Flagellation as a Criterion for the Classification of Bacteria

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INTRODUCTION

The fascination of Leeuwenhoek's motile 'animalcules' continues to beguile some of the most sophisticated 20th century microbiologists. Despite impressive technical advances which now make possible more refined anatomical, chemical, and physical analyses of motility at the molecular level, present hypotheses concerning the unique feature of the phenomenon are still almost synonymous with "vital forces." Our ignorance about the energy supply for motility, and the mode of conversion of chemical into mechanical energy, is profound. Evidence that energy sometimes seems to be generated in the flagellum itself is inferred from reports such as that of McKeen (99), who described the swimming movements of detached flagella of Phytophthora fragariae for 12 to 15 sec. Adenosine triphosphatase activity has been reported in both Polytomella and trout spermatozoid flagella (94, 130, 145), and apyrase was found in the flagella of some phytoflagellates such as Chlamydomonas (136, 145, 152). No enzyme activity has ever been detected in bacterial flagella, but there is indirect evidence that energy-yielding mechanisms involving adenosine triphosphate (ATP) are concerned in bacterial motility. For example, oxygen was found to be essential for the aerobic activity of Pseudomonas flagella (115, 128). Stanier et al. (136) reminded us of Engelmann's discovery in 1882 that bacterial motility is an extremely sensitive indicator of oxygen evolution from photosynthesizing chloroplasts of Spirogyra. Under anaerobic conditions, Pseudomonas motility was activated by arginine (115, 128) by its enzymatic conversion into citrulline and adenosine triphosphate. Citrulline and ornithine apparently elicited about one-fiftieth of the arginine activity, but 18 other amino acids were ineffective. Both flagellate and nonflagellate cells, however, utilized arginine at the same rate, and therefore it was concluded that the flagella...
did not metabolize it. Later, the arginine-citruline enzymes were separated from the particulate cell fraction, and were not detected on the external flagella.

An elementary fact which may be overlooked is that microorganisms which possess a great variety of energy-yielding systems, i.e., photautotrophic and chemosynthetic bacterial with considerable substrate specialization, aerobes and anaerobes, oxidizers and fermenters, nutritionally fastidious parasites such as Bartonella, as well as many saprophytic and parasitic fungi, pigmented and nonpigmented algae, and aerobic and anaerobic protozoa, are all able to supply energy for flagella movement. An energy-yielding system common to these, and absent in related nonmotile mutants, presumably exists but it has not yet been identified.

Advances in our understanding of motility were made from genetic analyses of nonfunctional "paralyzed" flagella. Lewin (84) described mating experiments with 13 ultraviolet-induced mutants of Chlamydomonas moewusi with paralyzed flagella; it seemed that at least 12 genes were concerned with normal motility. Likewise, Joys and Stocker (64) found that in Salmonella typhimurium many structural genes controlled the functioning of flagella, and more genes were also present which controlled flagella synthesis. Such facts emphasize that in microorganisms a functional flagellum is not a trivial appendage, but a morphological feature which represents the combined expression of many genetic loci, controlling many metabolic processes which may or may not be subject to various environmental influences.

Although this review is designed primarily to answer the questions and criticisms concerning the value of bacterial flagellation for both identification and classification purposes, I feel that an appreciation of modern knowledge of the fine structure, biochemistry, biophysics, and genetics of all types of flagellation is important in assessing the value of this feature. Many advances have been made since van Iterson discussed this topic 10 years ago (146, 147). Some bacterial systematists still regard flagellation as insignificant. I believe that a description of a microorganism is not complete without a detailed description of flagellation, accompanied by a good photomicrograph or electron micrograph showing this characteristic and other morphological details. Even bacterial flagella are complex and organized protein molecules which may constitute 2.0% of the total cell protein. To man, therefore, bacterial flagella surely merit serious appraisal; to the bacterium, they may well represent life or death, e.g., in a toxic or nutritionally dilute environment. Lacey (71) remarked that motile forms often outgrow nonmotile ones. Furthermore, motile cells may carry unsuspected pathogens; Meynell (101) described a lytic Salmonella bacteriophage adsorbed only by the host flagella, and Kassanis and McFarlane (65) revealed that virulent tobacco necrosis virus was transported by the motile zoospores of Olpidium brassicae. Finally, man's survival is ultimately dependent upon significant gene-carrying motile unicells, namely, spermatozooids.

**Fine Structure of Flagella**

*Comparative Anatomy of the Flagella of Bacteria and other Organisms*

It is misleading to regard as homologous the flagella of all microorganisms and the flagella of motile unicells of higher plants and animals, despite their common motility-conferring function. The fact that flagella and cilia other than those of bacterial cells frequently show the familiar, yet still intriguing, general fine structure of nine distinguishable peripheral fibrils [often double and with one set of nine fibrils showing characteristic "arms" in transverse section (42, 130)], plus two central fibrils of a different type, in an apparently uniform matrix (8, 28, 42, 119, 152), invites evolutionary speculations. Electron microscope studies have revealed a basic nine plus two fibril pattern in both flagellate and ciliate protozoa (41, 45, 119), green and brown algal swimmers (52, 97), fungal zoospores (15, 97, 152), bryophyte and pteridophyte spermatozooids (97), spermatozooids of the house cricket (with an additional ring of nine accessory fibrils; 66), and very many kinds of vertebrate and invertebrate ciliated epithelium and spermatozooids (8, 28, 119): the range of the latter includes flat and round worms, molluses, echinoderms, frogs, fleas, rams, boars, bulls, and man. This pattern is not explicable in either functional or phylogenetic terms. A recent study of the flagellar movements of four sessile flagellates (132) reminds one that such organisms are exceptionally dependent upon flagella-induced water currents to bring food particles to the exactly appropriate part of the cell body for ingestion. Sleigh (132) carefully observed and measured flagellar movements by use of stroboscopic illumination, and thus found that very different water current patterns were generated in the four organisms (Monas, Actinomonas, Poteriodendron, and Codonosiga) studied. He postulated that structural adaptations, possibly involving mastigonemes, may have caused the different kinds of flagellar beat observed, i.e., in the form of flat-plane sine waves in Actinomonas, Monas, and Poteriodendron, and in...
the form of a helical screwing rotation in Codo-
nosiga botrytis. In Poteriodendron, a particularly
narrow funneling of the water current downwards
to the base of the flagellum was noted. Such
unexpected nonuniformity is not yet explicable
in structural terms. Attempts have also been
made to associate the plane of beat and actual
bending movements of active flagella of bull
spermatozoids (85), which show slight asymmetry
in the nine plus two fiber complex (see also 42).
Such microstructural studies are technically
difficult, and there are disagreements in the
interpretation of protein structures deduced from
the high-resolution electron microscope and
X-ray diffraction pictures. Nevertheless, very
refined analyses, probably at the molecular level,
were described by Johnson, Sikorsky, and Speak-
man (61, 62); they usefully discussed various
causes of technical error prior to their conclusions
concerning the fine structure of suitably treated
(thioglycolate and sodium tungstate) contractile
α-keratin protein microfibrils of merino wool, a
protein similar to bacterial flagella protein.

No nine plus two fibril structure has been re-
ported for a bacterial flagellum, but this dis-
tinction is probably not significant in evolution-
ary terms. The difference may be more directly
related to size and secondary adaptations, be-
cause other evidence does unite bacteria, blue-
green algae, green algae, certain aquatic phy-
comycetes which show anteriorly flagellate
spores, and higher vascular plants in an “evolu-
tionary continuum,” e.g., on the basis of a
common characteristic pathway of lysine syn-
thesis involving α-β-diaminopimelic acid (Vogel,
quoted in 98).

