation between the two terminologies could be clearly determined.

The Methods have been collected from many sources. Most of them have been tested in the author's laboratory. In most instances the original procedure has been followed, but, particularly in the case of synthetic media for autotrophic bacteria, a complete new series of alternative media based on a common synthetic base has been provided in addition to the original methods. The methods are not to be regarded as "standard methods." It is hoped, however, that general application of them in conjunction with the Key will lead to a greater uniformity in descriptions at the generic level. In the presentation of the methods a few procedures for isolation of cultures, which have a direct bearing on the operation of the Key, have been included.

A fundamental objection to the use of a common procedure is that there is no guarantee that it is, in fact, the one used by the original author, or, if so, that subsequent authors have used a similar procedure. The objection is valid, but it is not impossible, even now, to re-examine species on a common pattern and continue to use such a pattern in the future.

The present indiscriminate use of tests is leading only to chaos. The adoption of a more uniform approach should result in a much clearer picture of the present state of knowledge and should act as a stimulus to more productive research.

It is very desirable that some measure of international agreement be reached on the matter of methods.

The Digest of Genera has been prepared from an analysis of data given in the *Manual*. In many instances, particularly in the case of older German literature on the sulfur or iron bacteria and with more recently described genera, a search of the original literature has been made. The object of the Digest is to present the facts relating to the descriptions of species in a manner which accentuates the deficiencies. With the exception of those organisms placed in the *Manual* in the Class *Microtatobiotes*, the digests are in two sections, namely, Differentiating Characters and Notes. The author has had no personal experience with the Class *Microtatobiotes* and has given under the heading of Differentiating Characters the generic description provided for each genus in the *Manual*. The differentiating characters given for the other genera are those derived by manipulation of the Key in reverse.

In the author's experience students automatically begin to trace keys backwards to determine the essential differentiating features. Because of numerous multiple insertions and the use of shunts in the Key, the practice is open to serious errors by those ignorant of the details of construction of the Key. For this reason the differentiating characters derived in this manner have been given. In the construction of the Key numerous assumptions had to be made to cope with lack of information. Where there has been any serious doubt the character has been treated as positive or negative with respect to the character. Where an assumption has appeared legitimate, such as the absence of chlorophylls from colorless organisms, it has been made. It is possible, of course, that some assumptions may not have been legitimate. For this reason the differentiating characters have in most instances been given in two parts. The first part includes those known characteristics, both positive and negative, which, in the author's opinion, most clearly define the genus. The second part includes those characteristics which have been assumed to be negative or are known to be negative and is introduced by the statement "do not or are not known to." The second part should not be regarded too

lightly. The absence of any reference to the liquefaction of inspissated serum by numerous organisms led the author to the wrong assumption that they were possibly negative. The application of this general comparative approach to identification has revealed numerous organisms to be positive in this matter. This information has never been recorded, hence the alteration made at H, **93**. It is not impossible that other corrections may need to be made.

The material included in the Notes should not be regarded as unimportant to the generic description. In some cases characters relegated to this section are uniform throughout the genus but have not appeared in the manipulation of the Key. In the compilation of the data in the Notes the number of species described has been listed, and for individual tests the number positive over the number tested is also given. For example, there may be 82 species in the genus. The indole test may have been cited for 47 and may have been positive for 28. In the text these facts would be summarized as "indole produced 28/47." A perusal of these data will give an idea of the extent to which species in each genus have been uniformly examined.

A Guide to Study has been included to aid students in taxonomic studies.

References to the higher taxa has been generally avoided. This has been done purposely to encourage a reconsideration of the evidence at the generic level.

TO MARGARET BREED

in appreciation of a silent service to microbiology

Library of Congress Catalog Card Number 59-13736
Made in the United States of America, at
Waverly Press, Inc., Mt. Royal & Guilford Aves.
Baltimore 2 Md., U.S.A.

CONTENTS

A COMPREHENSIVE KEY TO THE GENERA OF BACTERIA

USE OF THE KEY

First, determine the characters of the organism and then consult the Key, *always* commencing from the beginning. The Key poses a series of questions which can be answered in the affirmative or negative. Bold face numbers on the right hand side of the Key indicate the next number on the left to be consulted. The sequence should be followed until the right hand number is replaced by a generic name. Keys to the particular genus in the *Manual* should then be consulted for species identification.