Chemical and Physical Properties of Bacterial
Flagella

The anatomically simpler bacterial flagella
often consist almost solely (98.0% often quoted)
of protein (67), in continuity with the cell cyto-
plasm; this was convincingly proved by the re-
tention of flagella on naked lysozyme- or penicil-
lin-induced protoplasts. The protein is of low
molecular weight, ca. 40,000, and is chemically
unusual in that it usually contains only about
14 or 15 amino acids and no detectable histidine,
tryptophan, hydroxyproline, cysteine, or cystine.
Astbury and others (2, 153) determined such
data for Proteus vulgaris and Bacillus subtilis,
and Kerridge (67) cited similar data for other bac-
teria. The term “flagellin” was coined by Astbury
for bacterial flagellar protein, and many chemical
and physical properties of different flagellins
were described after standard protein purification
procedures. The protein nature of flagellins was
further confirmed by properties such as their
irreversible denaturation at pH values of <4.0
or >11.0, or on boiling. X-ray diffraction tech-
niques revealed a structural similarity with the
knee contractile proteins, and flagellin poly-
peptides displayed two molecular forms simulta-
aneously: the normal form was folded in the
α-keratin configuration, and the contracted
molecules showed cross-β-reflections. Astbury
and his colleagues believed that the mixture of
the two forms of flagellins was due to death
while changing their molecular configuration,
“molecular rigor mortis.” They hypothesized
that, during live movements, rhythmical un-
dulations accompanied visible changes of chain
length, and were associated with the two molec-
ular forms. Photomicrographs of cells of non-
motile variants of S. typhimurium, however,
showed that “paralyzed” flagella were morpho-
logically identical with the normal flagella of
the serologically identical parent strain (37).
Meynell (101) confirmed such observations: both
“paralyzed” nonmotile and motile flagella of
Salmonella, again morphologically and serolog-
ically identical, also had the same wave form and
X-ray pattern (101), suggesting the presence of
both molecular configurations. Thus, no mor-
phological or serological structure seems unique
to either active or inactive flagella.

Electron Microscope and X-Ray Diffraction
Studies of Bacterial Flagella

Direct electron microscope studies of bac-
terial flagella have recently shown dissimilar
ultrastructures, in accordance with our longer-
established knowledge of chemical and sero-
logical differences between strains and species.
Stocker (138), however, emphasized that flagel-
llins of different strains of, e.g., Proteus or B.
subtilis, showed only slight differences in anti-
genic specificity, and suggested that the wide
range of H antigens in Salmonella might be due
to different arrangements of amino acids, rather
than to gross qualitative or quantitative dif-
f erences. Some differing H-antigenic types do,
however, show physicochemical differences, such
as the optimal pH value for acid agglutination:
PH 3.8 for antigens b, k, r and pH 5.0 for antigens
g, o, q of Salmonella was cited by Stocker (138).
These values may reflect differences in the iso-
electric points of the flagellins, and it is ambitious
to attempt to reveal such differences pictorially.

Early pictures, e.g., those of Houwink and
van Iterson (53), showed that bacterial flagella
were not all alike. Especially thin flagella were
found in Bacterium herbicola, Serratia marcescens,
and Agrobacterium radiobacter; Alcaligenes faecalis
flagella were more substantial, and those of
Spirillum serpens were thick; Proteus flagella
were 12.0 μm wide and thinner than the polar flagella of *Pseudomonas*. Both Gray’s *Bacterium* and *Photobacterium splendidum* had thicker polar flagella together with thinner lateral flagella on the same organism. Pijper (107) also noted the thick polar flagella of *Vibrio* and *Spirillum* and thought that these flagella were a direct continuation of the cell wall, but that *Salmonella typhi* flagella were different. These contentions are supported by recent observations on *Spirillum serpens* (104), and by pictures showing definite sheathed flagella on *Vibrio* (146) and *V. metchnikovii* (35, 44). Glauert et al. (44) found that the central core was ca. 120 A in diameter; they believed that it was proteinaceous and had a parallel subfibrillar structure, and possibly had a central hollow 30 A in diameter. The latter is relevant when considering bacteriophage penetration in those cases where the receptor sites are definitely located on the host flagella (101). The whole sheathed flagellum of *V. metchnikovii* was ca. 270 A thick, and Glauert et al. confirmed the continuity of the sheath and cell wall (same kind of layered structure in both). Fullett and Gordon (35) found that *V. metchnikovii* flagella showed only one antigenic specificity; they discussed the possibility that the sheath was only physically different from the core, but electron microscope studies led them also to believe in a flagellin core covered with a sheath-cell wall origin. The sheaths of *Vibrio* and *Spirillum* (104), together with the sheathed stalks of *Caulobacter*, clearly shown in 1950 by Houwink and van Iterson (53) and by Poidexter and Cohen-Bazire (112, 113), perhaps suggest taxonomic relationships. The facts that the sessile, stalked *Caulobacter* spp. produce vibrioliike motile swarmer cells (112), and that there is a similarity of deoxyribonucleic acid (DNA) base composition values and other physiological properties, are pertinent. Poidexter distinguished caulobactera, pseudomonads, and vibrios by means of specific bacteriophages, and by the recognition of the unique cellular differentiation into stalked cells and swarmer cells in caulobacters (112). When assessing the taxonomic significance of sheathed flagella, it is well to remember that sheaths have also been reported for *Proteus* (146) and *Bacillus brevis* (27).

Electron microscope studies of spirochete flagella (6, 86, 122) have clarified many controversies about the relationship between spirochetal axostyles (axial filaments) and flagella. The location of axial fibrils alongside the protoplasmic cylinder, and internal to a three-layered outer envelope, the cell membrane or “sheath,” is clear in transverse sections of *Treponema microdentium* (86) and *Leptospira pomona* (122). Listgarten and Socransky (86) emphasized the resemblances between axial filaments and bacterial flagella, namely, (i) α-keratin fibril structure, (ii) size, (iii) “hooklike bends” near the site of insertion, and (iv) special basal attachment structures in the cytoplasm. A specialized complex structure, a “terminal organ,” to which the axostyle is attached in *Leptospira pomona*, was described and clearly illustrated by Ritchie and Ellinghausen (122). The chemical composition of axial filaments is not yet known, but homology with bacterial flagella is a reasonable assumption (86, 122). The fact that the spirochete fibrils are positioned alongside the cell cytoplasm, whereas bacterial flagella pass through the cell wall, is not a unique situation. Some protozoan trypanosomes also show such enclosed flagella, and it is interesting to speculate on possible parallel evolution to explain these and other similarities between protozoan flagellates and the bacterial spirochetes. Organisms with enclosed flagella are not necessarily nonmotile; a simple model suggesting a new type of locomotion, mediated by the axial filament, was discussed by Ritchie and Ellinghausen (122).

Flagellins have, in a few cases, been examined by X-ray diffraction techniques as well as with the electron microscope, and some finer, regular structures have been described. van Iterson (146) reported that a diphtheroid flagellum 190 μm in diameter was composed of three strands, forming a triple-threaded helical screw. More recently Lowy and Hanson (90) illustrated that the flagella of *Pseudomonas* and *Proteus* in the type 2 phase (only) showed five plus five and four plus four helical fibrils, respectively. Two forms of fine structure were also demonstrated in attached flagella of *S. typhimurium* (91); in *Pseudomonas rhodos*, both types of fibril structure were, however, found in different parts of the same flagellum. Dimensions of the globular subunits composing these fibrils have been given by the various authors. *Leptospira* and *Borrelia* fibrils had a different spacing of the ca. 80-A diameter polygonal subunits compared with *Proteus* (90) or the 50-A globule of *Salmonella* (91). Sometimes the globules are thought to lie in rows parallel to the flagellum axis; in other cases, a helical arrangement is discernible (90, 91). Current data on this point were cited and discussed for a variety of microorganisms in a recent paper by Lowy and Hanson (90a). Obviously, we should like to know whether such ultrastructural differences may be correlated with chemical or antigenic differences, and thus be of greater taxonomic value. Much of this research, however, is directed towards the detection of possible structural changes associated with the contraction of
muscle fibers or flagellin molecules during movement. Speculations on flagellar movement at the molecular level were fully discussed by Silvester and Holwill (130) and Lowy and Hanson (90a). Acute Fleming-like observation of an accidental contamination of certain experimental material, namely, muscle fiber preparations at 4 C, is interesting. Fortunately the contaminant was that ubiquitous flagellate, *Pseudomonas fluorescens*, and the similar fine structure of its flagella and the muscle fibrils apparently diverted Lowy and Hanson to their profitable findings (90, 90a, 91). The microscopic and submicroscopic helical forms of active proteinaceous flagella, both in the 11 fibrils and in the matrix (152), or in the bacterial flagellins (90, 90a, 91, 130), are relevant to Pirie’s (152) stimulating suggestions concerning the problem of the origin of mechanical energy. He cited, for example, the hypothesis of Szent-Gyorgi that proteins may act as semiconductors which lead electrons from one site to another, energy being delocalized as a wave, and Marshak’s postulations that various helical structures seen in cells might produce biologically important fields which absorb electromagnetic radiations.

In the more structurally elaborate flagella of algae and protozoa, considerable amounts of lipid and nucleic acid may also be present. The flagella of *Crithidia* (Strigomonas) *oncopelti*, for example, consist of 72.0% protein, 27.0% lipid, 0.5% nucleic acid, and 0.5% carbohydrate (17), with the proviso that the latter two components might represent cytoplasmic contamination. The significance of the nonprotein components is not known.

**Synthesis and Organization of Flagella**

Some of the factors governing the actual synthesis of flagella are now being defined, especially since biochemists exploited the easily separated and highly pure flagellins for studies of protein synthesis. This aspect was reviewed by Kerridge (67), who emphasized that factors responsible for the organization of synthesized flagellin molecules into functional flagella were not known. This problem has been studied mainly anatomically in some of the larger algae and protozoa where various essential accessory structures are visible. Elaborate networks of fibrils linking basal granules, and forked roots of flagella and cilia, are revealed in suitably fixed and sectioned material. The complex infraciliatures which appeared to function as a "primitive nervous system" for the coordination of ciliary movement in some protozoa were reviewed by Weiss (155) and Grimstone (46; see also 28, 119). Differentiated structures joining together the roots of a subpolar ring of ca. 120 flagella on an *Oedogonium cardiatum* zoospore are clearly seen in recent pictures from the laboratory of I. Manton (52), to whom we are much indebted for her pioneering electron microscope studies of a commendably wide range of motile plant cells (see 97 for these references).

Stocker (138) realized the non-necessity of similar elaborate internal structures to coordinate the movements of bacterial flagella, because hydrodynamic forces alone would ensure "in-phase" aggregation of separate, active, bacterial flagella. A purely mechanical explanation was offered. Caution is always prudent when comparing the behavior of any nucleated organisms and bacteria. Besides the different arrangement and behavior of the chromatinc material, in bacterial reproduction cytoplasm is transmitted at every fission, and cytoplasmic factors such as episomes are known to govern the expression of such features as maleness and drug resistance in *Escherichia coli*, and tyrosinase inheritance in *Streptomyces*. Stocker, Quadling, Kerridge, and others have been compelled to conclude that an intracellular "system" or "motility-conferring particles" govern the functioning of flagella (to be discussed later); these might be located in the cytoplasm as cytoplasmic genes or episomes. They may also occur in higher organisms, in addition to blepharoplasts and other flagella-associated structures.

Recent confirmations of earlier observations (see 28 and 152 for bibliographies) describing the origin of the basal granule (blepharoplast) of the flagellum from the divided centriole of the nucleus of some algae and fungi are interesting. A "rhizoplast" structure seemed to maintain a connection between the blepharoplast and the nucleus (152). This has also been reported in the protozoan *Naegleria grübei* (111, 158). This ancillary structure is probably concerned with flagellar motility, and may eventually be isolated and studied further. The remarkable ability of a single cell of *Naegleria* to exist in either the flagellate or amoeboid form was described in some detail by Willmer (158) and Pittam (111). The phenomenon has obvious intrinsic interest and is also taxonomically important, because the protozoan classes of amoeboid Rhizopoda and flagellate Flagellata are so widely accepted. The conversion of the amoeboid form to the flagellate form by transfer of *Naegleria* from a solid medium to distilled water occurred in 30 min. Similarly, certain myxomycete plasmodia, *e.g.*, *Arcyria cinerea*, produced pseudopodia which, after constriction from the parent thallus, rapidly (within about 30 min) developed into motile flagellate swarmer cells (68). During these processes, cell
surfaces were obviously changed, flagella components both synthesized and organized, and in *Naegleria* the contractile vacuole and mitochondria also showed an associated pattern of change and activity. Vitamin A also affected the formation of the flagellate phase. In the absence of vitamin A, mucin was formed instead of flagella by *N. grüberi*. In the respiratory epithelium of developing vertebrates, there is also a reported antithesis between the formation of mucin and flagella (158), no single developing cell being able to form both. Perhaps it is pertinent that many motile but nonflagellate blue-green algae and some bacteria, especially the trichomatous types such as *Oscillatoria* and *Beggioa*, and also the swarming flexuous myxobacteria, secrete polysaccharide mucilages. In the *Naegleria* studies, the importance of the blepharoplast was again emphasized, and thus catalyzes the search for an equivalent structure at the base of bacterial flagella. Basal granules have been convincingly demonstrated in several bacteria; e.g., *Proteus vulgaris* and *Vibrio metchnikovi* (53); in *Vibrio* and *Spirillum* (146); in *Spirillum* spp. (156), where sometimes there was a granule at the base of a polar tuft of flagella (in marine and fresh water species) and sometimes only one basal body (in one marine species); in *Clostridium* (123), in *V. comma* (142), and in *V. metchnikovi* by Glauert et al. (44), who depicted a distinct basal disc about 30 A wide. These authors (44) did not believe that basal granules were always present; they suggested a simpler hooklike attachment in *Proteus*, such as was postulated for *Agrobacterium radiobacter* by Houwink and van Iterson (53), with supporting pictures of detached flagella with terminal hooks (claimed to be abundant in the upper layers of broth cultures). Distinct blepharoplasts are often only clearly visible in old and autolyzing cultures, so they may be artifacts caused by cytoplasmic coagulation. Many attempts to show them in sectioned material have only just begun to yield pictures of slightly specialized zones at the probable point of flagellum insertion. Murray and Birch-Andersen (104) depicted a more electron-transparent polar region with modified membrane structures in *Spirillum serpens*, but the detailed mode of anchorage of the flagellum was not visible. van Iterson and Leene (148, 149) located sites of tellurite-reducing activity at possible speculated sites of flagella origin or synthesis, in sectioned glutaraldehyde-fixed material, and showed zones of reduced tellurite of very different appearance in *B. subtilis* and *P. vulgaris*. Two chondrioid organelles were obvious in *Bacillus*, but a delicate matrix of reduced tellurite granules contiguous with the cytoplasmic membrane was evident in *Proteus*; these zones were possibly concerned with protein (flagellin) synthesis. Kerridge (67) believed, on the evidence of the incorporation of labeled amino acids into the flagella of *Salmonella*, that both the cytoplasmic membrane and the 70 to 80S ribosomes (which may have been associated *in situ*), and possibly also an associated protein, were concerned in the process. DNA was apparently unnecessary for the formation of the flagellin-synthesizing system, because he obtained an increase in the number of flagella per bacterium after flagellar regeneration in the presence of DNA-inhibitory 5-fluorouracil and fluordeoxyuridine. The synthesis was not penicillin-sensitive, although no synthesis was detected in penicillin-induced protoplasts. Therefore, it seemed that the absence of the cell wall prevented the organization of flagellin molecules into flagella. This and other evidence led Kerridge to believe that a "flagella-forming system" must be synthesized (see 67 for full discussion). It seems possible to me that different factors might be involved in the formation of polar and peritrichous flagella. Polarity flagellates cells show a definite antero-posterior differentiation, and therefore their growth-regulation mechanisms may be very different. New polar flagella seem to arise rapidly after cell division [within 30 see in *Pseudomonas aeruginosa* according to Jacherts (59)], and during personal investigations I never detected young *Pseudomonas* rods without any flagella. Young cells bearing short, straight flagella have been recorded for a few peritrichous flagellates, e.g., *Salmonella* (116), and 4-hr *Proteus* (53), and in young cells of aeromonads and chromobacteria showing "mixed" polar and lateral flagellation. The locations of general growing points or the more specific zones of flagellin synthesis in all bacteria are still largely undetermined. The distribution of tritium-labeled compounds in peritrichous *Salmonella* supported the hypothesis of intercalary growth; Kerridge discussed this (67). There is still little information concerning the presence of any polar flagella in peritrichously flagellate bacteria (67), and, when present, whether they are the same as the lateral flagella or not. For example, Bisset and Hale (4) claimed that in actively growing "budding" cultures of *B. subtilis* there was a concentration of flagella at one pole, and that new flagella originated later at the opposite pole, but there was no description concerning the origin of the numerous lateral flagella. Houwink and van Iterson (53) showed clear pictures of thicker polar flagella and thin lateral flagella in Gray's *Bacterium* and in *Photobacterium*. This will be discussed later in connection with the findings that a pure isolate may
contain organisms with polar flagella only, or, simultaneously, cells with both polar and lateral flagella (80, 134). Perhaps some of the growth zone problems might be investigated by use of highly specific fluorescent flagellar antisera and living cells, such as described by Walker and Batty (150, 151) to follow antigenic changes on the surfaces of Clostridium sporogenes during spore formation and germination, and to type the flagella of different types of C. botulinum. Different-aged zones in the cell walls of E. coli and Streptococcus faecalis were similarly revealed by Chung et al. (18); maybe such specific techniques will eventually be applicable to thin sections.