1. Organisms green, blue-green or yellowish green, brown or red, containing chlorophyll "a" either in well defined chloroplasts or in the cytoplasm..... **Algae**
Organisms colorless or pigmented; if the latter, green pigments do not have the characteristics of chlorophyll "a"..... **2**

2. Diameter or width of cells exceeds 2.0 μ; proceed to.....Section A p. 2
Diameter or width does not exceed 2.0 μ; proceed to.....Section B p. 7

Note: a. In the assessment of diameter or width, measurement must be made of the cells themselves and not of any capsular structures or sheaths which may surround them. In Section A some cells have widths up to 100 μ and are clearly visible to the naked eye.

2. b. To avoid confusion the following terms have been used in the sense indicated below (for comment on the use of them see the Introduction):

Multicellular Organism: a group of cells, arranged uniseriately, which are joined for the whole or major part of their width and result in an organism having a lateral wall with little or no indentation and lack of articulation at the septa. The septa are clearly visible in unstained cells after the removal of inclusion substances such as sulfur or fat.

In the Key, small organisms which appear to be unicellular in unstained preparations and in preparations stained by Gram but which are distinctly multicellular after being stained to show cell walls are treated with the unicellular organisms. A note to this effect appears at the appropriate place.

Unicellular Organism: an organism which shows no evidence of dividing septa, other than those involved in a normal division, in unstained preparations or in preparations stained to reveal cell walls.

Cell: a unicellular organism.

Filament: an elongated rod which shows no evidence of multicellularity without the application of special techniques designed to demonstrate cell walls.

Chain of Organisms: a group of unicellular or multicellular organisms, arranged uniseriately, which are completely separated or are attached for only a minor part of their width and are freely articulate at the points of attachment.

The presence or absence of a sheath should not be taken into consideration.

Trichome: this term as used in the Key may refer to (*a*) multicellular, nonflagellated, gliding organisms; (*b*) chains of unicellular, nonflagellated, gliding organisms; (*c*) nonmotile, multicellular organisms; and (*d*) multicellular flagellated organisms. These cover the different kinds of bacteria for which the term has been employed in descriptions in the *Manual*.

The presence of a sheath should not be taken into consideration.

Sheath: a hollow structure surrounding a chain of cells or a trichome. It may be close fitting but is not in intimate contact with the cells. Sheath-forming organisms usually produce a gum-like holdfast and a gum-like secretion resembling a capsule, but as the length of the chain or trichome increases, the gum-like secretion resolves into a hollow structure, the sheath, which lacks intimate contact with the cells.

Capsule: a substance secreted by microorganisms which forms an envelope around the cell and remains in intimate contact with it. Its margin may be sharply defined or, owing to its relative solubility in water, merge imperceptibly into the surrounding fluids.

SECTION A

1. Multicellular organisms with or without a sheath *or* unicellular organisms arranged in chains and surrounded by a sheath... **2**
Unicellular organisms..... **18**

2. Multicellular organisms not in a sheath **3**
Organisms in a sheath..... **11**

3. Both trichomes and abstricted cells are nonmotile and are not flagellated..... **4**
Either trichomes or abstricted cells or both are motile. Motility may be either by gliding on solid surfaces (nonflagellated) or by means of flagella. (Apparently nonmotile but flagellated cells are included in this section)..... **5**

4. Trichomes up to 5000 μ in length attached basally by means of a globular holdfast; endospores produced in any or all cells within the trichome and obliquely situated; recorded from the alimentary canal of millipedes, cockroaches, and toads
Arthromitus p. 128
M p. 835
Long trichomes arranged in bundles; each cell contains one or more gas vacuoles which gleam reddish in transmitted light; do not deposit sulfur internally
Peloploca p. 81
M p. 270
Trichomes limited to four cells, the end ones being rounded; may be arranged in chains; recorded from the buccal cavity
Simonsiella p. 128
M p. 833

5. Trichomes flagellated, peritrichous.. **6**
Trichomes or abstricted cells not flagellated; motility of a gliding type on solid surfaces or along adjacent cells..... **7**

6. Organisms approximately 5 μ in width and straight to curved; develop a large endospore apparently by fusion of several cells within the trichome. The spore is normally centrally located. Division of the organisms is preceded by formation of biconcave discs within the trichome somewhat similar to those produced by the blue-green alga *Oscillatoria*; found in large numbers in the cecum of the guinea pig
Oscillospira p. 128
M p. 834
Organisms 3 μ or more wide; actively motile; do not produce endospores; common in fresh dung..... **Caryophanon** p. 127
M p. 831

7. Spiral cells.. **Thiospirillopsis** p. 129
M p. 840
No spirals..... **8**

8. Entire trichomes are motile..... **9**
Entire trichomes are not motile; attached by means of a holdfast; taper from the base to the tip; single cells formed by abstriction from the tip are motile by a gliding motion on a solid surface; trichomes characteristically arranged in rosettes but may occur singly; do not deposit sulfur internally..... **Leucothrix** p. 131
M p. 850
Entire trichomes are not motile; attached by means of a holdfast; taper from the base to the tip; single cells formed by abstriction from the tip are motile by a gliding motion on a solid surface; trichomes characteristically arranged in rosettes but may occur singly; deposit sulfur internally when growing in sulfide-containing waters
Thiothrix p. 129
M p. 842

Note: Harold and Stanier (Bacteriol. Revs., *19*, 1955, 49) were unable to find a

sheath on *Leucothrix* although the original description cited a prominent sheath. Pringsheim (Bacteriol. Revs., *21*, 1957, 69) reports that a prominent sheath does exist but cannot be demonstrated by means of nigrosin. It stains pink with methylene blue whereas the cells stain a dark indigo.

Winogradsky (Schwefelbacterien, Leipzig, 1888) also notes that the sheath of *Thiothrix* is almost indistinguishable in living specimens but becomes visible at the tip where hormogonia are separating or may be observed when degeneration occurs after removal of sulfide.

The insertion of *Leucothrix* and *Thiothrix* at this point in the Key is made to cover the possibility that sheaths may be overlooked.

9. Elemental sulfur is deposited in a globular form in the cells when growing in waters containing hydrogen sulfide

Beggiatoa p. 129
M p. 838

Elemental sulfur is not deposited internally..... **10**

10. Cells within the trichomes contain one or more gas vacuoles which gleam bluish or reddish in transmitted light; described from surface films in pond waters

Pelonema p. 81
M p. 271

Note: Pelonema is described as having a thin sheath and as possibly being motile. It seems unlikely that floating motile filaments would be ensheathed, and for this reason *Pelonema* is included here.

Not as above..... **Vitreoscilla** p. 130
M p. 845

11. Width of sheath increasing from base to tip; cells within the sheath divide transversely and longitudinally towards the tip to produce large numbers of coccoid elements; attached by means of a holdfast. **12**

Width of the sheath uniform or variable; division of cells in transverse direction only **13**

12. Cells within the basal portion of the sheath longer than wide; when growing in iron-bearing waters, the sheath becomes heavily impregnated with iron

Crenothrix p. 82
M p. 272

Cell within the basal portion of the sheath much wider than long; sheaths remain colorless in iron-bearing waters

Phragmidiothrix p. 82
M p. 273

13. Cells within the base of the sheath 2 by 10 μ with rounded ends; divide transversely near the tip to produce spherical, nonmotile cells which are extruded either singly or in chains. The sheath becomes heavily impregnated with iron or manganese, becoming wide at the base and tapering towards the tip; attached by a holdfast; false branching is common

Clonothrix p. 82
M p. 274

Spirally wound to straight chains up to 250 μ long; sheaths heavily encrusted with iron

Leptothrix p. 80
(*L. pseudovacuolata*) *M p. 264*

Note: The single species may be a *Sphaerotilus*.

Not as above..... **14**

14. Chains of cells enclosed in a sheath of uniform width; attached by means of a conspicuous holdfast; free cells motile by means of subpolar flagella

Sphaerotilus p. 79
M p. 263

Note: Species of *Sphaerotilus* have been shown to precipitate iron in the sheath, in which state they strongly resemble species of *Leptothrix*. Skerman, Dementjeva, and Carey (J. Bacteriol., *73*, 1957, 504) have shown that *S. natans* will also deposit sulfur internally. Although it has a superficial resemblance to *Thiothrix*, it differs in having flagella.