At this stage, we have insufficient data to assess the taxonomic value of any of the foregoing indications of grosser or finer structural differences in bacterial flagella; one hopes that some of the well-established and stable differences in amino acid composition (1, 67), and specific antigenicity of the flagella of different bacteria, will eventually be confirmed independently in terms of structural differences visible in high-resolution pictures. The prospects are encouraging.

**Flagellation as a Criterion for Classification of Higher Protista**

Details of flagellation, such as the number, length, site of origin on the organism, presence or absence of one or two rows of lateral hairlike mastigonemes, tapering or blunt ends, are still very widely and unquestioningly accepted as of great value for the classification of many algae, protozoa, and the two groups of phycocyanetes, Uniflagellatae and Biflagellatae, which have motile reproductive cells. Historically, the first algal classifications which utilized the more obvious morphological and reproductive characteristics caused much confusion and many inconsistencies. Then it was realized that the different pigments (photosynthetic and accessory), the different kinds of major intracellular storage products, and some cell-wall characteristics exhibited by different kinds of algae were all closely correlated with the various types of flagellate cells (mature unicells or the motile reproductive cells). Therefore, these joint, correlated criteria were used to differentiate major groups of algae (classes) much more satisfactorily (38). It is now also generally agreed that the two classes of algae showing no flagellate members, i.e., the Rhodophyceae and the Myxophyceae, each show clear evidence of a separate ancestry. Algal classification was fully discussed by Lund (92) in a most useful illustrated review. He cited many examples which showed that detailed flagellation structures were often very reliable generic characters. Bourrelly’s 1957 (7) suggested revised classification of the Chrysophyceae, for example, was based upon flagellation differences, such as the number of flagella, their individual lengths (which may or may not be the same), whether they are naked (acronemate) or covered with fine lateral mastigonemes (pantonecous), or whether an additional filiform appendage (a haptonema) was present. These criteria enabled a demarcation of several flagellately uniform series within the class Chrysophyceae. These series were ranked as orders by Bourrelly, an elevation disputed by other phycologists. Many, e.g., Lund, feel that, although flagellation details are of great usefulness to differentiate genera of either adult or reproductive cells in the Chrysophyceae, Xanthophyceae, Chlorophyta, Euglenophyta, and Cryptophyceae, other closely correlated features are desirable before assigning such high taxonomic status to flagellation details. Nevertheless, the usefulness of the recognition of relationships between certain adult nonmotile algae, e.g., palូmellloid forms embedded in mucilage, which produce motile gametes showing a recognizable type of flagellation, has been proved. This criterion has also enabled the recognition of pleomorphic coccal, filamentous, and flagellate forms of the same organism; sometimes these had been assigned to different genera. Naturally-occurring colorless variants of many algae may also be more easily identified and classified if they are flagellate, as exemplified by the recent illustrations of the two dissimilar flagella of the colorless biflagellate protonad, Bodo (12).

Ciliary or flagellate organelles have also long served as “key” characters for the differentiation of the two classes, Flagellata and Ciliata, of the protozoa. This was discussed by Corliss (24), and, in modern revisions of both ciliate and flagellate taxonomy, cilia and flagella and their associated structures (“infraflagellate” and “mastigon”” systems) are still accepted as fundamental and primary features.

**Criticals Concerning the Taxonomic Value of Bacterial Flagellation**

Because bacterial flagella are structurally and chemically much simpler structures and are not strictly comparable with those already discussed, it is wise to question whether flagellation is an equally fundamental characteristic for bacterial classification, or whether by utilizing this feature we are being misled by the opinions inherited from our pioneer botanically trained taxonomists. The records of Migula, Beijerinck, Winogradsky,
Smith, and many others leave us in no doubt that they considered nonmotility, polar flagellation, or peritrichous flagellation of supreme importance. It was a prime determinant in the classification systems of Migula, Lehmann and Neumann, and Erwin Smith. Little sympathy was afforded to those pre-electron microscope systematists who were incapable of determining both the presence and location of bacterial flagella. Need more be said in answer to current complaints that flagella-staining techniques are difficult and time-consuming (frequently with the reprehensible rider that, therefore, they are of little value for classification purposes)? A second reason, rather more commendable, which is often given in denial of the usefulness of flagella-staining for identification or classification purposes, is that the results are variable. The third reason commonly proffered is that the feature is of little taxonomic value. These three criticisms are considered below.