Not as above; if motile, not flagellated. **15**

15. Sulfur deposited internally when grown in water containing hydrogen sulfide... **16**

Sulfur not deposited internally..... **17**

16. Several trichomes within a common sheath..... **Thioploca** p. 129
M p. 841

A single trichome within each sheath; usu-

ally attached by a holdfast

Thiothrix p. 129
M p. 842

17. Colorless trichomes, attached at the base, tapering from the base to the tip; most characteristically arranged in rosettes but may occur singly. Constriction of the outer wall near the tips produces a beaded appearance. Single cells are abstricted and may exhibit a gliding motility on a solid surface. The trichomes themselves are immobile..... **Leucothrix** p. 131
M p. 850

Note: Harold and Stanier (Bacteriol. Revs., *19*, 1955, 49) were unable to find a sheath on *Leucothrix*, although the original description cited a prominent sheath. Pringsheim (Bacteriol. Revs., *21*, 1957, 69) reports that a prominent sheath does exist but cannot be demonstrated by means of nigrosin. It stains pink with methylene blue whereas the cells stain a dark indigo. Colorless trichomes up to 500 μ in length; each cell contains one or more gas vacuoles which gleam reddish or bluish in transmitted light; enclosed in a thin transparent sheath; occur singly... **Pelonema** p. 81
M p. 271

Note: Peloploca, which has a similar cellular morphology although described as "no sheath evident," and which occurs in bundles, should be compared carefully with *Pelonema*.

18. Spiral cells..... **19**
Not as above..... **23**

19. Cells contain bacteriochlorophyll and carotenoid pigments; cell masses various shades of red or purple..... **20**
Not as above..... **21**

20. Oxidize hydrogen sulfide, depositing sulfur internally... **Thiospirillum** p. 51
M p. 46

Do not oxidize hydrogen sulfide

Rhodospirillum p. 54
M p. 58

21. Rigid cells 6 to 50 μ long; actively motile by means of polar flagella; deposit sulfur internally when growing in waters containing hydrogen sulfide

Thiospira p. 59
M p. 82

Flexible cells; not flagellated; do not deposit sulfur internally..... **22**

22. Large, spiral cells with tapered ends, up to 100 μ long; protoplast wound spirally around a well defined axial filament; no cross striations; motile by means of a flexuous movement.... **Spirochaeta** p. 136
M p. 893

Spiral cells with a round cross section and blunt ends; up to 60 μ long; cells have a ridge or crista composed of numerous fibrils running along one side of the spiral; cross striations distinct; found in the intestinal tract of molluscs

Cristispira p. 136
M p. 895

23. Stalked cells; aquatic in habit..... **24**
Cells not borne on stalks..... **25**

24. Cells rod-shaped; 2 by 6 to 12 μ; single cells attached terminally and at right angles to branches of a lobose, dichotomously branched stalk; form globular bush-like or plate-like growths on the surface of waters..... **Nevskia** p. 84
M p. 216

Cells pear-shaped to spherical; multiply by budding; cells attached by a long slender stalk to a holdfast with several stalks frequently arising from one holdfast. (This organism has so far been found only in lake waters where the temperature does not exceed 23° C.)..... **Blastocaulis** p. 84
M p. 279

Cells pear-shaped; borne on a very short stalk; cells grow attached to each other in a cauliflower-like mass and reproduce by longitudinal division and budding. Colonies break up at intervals, and liberated cells start new colonies. Cells and methods of reproduction resemble those found in *Chaemosiphon*, a blue-green alga; discovered in the body cavity of fresh water crustaceans..... **Pasteuria** p. 84
M p. 279

25. Endospores produced..... **26**
No endospores produced..... **27**

26. Spherical cells in cubical packets
Sarcina p. 101
M p. 467
Rod-shaped cells... **Clostridium** p. 116
M p. 634

27. Cells contain bacteriochlorophyll and carotenoid pigments; cell masses are various shades of red, brown, and purple; proceed to..... Section J p. 43
Not as above..... **28**

28. Iron deposited on the cells or in capsules..... **29**

Note: In the absence of further information, these organisms are identified on their iron-depositing characteristics. Most iron organisms studied in pure culture metabolize the organic compound which forms the iron chelate, and the liberated iron chelates with some cell component. Citrate-utilizing organisms will, for example, release iron from ferric ammonium citrate. Accumulation of the iron in or on the cell may depend only upon the nature of the cell substance. Pure culture studies may place these organisms in more commonly recognized genera. Many more organisms, if tested, may fall into the following genera. They should also be treated as non-iron-depositing cells and should be followed through the Key.

Not as above..... **32**

29. Iron deposited as a torus, a solid ring partially or completely surrounding the cell in one area only, giving the cells the appearance of open or closed links of a chain **30**
Iron deposited uniformly over the cells or capsules..... **31**

30. Cells completely surrounded by a torus
Naumanniella p. 69
M p. 223
Cells only partially enclosed, appearing like a horseshoe. Flagella of unequal length borne at the open end
Ochrobium p. 69
M p. 225

Note: The type of flagellation suggests that this may be an alga.

31. Spherical cells 1 to 2 μ in diameter, 2 to 60 or more cells occurring in a primary capsule 10 to 20 μ wide; secondary capsules unite to form a mucilaginous colony; iron or manganese compounds are stored in the secondary capsules.. **Siderocapsa** p. 67
M p. 218
Cells coccoid to ovoid, 4.8 to 5.0 by 6.5 μ, forming short chains embedded in a thin mucilaginous layer; iron compounds stored in the surface membrane of the cells
Sideronema p. 68
M p. 220
Cells rod-shaped, 2.5 by 6 to 15 μ, straight or slightly bent; not encapsulated; iron or manganese stored on the surface or in the membrane of the cell
Siderobacter p. 69
M p. 226

32. Strict intracellular parasites occurring in the cytoplasm of conjunctival cells in cattle, goats, and sheep. Elliptical, coccoid, rod-shaped, and comma-shaped cells occur
Colettsia p. 40
M p. 961
See also Section K p. 45
Spherical cells produced in macroscopic fruiting bodies on decaying vegetable material or in culture; fruiting bodies sessile or nearly so. The cocci germinate to produce rod-shaped cells which glide on a solid surface; not flagellated
Myxococcus p. 135
M p. 883
Or..... **Chondrococcus** p. 135
M p. 886
See Section L for criteria for separation. p. 135
Pleomorphic cultures consisting of large and small cocci and small rod-shaped cells which are motile by means of a single polar flagellum; strongly halophilic, requiring 20 to 30 per cent salt for optimal growth; Gram-negative... **Halobacterium** p. 66
(*H. cutirubrum*) *M p. 207*
Not as above..... **33**

33. Cells spherical to ovoid, varying from spheres 5 μ in diameter to large cylindrical organisms 35 to 100 μ long; sulfur deposited internally when growing in the presence of hydrogen sulfide. In one of the two recorded

species, large crystals of calcium carbonate fill the cells; motile with a slow jerky rotating action when in contact with solid surfaces..... **Achromatium** p. 131
M p. 852

Cells spherical to ovoid, 5 to 20 μ in diameter, with the cytoplasm compressed in one end of the cell; sulfur deposited in the cytoplasmic layer; exhibits an extremely rapid darting motion in free solution suggestive of flagella, which have never been demonstrated; found in waters containing hydrogen sulfide, forming a tenacious web-like growth in a zone of critical hydrogen sulfide-oxygen concentration
Thiovulum* p. 59
M p. 81

Not as above..... **34**

34. Cocci varying in diameter from 0.5 to 4.0 μ; grow in a mineral salts-bicarbonate medium with formate as the only known source of available carbon, fermenting it to methane, CO_2, and possibly hydrogen; pH range, 7.4 to 9.2. **Methanococcus** p. 102
(*M. vannielii*) *M p. 473*