A short digression is perhaps necessary. The structures to be discussed are those actively concerned with the locomotion of bacterial cells, and not the once-confusing surface structures variously named "fimyel," "pili," or "fimbriae." These are much thinner, straighter, and more numerous structures visible only under the electron microscope (see 30, 31, and 144 for pictures and further information). They occur independently of flagella, and, unlike flagella, are resistant to proteolytic enzymes, ribonuclease, deoxyribonuclease, and low pH values (11). Neither are strands of hardened slime significant, and we owe much to Piper (107), who provokingly compelled us to study bacterial flagella much more carefully to distinguish among true flagella, fimbriae, and random strands and twirls of fixed and stained polysaccharide.

**Criticism of Flagella-Staining Techniques as Difficult and Time-Consuming**

Techniques involving the use of the light microscope, after the growth of motile organisms and suitable fixation, mordanting, and staining procedures, are very simple and certainly not more time-consuming than serological or pathogenicity test methods, or indeed many routine biochemical tests. Leifson's modified method (73) is widely used, and his many publications, including his recent *Atlas of Bacterial Flagellation* (75), together with his legion of disciples, are sufficient testimony to the satisfactory nature of the method and the value of the results, with a very wide range of microorganisms. I have used Leifson's method (and others) and found it satisfactory, but I prefer an equally simple silver-plating technique (121).

With electron microscope facilities, flagella are relatively easy to see by use of either shadowing techniques or negative-staining methods. The emphasis of Rhodes (121) in the case of light microscopy, and Hodgkiss (49) who used the electron microscope, concerning the impossibility of viewing accurately a three-dimensional body in either microscope, cannot be over-emphasized. The remedy is simple; errors are avoidable if a sufficiently large number of flagellate cells are examined.

**Criticism of Flagella-Staining Results as Variable, Unreliable, or Unrepeatable**

The above criticism implies many things, and is too general to answer; thus, more precise meanings are discussed below.

One criticism of flagella-staining results is that flagella are not seen on stained preparations although the organisms were motile. The reasons for this are often technical. From personal experience, I submit that failure to see flagella is very often due to the lack of appreciation that there is an obligate need to focus, exactly, the condenser of a light microscope in order to resolve flagella, which may be extremely fine even in mordanted and silver-plated preparations. Patience is also needed to search sufficient fields; the edge of a dried drop is usually the best area for aerobes. The staining technique may have been faulty, especially if the excess mordant is not thoroughly removed by repeated washing with distilled water before silver-plating (121). A too dense background may result if a broth instead of a nutrient agar-water slope culture is used as a source of motile organisms, unless adequate time is spent on the essential preliminary washing and centrifuging procedures advocated by Leifson (73).

Occasionally, the unthinking worker may profit in that apparently nonmotile organisms may show flagella after suitable staining. Such a situation may be fairly common; it was reported (102) that six of seven strains of *E. coli* had flagella, although the cells were nonmotile, when grown for more than 18 hr at > 23 C. This effect of higher incubation temperatures on motility was fully discussed by Kerridge (67). He concluded that in *S. typhimurium* the presence of nonfunctional flagella and consequent nonmotility at 44 C, compared with normal motility at 37 C, was a phenotypic effect, caused by failure to form some factor, such as a particularly heat-labile enzyme, which was necessary for the synthesis of a system able to confer motility upon the reversibly paralyzed flagellins. Morrison and McCapra (102), using absorbed sera,
found antigenic differences between paralyzed and normal flagella in 7 of 16 strains of E. coli Type 1, so this phenomenon may be of considerable theoretical and diagnostic importance if it is widespread.

A second criticism of flagella-staining results is that the same isolate showed different or no flagellation when the conditions and techniques were exactly repeated. Within my limited experience of about 500 Pseudomonas isolates and a variety of cultures used for teaching purposes, I have never found significant differences in the flagellation pattern when the environmental conditions were exactly repeated, although there are many claims to the contrary. It would be valuable to study this point with more precision with the use of cultures derived from single cells, isolated perhaps by the simple-sounding technique described by DeVay and Schnathorst (29). There are reports (117) that even in genotypically homogeneous Salmonella not every isolate was motile, and also that isolates phenotypically nonmotile could give rise to motile mutants. Meynell (101) induced motility in a nonmotile mutant by the elimination of a lysogenic bacteriophage. This DNA effect and many other genetic factors affecting flagellation are now better understood, but much care is needed in the interpretation of genetic experiments. Even in genetically homogeneous populations, the expression of genetic material is subject to many kinds of very complex regulation. For example, both structural and regulatory genes can themselves mutate; gene action may be blocked by "feedback" inhibitory or other repressive mechanisms, which may themselves be activated or inactivated by very small amounts of compounds, including those of low molecular weight or even ions, such as iron or aluminium in the case of flagella. Also, a very transitory environmental influence may result in flagellation differences which may be perpetuated for many generations. An excellent general discussion of such phenomena was given by Pontecorvo in the 1963 Leeuwenhoek lecture (114). Genetic studies affecting various aspects of flagellation will be discussed in later sections.

A third criticism of flagella-staining results is that minor changes in environmental factors result in different types of flagellation, or loss of flagellation. The "minor change" implied might be a difference in the detailed composition of the medium, temperature, pH value, or degree of aeration. Such critics imply that the character is therefore too transient to be "basic," "fundamental," or even "useful." Vague generalizations such as this are again best answered by considering specific examples.

Loss of flagellation. This phenomenon discourages many, but, to decide whether the loss is permanent, a few elementary points must be remembered. Motile cultures require an adequate liquid phase; "sloppy" agar has long been used to encourage motility for serological typing of flagellar antigens, but this, or the syneresis water of an agar slope, is often not of sufficient volume. Organisms should be in the logarithmic phase and actively growing, because flagella have a limited life span. Quadding (116) showed that S. typhimurium flagella were not synthesized continuously but in bursts, and it was estimated (139) that even in a logarithmic-phase culture only about 50.0% of the organisms might be fully flagellate at one instant. A too acid or too alkaline medium will cause flagellin disintegration. Also, it still seems not to be widely appreciated that the temperature for optimal flagellation is frequently much less than that for optimal (most rapid) growth; there are many reports of abundant flagellation at <25 °C, whereas replicates at >30 °C showed cells with few or no flagella. Abundant peritrichy was evident, for example, on 81 strains of Listeria grown at 20 °C, but there were few flagella and poor motility at 37 °C (81). A useful comprehensive table with full references to data of this kind was given by Lacey (71). Aeration is certainly essential for the motility of aerobes (115, 128, 136), and, as already discussed, anaerobic motility may be stimulated by arginine (128). Probably the oxygen and temperature factors are closely related, because oxygen is 46.0% more soluble in water at 10 °C than at 30 °C. The detailed composition of the growth medium is also important. Both iron and aluminium can suppress flagellation, as shown by Weinberg and Brooks (154) in B. megaterium and B. subtilis, and by others (135) with Bacillus and Proteus. Lacey (71) fully discussed nongenetic variation of flagella with special reference to their antigenic properties; his comprehensive Table 1 summarized the effects of such factors as temperature, light, surface-active compounds, drying, acid, salt balance, ammonium nitrogen as sole nitrogen source, 0.1% phenol, and penicillin on the flagella of a wide range of flagellate bacteria and algae.