Not as above..... **35**

35. Spherical cells..... **36**

Rods, curved or straight..... **43**

36. Arranged in cubical packets
Sarcina p. 101
M p. 467

Not as above..... **37**

37. Motile by means of peritrichous flagella..... **38**

Nonmotile..... **40**

38. Gram-positive; cells occur in irregular clusters..... **Micrococcus** p. 99
(*M. cryophilus*) *M p. 455*

Gram-negative..... **39**

39. Cells coccoid only at pH 7.0 on peptone yeast extract acetate agar; develop into multicellular rods with peritrichous flagella under other conditions; do not fix atmospheric nitrogen... **Caryophanon** p. 127
M p. 831

Cells grow in nitrogen-free mineral salts media containing a suitable source of carbon, fixing atmospheric nitrogen
Azotobacter p. 84
M p. 283

40. Aerobic..... **41**

Anaerobic..... **42**

41. Gram-positive; cells occur in irregular clusters..... **Micrococcus** p. 99
M p. 455

Gram-negative; fix atmospheric nitrogen. The coccoid form is only part of a cycle of morphological forms, the initial stage of which is a large rod.. **Azotobacter** p. 84
M p. 283

42. Large cocci, 3 to 4 μ wide, sometimes bearing rod-shaped protuberances on opposite sides and at an obtuse angle to one another—a pleomorphic stage of a rod-shaped cell 0.8 by 2.4 to 10 μ; produce copious gas from peptone
Sphaerophorus p. 97
(*S. ridiculosis*) *M p. 441*

Spherical cells; pleomorphic, ranging in diameter from 0.7 to 2.5 μ; occurring in pairs, short chains, and in irregular groups; dependent upon glycine for growth in organic media. Glycine is decomposed to CO_2, NH_3, and acetic.. **Peptococcus** p. 102
(*P. glycinophilus*) *M p. 474*

43. Large, cylindrical, pear-shaped or slightly curved rods 3 to 14 μ wide; actively motile by means of a single polar flagellum; contain large spherules of calcium carbonate and may also contain sulfur..... **Macromonas** p. 59
M p. 80

Not as above..... **44**

44. Curved rods..... **45**

Straight rods..... **46**

45. Curved rods with a bunch of flagella inserted laterally in the concave part of the cell; anaerobic; recorded from the cecum of the guinea pig, the buccal cavity of man, and the rumen of the herbivore
Selenomonas p. 73
M p. 258

Curved rods with polar flagella; 1.7 to 2.4 by 6.6 to 14.0 μ; contain small globules of sulfur in the center of the cell and a single

* See notes on *Thiovulum*, p. 59

large volutin granule at each end
Thiospira p. 59
M p. 82

46. Cells 1.4 to 2.0 by 4.0 to 5.0 μ; motile by means of polar flagella; anaerobic to microaerophilic; ferments glucose, producing ethyl alcohol, carbon dioxide, and lactic acid..... **Zymomonas** p. 65
M p. 199

Motile by means of peritrichous flagella; grow in a nitrogen-free mineral salts medium, fixing atmospheric nitrogen
Azotobacter p. 84
M p. 283

SECTION B

Note: Criteria for the separation of the small, colorless flagellated protozoan forms from bacteria are very limited. Organisms which (*a*) when stained with Giemsa stain show a clearly differentiated nucleus and cytoplasm without preliminary acid hydrolysis, (*b*) divide along the longitudinal axis, and (*c*) possess flagella or cilia which are clearly discernible without staining are possibly protozoa. A cross section of the flagella of protozoa examined with the electron microscope shows a structure quite unlike that of bacterial flagella. The flagella of protozoa consist of a pair of central fibrils surrounded by nine pairs of peripheral fibrils all enclosed in a sheath (C. K. Pine, Exptl. Cell Research, *14*, 1958, 388).

1. Ultramicroscopic and filterable forms; strict intracellular parasites of animals and plants not cultivable on artificial media but transferable by contact or by arthropod vectors..... **Viruses**

2. Strict parasites occurring within tissue cells of animal hosts or on or in erythrocytes. With few exceptions, which have been treated under Section H, they cannot be or have not been cultivated in artificial media. Some can be cultivated in chick embryos or in tissue cultures. In the tissues or blood stream they occur *either* as spherical elementary bodies and initial bodies from 0.2 to 2.0 μ in diameter or slightly larger (usually 0.20 to 0.35 μ), singly or in aggregations in plaques several microns in diameter *or* as bacillary, triangular, ring-shaped, horseshoe-shaped, and other pleomorphic forms. Bacillary forms may be as long as 3 μ. Stain with Giemsa's or Macchiavello's stain without differentiation into cytoplasmic and nuclear structures, a condition which would be suggestive of protozoa..... Section K p. 45

3. Small, spherical bodies, 150 to 300 mμ in diameter, which germinate to produce filaments approximately 0.2 μ wide and from 2 to 50 μ long, sparsely or richly branching. At a later stage of growth small endomycelial corpuscles develop in the filaments by a process of successive condensation and constriction. As a result the homogeneous filaments are retransformed into chains of close-set spherical bodies which are released by fragmentation; highly resistant to penicillin and sulfathiazole; colonies on agar have a dense granulated central area which penetrates into the agar and which is surrounded by a translucent, flat, peripheral zone or consist of a pearly film containing numerous spots due to calcium or magnesium soaps; do not ferment lactose, sucrose, mannitol, or dulcitol
Mycoplasma p. 138
M p. 914

Note: L-phase colonies of some bacteria bear a strong resemblance to the colonies of *Mycoplasma*. They are generally more opaque, more heavily marked on the surface, tend to revert to the normal bacillary form in penicillin-free, semisolid media, are more difficult to subculture, do not require cholesterol for growth, and ferment the same carbohydrates as the parent organism.

4. Spiral cells; proceed to
Section C p. 9

This section does not include (*a*) all forms like *Vitreoscilla*, which, through their great length and extreme flexibility, are apt to coil in one plane in watch spring fashion; (*b*) spiral cells of the streptomyces type which arise from branching

Gram-positive filaments; or (*c*) chains of vibrios. The latter do not possess the true helical twist of the spiral organisms.

5. Spherical to ovoid cells which reproduce by production of a tubular outgrowth, 0.2 to 0.3 μ wide, from the cell on the end of which a daughter cell is formed. The tubular outgrowths may be simple or branched. Daughter cells are initially spherical but are later ovoid to rod-shaped; colorless or contain photosynthetic pigments.

Colorless cells, ovoid, 0.5 by 1.0 μ when mature; motile by means of a single polar flagellum; daughter cells may break loose from the tubular outgrowth and form tubes of their own while still actively motile

Hyphomicrobium p. 55
M p. 277

Cell masses salmon pink to a deep orange-red; cells ovoid, 1.2 by 2.8 μ; nonmotile; contain photosynthetic pigments; grow only under anaerobic conditions when exposed to light.. **Rhodomicrobium** p. 55
M p. 277

6. Spherical cells which reproduce by binary fission or by budding. Well defined stalks are secreted by some species, the budding form of reproduction being confined to the stalked types; proceed to

Section D p. 11

See also Section B, **10**..... p. 8

7. Vegetative cells, rod-shaped, not spirally twisted; Gram-negative. Microcysts produced in macroscopically visible fruiting bodies or occur loosely among elongated S-shaped, twisted or straight, flexible Gram-negative rods; germinate to produce rod-shaped cells which are motile only by a creeping action on solid surfaces. These rods may contract to form spherical microcysts or may combine in groups to form fruiting bodies in which the spherical or rod-shaped microcysts are formed

Section L p. 47

8. Rod-shaped cells, 0.5 to 1.5 by 2 to 5 μ, which grow in colonies on the surface of water containing sulfide and which deposit sulfur either inside or outside the cells. One species forms bladder-like gelatinous colonies with the bacteria embedded in the surface..... **Thiobacterium** p. 59
M p. 79

Note: This very poorly defined group is separated here because of a complete lack of information of other properties. It is suggested that any such forms, if found, should be keyed out in the section on rods to determine their possible taxonomic relationship. The presence of the sulfur around the cells in such a location may not be significant.