Concerning genetic determinants of flagellation, especially in Salmonella, Stocker, Zinder, and Lederberg (140) found that they could induce motility in nonmotile strains of S. typhimurium by means of bacteriophage-induced lysates derived from motile hosts. The flagellar type thus induced was that of the recipient strain, a most useful discovery which enabled flagellar typing of the taxonomically difficult nonmotile salmonellae. These workers deduced that at least six
nonhomologous genetic factors were involved in the flagellation of *S. typhimurium*, and that the mutation of any one of them might result in nonmotility. They also concluded that at least two additional genes were directly concerned with the locomotor function. Later studies of bacteriophage-induced motility by Stocker and Quadling (see 118 for full references to many earlier papers), who used *Salmonella*, resulted in the hypothesis of the "unilinear transmission of motility," because they discovered that the flagella on a dividing cell were shared equally during cell division under conditions which prevented the synthesis of new flagella (i.e., growth at 44°C). Within three generations, the average number of flagella per bacterium fell from 8.2 to <1.0, and the proportion of nonflagellate cells increased from <5.0 to >70.0%; soon the whole culture became nonmotile. Stocker first suggested that each active normal flagellum was associated with a "motility-conferring particle." Such particles were thought not to be replicated, but to be retained by one of the daughter cells during fission and thus passed down in a unilinear manner for at least 20 generations (60 reported by Lederberg). Therefore, if growth conditions precluded the synthesis of these particles, or the flagellins themselves, nonmotile clones could arise in a purely phenotypic manner, as indeed they do at higher temperatures. One might hypothesize further in terms of cytoplasmic genes or epismes, and such suggestions naturally account for the continued interest in basal granules and other intracellular structures specifically concerned with functional flagella.

Transmission of motility by means of purified DNA from motile strains to nonmotile strains of *B. subtilis* was described by Nasser and Koffler (105). The activity was destroyed by deoxyribonuclease but not ribonuclease. Here there was evidence, obtained with labeled amino acids, that the nonmotile mutants were unable to synthesize flagellins; by Ouchterlony serological techniques, Nasser and Koffler were unable to detect any (or <0.0025% of cell dry weight) flagellins in the lysozyme-induced spheroplasts of the nonmotile strains. Neither could Kerridge (68) detect intracellular precursors of flagella by use of methyl-labeled ε-methyllysine, the unusual amino acid unique (?) to flagella.

The above considerations reveal some of the complexities associated with nonmotility; active flagellation therefore presumably provides us with information about many extracellular and intracellular factors of considerable present and future taxonomic value.

**Constancy of the point of origin of the flagella on the organism.** True and constant polar flagellation has long been recognized as a characteristic of bacterial pseudomonads, vibrios, and spirilla. The unique (bacteriologically) lateral tuft of ca. 12 flagella of *Selenomonas* was described by Boskamp in 1911, and most usefully served for the later recognition of these organisms despite their varied ecological habitats (82, 147). The peritrichous flagellation of many *Enterobacteriaceae*, e.g., *Proteus*, *Serratia*, *Escherichia*, *Aerobacter*, *Salmonella*, and *Bacillus*, and also the *Bacillaceae*, and the sparse or "degenerate" peritrichy of many *Agrobacterium* and *Rhizobium* strains, has proved of considerable diagnostic value both at the microscopic and serological levels. However, the critics who point out the very real taxonomic problems of the difficult-to-determine subpolar or "shoulder" flagellation, reported, for example, in some yellow xanthomonads (?) (78) and rhizobia (79), must be answered. Likewise, the confirmed reports of both polar and peritrichous flagellation in single isolates of, e.g., *Chromobacterium*, *Alcaligenes*, *Flavobacterium*, *Achromobacter*, and *Aeromonas* (76, 80, 134), merit discussion.

Subpolar flagellation is difficult to differentiate from either true polar or sparsely peritrichous flagellation. The problem of the cell body obscuring the actual origin of polar or subpolar flagella is ultimately a statistical one, and is overcome in simple practice by examining a suitably large number of flagellate cells. Leifson thought that true subpolar flagellation was constantly found in certain bacteria, e.g., yellow-pigmented marine rods with a single flagellum or a tuft inserted at right angles (78), and it was also characteristic for certain subgroups of *Rhizobium* (79), the latter confirming the findings of Conn and Elrod (23) in some clover and alfalfa strains of *Rhizobium*. Apart from the striking subpolar multitrichous flagellation of *Sphaerotilus natans* swarmers (9), and the subpolar flagellation of the caulobacter *Asterionella* (113), all other claims of subpolar flagellation are, to my knowledge, confined to the two sparsely peritrichate and sparsely studied families, *Rhizobiaceae* and *Achromobacteraceae*.

The phenomenon of "mixed flagellation," i.e., organisms of a single isolate showing cells with either polar or lateral flagella, or both, was first fully described by Leifson and Hugh (80), and was noted to be particularly conspicuous in *Chromobacterium* and *Aeromonas*. Organisms often showed both polar and lateral flagella when young, but older cultures normally showed only polarly flagellate cells. Whether the cultures were broth or agar slants was also important; broth cultures tended to contain only polarly flagellate cells, whereas the organisms from slants exhibited the mixed type of flagellation, i.e., a normal polar
flagellum, plus lateral flagella much shorter in length and also with a much shorter wavelength. The lateral flagella were also more easily detached by mechanical methods (relevant to earlier discussions regarding the mode of attachment). In diagnostic practice, therefore, one should examine washed cells from both broth and slope cultures of young and older cells to detect this phenomenon. Induction of lateral flagella by growth on solid surfaces is an old observation (see 53 for earlier references); Houwink and van Iterson (53) thus induced them in Photobacterium splendidum (now known to be similar to some Aeromonas, 135a), and Pseudomonas pyocyanea, and suggested a nutrient-absorbing function because more were formed if the nutrient content of the agar was low. In Aeromonas, mixed flagellation is so common that some feel that it is a most useful diagnostic characteristic (33, 76, 78).

Mixed flagellation was also investigated in detail by Sneath (134) in Chromobacterium with the use of many experimental approaches. He also concluded that the transient lateral flagellation was phenotypically induced. Many environmental variables were investigated, and it was ironic indeed that his careful trace element studies included neither iron nor aluminium. Sneath’s finding that the polar and lateral flagella of Chromobacterium were not identical serologically is significant. He made the pertinent suggestion that perhaps the mysterious phase-1 and phase-2 flagellar antigens of Salmonella might not all be located on peritrichous flagella; the poles of peritrichate rods merit careful scrutiny. Another observation made by Sneath was that lateral flagellation was not associated with a multi-“nucleate” (chromatinic material) condition, indicative of a transitory coenocytic state, a situation which was demonstrated in the long flagellated filaments of Clostridium acidiurici (143). It is interesting that growth at 44°C will repress cross-wall formation as well as the functioning of flagellae, and that wall-less spheroplasts may bear inactive flagella. Factors affecting the sites of synthesis and organization of both wall and flagella polymers might still explain mixed flagellation in terms of organizing factors and specific growth zones. Concerning nonhomogeneous flagella, Leifson and Hugh (81) claimed that their yellow xanthomonads (?) gave comparable flagellar antisemum titer when the flagella were polar only (wavelength 3.0 μ), lateral only (wavelength 1.0 μ), or mixed, with the logical deduction that the flagella were therefore antigenically identical. However, in V. percolans which showed mixed flagellation, there were some serological differences between polar and lateral flagella, demonstrable without ambiguity, because here purely polar and purely peritrichous stable clones were obtained (80). Their indications that mixed flagellation was sometimes temporary and sometimes permanent is taxonomically important, especially if their reported production of a stable peritrichous and a stable polarly flagellate variant is a frequent natural occurrence. Indications of this were also reported by Ewing et al. (33) in Aeromonas shigelloides.