9. Rod-shaped and filamentous forms reproducing by binary fission, by fragmentation of the mycelium, by the production of endospores or conidia or by the production of microcysts; proceed to

Section E p. 14

10. Colorless, spherical cells 0.8 to 1.0 μ in diameter, arranged in parallel rows in flat sheets on the surface of liquid manure and culture media. The sheets break characteristically into squares, each of 16 cells. Grows well on acetate plus beef extract plus yeast extract agar. Single cells are rare

Lampropedia

Note: This genus does not appear in the seventh edition of the *Manual.* The description given by Pringsheim (J. Gen. Microbiol., *13*, 1955, 285) does not give a clear indication of the shape or size of the cells, emphasis being placed on the peculiar colony form of which an excellent illustration has been given. The information on morphology given above was obtained by phase contrast examination of a culture kindly supplied by Dr. Pringsheim. In preparations heat-fixed and stained by Gram, the cells are Gram-negative and appear to be lenticular in shape.

11. *Note:* Attention is drawn to a new group of microorganisms described by A. E. Kriss and I. N. Mitzkevich (J. Gen. Microbiol., *20*, 1959, 1) in a paper entitled "Krassilnikoviae: A New Class of Microorganisms found in the Sea and Ocean Depths". The authors give the following diagnosis: "filaments, non-septate, non-

ramified, diameter 0.4–0.5 μ may be enbe enclosed in a sheath. At one end form a cluster-shaped head, consisting of rounded bodies of diameter 0.5–2.0 μ. The number of round bodies in a cluster on one filament may amount to several scores. The organisms are widely spread in seas and oceans. May be found in considerable number at deep horizons. Have not been obtained in laboratory culture. Do not grow in conventional media or in isolated sea water samples. Rapidly develop on submerged fouling slides."

SECTION C

1. Organisms contain chlorobium chlorophyll *or* bacteriochlorophyll with carotenoid pigments 2

Organisms do not contain photosynthetic pigments 4

2. Nonmotile cells containing only chlorobium chlorophyll; appear distinctly green even under a microscope; may be found in *pure cultures* associated with other morphological forms such as rods and streptococcal forms with the latter often predominating; strictly anaerobic cells which oxidize sulfide, depositing sulfur outside the cells

Chlorobium p. 52
M p. 62

Note: This pleomorphism, recorded by van Niel, has been disputed by later investigators.

Cells contain bacteriochlorophyll and carotenoid pigments; red or purple in masses of cells; actively motile by means of polar flagella 3

3. Organisms grow autotrophically under anaerobic conditions exposed to light; oxidize sulfide and thiosulfate to sulfur, which is deposited inside the cells

Thiospirillum p. 51
M p. 46

Organisms will grow anaerobically when exposed to light but will not grow under strictly autotrophic conditions; require growth factors available in yeast extract; may oxidize sulfide but do not oxidize thiosulfate; sulfur is not deposited in the cells

Rhodospirillum p. 54
M p. 58

4. Uniseriate chains of cells enclosed in a sheath; impregnated with iron when in iron-bearing waters; spirally wound around themselves or algal filaments

Leptothrix p. 80
M p. 264

Note: Species of *Sphaerotilus*, considered by Pringsheim (Phil. Trans. Roy. Soc. London, Ser. B, *233*, 1949, 453) and others as identical with *Leptothrix*, frequently show spirally twisted, sheathed forms among normally straight ones.

Chains of curved rods wound into a ball within a nearly spherical capsule; do not store iron or manganese

Myconostoc p. 73
M p. 260

Spiral cells bearing a torus of iron hydroxide

Naumanniella p. 69
M p. 223

Very thin cells wound into tight cylindrical coils, 15 to 20 μ long; may be embedded in a capsular material when grown on silica gel. Slowly oxidize ammonia to nitrite

Nitrosospira p. 56
M p. 70

Not as above 5

5. Nonmotile trichomes spirally wound around each other in bundles; not ensheathed; cells within the trichomes contain gas vacuoles which have a reddish gleam in transmitted light

Peloploca p. 81
M p. 270

Motile trichomes having a slow, creeping, rotating type of motility on solid surfaces with the tips of the filaments oscillating; no flagella; deposit sulfur internally from sulfide-containing waters

Thiospirillopsis p. 129
M p. 840

Not as above 6

6. Cells parasitic on the protozoan, *Paramecium* 7

Not as above 8

7. Cells contain 1.5 to 2.5 spiral turns;