The speculations of Leifson and Hugh (80) and others that polar to peritrichous flagellation perhaps indicates an evolutionary sequence of change from a primitive, aquatic, polarly flagellate uniceil to a more advanced peritrichate and multicellular state, has interesting parallels in the algae and fungi (and rather more supporting evidence in the larger protista). The bacterial phenomenon of young cultures containing organisms with mixed flagellation giving rise to cells with only polar flagella on further incubation, is, however, possible ontogenic evidence which fails to support the above hypothesis (ontogeny recapitulating phylogeny). Bisset and Hale’s report (5) that the more highly specialized sporeforming B. cereus produced motile gram-negative swarmer with polar flagella, which subsequently developed into multicellular gram-positive bacilli with peritrichate flagella, does lend support to the hypothesis if their observations can be confirmed. Furthermore, polar flagellation of gram-positive cells is rare [to my knowledge reported only for the reproductive swarmer of Actinoplanes, which has a gram-positive mycelium (9)]. Several arguments support the views that gram-positive bacteria are more specialized; e.g., they have more complex morphology such as multicellularity; mycelium formation; oidium, conidium, and endospore formation; and even sporangia. Many show devices and behavior indicative of decreasing dependence upon environmental water, and adaptations to some extremely specialized ecological niches such as salt pans, hot localities (compost and silage, pasteurizing vats), and very specialized pathogenic sites, e.g., the varieties of Mycobacterium tuberculosis. The possibility of secondary adaptations of terrestrial organisms to an aquatic medium should not be ignored; the Actinoplanaceae may be an example of this, especially Actinoplanes with polarly flagellate reproductive swarmer.

Another speculation derives from the fact that many flagellate algal and fungal swarmers are haploid cells, and are effective agents of asexual reproduction in an aqueous environment. Possibly, as in the Fungi Imperfecti, bacteria are so
ably propagated asexually by means of unspecialized products of binary fission (flagellate or nonflagellate) that methods for their sexual reproduction have never been evolved, or are rare, lost, or very specialized. This seems to be an accurate description of the sex life of bacteria, as we understand it today.

It is ecologically interesting that many of the gram-negative, polarly flagellate vibrios, aeromonads, and pseudomonads are so abundant in the nutritionally dilute and colder natural habitats, both freshwater and marine, and in the alimentary tracts of both vertebrate and invertebrate aquatic animals. This was indicated by Leifson et al. in their preliminary studies of 600 marine isolates (76, 78). Of all the motile isolates from vertebrate and invertebrate intestines, 24.0% were Aeromonas spp. and other gram-negative rods with mixed flagellation. Thus, there is again an indication that mixed flagellation may be an ecological adaptation. Such findings certainly reinforce Sneath's emphasis that the precise conditions of culture must be stated and standardized if the location of flagella is to be used for classification purposes. The rarity of peritrichously flagellate bacteria among their 600 isolates originating in the sea, or in the alimentary tracts of marine vertebrates and invertebrates, was emphasized in Leifson's survey (78); <1.0% of all the motile bacteria present were peritrichate. To account for this, one should perhaps recall the intensely anthropocentric and terrestrial nature of much of our present knowledge, concerned as we have been with mammalian diseases and the growth and spoilage of human food. Sea-cows aside, one would scarcely anticipate large populations of marine lactose-fermenters, nor many marine organisms with a conspicuous glucose-starch-type carbohydrate utilization associated with green-plant photosynthesis. Marine algal photosynthates include abundant mannitol, fucose, and rhamnose polymers, "Floridean starch" (Rhodophyceae), leucosin (Chrysophyceae), and often abundant oils and lipids.

Flagellar shape, thickness, and length: some relevant genetic studies. The illustrations of Fischer and Migula and other pioneer bacteriologists indicated that certain bacterial genera or species had flagella of a very constant shape, with reference to the wavelength and amplitude of the spiral helices seen in suitable dead preparations. In 1909, Reichert (120), using dark-ground illumination, measured these values on living cells, and found that the pitch of the screw of Salmonella typhi was constantly 2.5 μ; that of Proteus vulgaris, 2.0 μ; that of Serratia, 2.0 μ; and that of Sarcina, 2.0 or 3.0 μ. Weibull (153) found that this character was maintained even in dead (ammonium sulfate-precipitated) preparations of purified flagellins; for Proteus the wavelength was again 2.0 μ, and for Bacillus subtilis the value was constantly 2.5 μ. The apparent constancy of this feature was therefore promising for diagnostic purposes.

Many further measurements of wavelengths and amplitudes of flagella on living cells were published by Pijper et al. (108, 109) from photographs of cells which had been slowed down in a viscous mounting medium. They concluded that under standard conditions every strain appeared to have a constant wavelength, but that this was not correlated with any other known character. There were, for example, three values for Serratia and at least two values in Pseudomonas (1.53 μ for P. aeruginosa, 1.32 μ for another Pseudomonas sp.), and 0.60 μ for P. diminuta). Furthermore, not all strains of the serologically homogeneous Salmonella dublin had the same flagellar dimensions. Occasionally there was a tendency to constancy, e.g., in P. aeruginosa and P. diminuta, but these authors concluded that in general there was too much overlapping. The range which could be exploited taxonomically was not great, and the "biplicity" phenomenon, i.e., flagella of two wavelengths, one twice the value of the other, was frequent and sometimes occurred even on the same rod. They emphasized that flagella wavelength was certainly affected by pH values; in Salmonella, Sarcina, and Proteus, for example, wavelength was at a minimum at pH 7.0, and any departure from this value caused an increased wavelength. Between pH 7.0 and 5.0 the wavelength changes were freely reversible. Pijper claimed that the temperature and viscosity of the medium markedly affected wavelength, and so he constantly reiterated that measurements made from dead, stained, cells were useless artifacts.

Leifson and his colleagues have nevertheless produced a wealth of such measurements of the flagella of a commendably wide variety of dead and variously fixed bacteria from all kinds of habitats [see Atlas of Bacterial Flagellation (75)]. Many recognized variables and anomalies have been investigated by workers in his laboratory. In one of their earliest papers (77), they found, for example, that 60 strains of Proteus-Providence organisms could show four definite types of flagellar curvature. Some individual organisms showed more than one type, sometimes even in one individual flagellum. Curvature again varied according to the pH value of the medium. One strain of P. mirabilis was induced to change from 100% "normal" type of flagella, with a wavelength of 2.2 μ, to 100% "curly" flagella,
with a wavelength of 1.1 \( \mu \), if the pH value of the medium was changed from 8.0 to 6.0; another strain showed no such effect. They too [compare with Fijper et al. (108, 109)] found Salmonella 

\textit{wichita} substrains with flagella of long or short wavelengths, and also pictured one cell bearing both types, or even a single flagellum with segments of different curvatures (see p. 115 of the Atlas for illustrations). Nevertheless, provided that sufficiently large numbers of flagella were measured, Leifson et al. concluded that the mean wavelength and amplitude was significant for a strain, although different strains of a species showed different values; the mean value for a genus, in fact, included 95.0\% of the strains, even in the Proteus-Providence group in which this characteristic was "exceptionally mutable." In a study of >100 strains of 

\textit{Pseudomonas aeruginosa}, a flagellar wavelength of ca. 2.0 \( \mu \) was constantly found, and this value also applied to \textit{P. stutzeri}, \textit{P. fluorescens}, \textit{P. saccharophila}, \textit{P. mucidolens}, and \textit{P. marginalis}. The present writer is in agreement with this 

\textit{Pseudomonas} value for \textit{P. aeruginosa} and \textit{P. fluorescens} and some phytopathogenic species. The flagellation of other phytopathogenic pseudomonads seems, however, to be less abundant and more variable; this seems taxonomically promising.

Many of the "genera" and "species" for which variable flagellar wavelength has been reported are often classified on equally variable or superficially investigated criteria, and the range of these categories is frequently very inconsistent. I agree with Leifson et al. that flagellar wavelengths and amplitudes are useful measurements, even when determined from superficial preparations. Many more studies are necessary; some conclusions have been drawn from far too few isolates and numbers of flagellate cells of one isolate, but this is understandable when the range of organisms investigated has been sufficiently wide. A practical point is that formalin fixation [recommended by Leifson (73, 75) in his technique, especially when dealing with pathogens] sometimes causes a straightening of flagella, confirming the chemical studies of flagellins by Astbury et al. (2). Leifson realized this, and suggested that possibly formalin sensitivity or formalin resistance might have taxonomic or chemical significance. Houwink and van Iterson (53) showed that 5.0\% formalin caused an aggregation of, for example, \textit{Proteus vulgaris} flagella into thicker but uniform bundles, "Riesenköpfe," with the flagella all in perfect phase, helical, and thus resembling a single flagellum. Other agents known to straighten some flagella are specific flagellar antisera (100), potassium phosphate (77), changes in the viscosity of the medium (108, 109), or any fixative which involves dehydration. A careful investigation to compare the effects of many protein fixatives upon many flagellins would be most valuable at the microscopic, biochemical, and biophysical levels. One must remember that flagella are very hydroscopic, and the technical suggestion of coating slides with water-repellant silicones, such as dichloro-dimethyl-silane (2), may merit further trial.

The biplicity phenomenon, i.e., strains or even single organisms showing flagella of two wavelengths, one twice the value of the other, is strange. Kerridge (67) found that the abnormal "curly" flagella with only half-normal wavelength, inducible in \textit{S. typhimurium} by allowing mechanically deflagellated cells to regenerate new flagella in a medium containing \( p \)-fluorophenylalanine, were, however, nonfunctional. Thus, it seemed that the synthesis of abnormal flagellins containing abnormal amino acids caused an alteration of flagellar curvature. Later workers clarified these observations in genetic terms. Iinio (57) deduced that the normal wavelength (2.2 \( \mu \)) or the curly wavelength (1.1 \( \mu \)) of flagella of \textit{S. typhimurium} and \textit{S. wichiita} was associated with a phase variation in flagellar antigens. Curly flagella were always in phase 1 and contained antigen i, whereas normal flagella were in phase 2 and contained antigen 1,2. Phase-1 curly mutants were relatively stable and only rarely (<10^{-4} per divisions) yielded both curly and normal subclones. Further analysis with \textit{S. typhimurium} mutants and transducing bacteriophages revealed that the phase-1 curliness was dependent upon a genetic determinant, \( H_1 \), for the phase-1 antigen type, and that this determinant was transducible. Similarly, the phase-2 antigen type was controlled by \( H_2 \), inferring that \( H_1 \) and \( H_2 \) controlled the type of flagellin in phase-1 and phase-2 flagella, respectively. So long as the curly genotype was retained, there was a phenotypic expression of curly flagella. No antigenic differences between normal-\( i \) and curly-\( i \) mutants of \textit{S. abortus-equus} were detectable, i.e., the \( i \) antigen of both curly-\( i \) and normal-\( i \) flagella was identical. Mutation in \( H_1 \) or \( H_2 \) might be the reason for structural differences which might result in, (i) a different flagellar shape (possibly due to changes in the amino acid composition or sequence), (ii) a changed antigenic type, (iii) nonmotile flagella, or (iv) inability to form flagella. Other genes not concerned with phase specificity are known to be necessary for the complete synthesis of flagella: e.g., a specific gene was postulated to be responsible for the methylation of lysine in flagellin molecules.

Another study which revealed the genetic
complexities of flagellation in *S. typhimurium* was reported by Joys and Stocker (64), who also used various mutants in recombination experiments involving the antigen i. They tried to correlate changes in the amino acid sequence in the flagellin of antibody-induced mutants, namely, microscopically detectable curly type mutants with flagella of a shorter wavelength, of the phase-1 flagellar antigen. They concluded that there were at least 13 subfactors in antigen i, and that each mutant lacked a different subfactor(s) which was present in the wild type. At present, the amino acid sequence in the flagellin polypeptide is not known, so the already-known and mapped gene sequence in *S. typhimurium* cannot be compared with it. At least two more genes were concerned with the functioning of flagella.

Of more direct taxonomic importance, however, is the increasing use of flagella markers in mating experiments involving the hybridization of DNA molecules from organisms which are commonly accepted as members of different genera, e.g., *Escherichia*, *Salmonella*, and *Shigella*. Mäkelä (95) succeeded in mating Hfr *Salmonella* abony with F"* E. coli", and demonstrated the transfer of flagellar antigens from the former to the latter. Thus, hybrids with flagellar antigens of both parents were obtained. The complex behavior of *Salmonella*, with its ability to produce two possible flagellar antigenic states (each determined by two series of alleles at the H1 and H2 loci governing the exhibition of either phase-1 or phase-2 antigens), was known (57). *E. coli*, on the other hand, normally exhibits one flagellar antigenic state only, although the specificity of it is determined by a series of alleles at one locus. Mäkelä answered the question concerning possible allelism between the single flagellar gene of *E. coli* and the H1 or H2 genes of *Salmonella*, by successfully introducing a short length of *Salmonella* chromosome containing the “factor,” known to be associated with H2, into *E. coli*, and thereby making it also capable of diphasic variation (it showed the phase-2 antigens of the *Salmonella* parent). The complementary transference of some *E. coli* genome into *Salmonella* has not yet been achieved, and apparently many *Salmonella-Escherichia* combinations are infertile. Mäkelä’s evidence suggests that the H genes of *E. coli* and the H1 genes of *Salmonella* represent a series of alleles at the same locus, and thus provides further independent evidence of the close relationship of certain members of these two genera.

Also of potential taxonomic importance is the report that chi phage could attack only motile *Salmonella* (57), with the inference that the bacteriophage receptor was a specific flagellin. Thus, bacteriophage susceptibility, so often used for subgeneric classification, would in such cases be correlated with certain flagellar antigen types determined by the H locus. A *Salmonella* phage, φX, which attacked only motile strains, was studied by Meynell (101), following up earlier observations by Sertic and Botulakov in 1936 (124, 125). Meynell’s electron micrographs showed bacteriophages attached by their tails to the distal parts of the host flagella, apparently randomly, but occasionally in small clusters at a specific zone (not the basal granule). Techniques known to prevent flagellation, such as growth on 0.2% phenol agar or growth at 44 C, prevented lysis. Decreased adsorption of bacteriophage on deflagellated cells with flagellar stumps only, was confirmatory evidence, and Meynell found that paralyzed flagella were also phage-resistant. Her exhaustive serological and genetic analyses with 524 naturally occurring *Salmonella* strains, together with her artificially acquired serotypes (obtained by the transduction of flagellar antigens), led to the discovery that *Salmonella* with antigens of the g complex were resistant to φX phage. Only hosts with H antigens l, e, h, or Arizona B were sensitive. Thus, active flagella of the correct antigenic type were essential; detached flagella never adsorbed the phage. After phage adsorption, a host flagellum was rapidly immobilized, possibly even by one adsorbed phage particle, although more than one phage was necessary to effect lysis. Although the H antigen clearly controlled bacteriophage susceptibility, other factors were also involved, possibly to control the injection of the phage DNA and its passage to the host genome. When a nonmotile isolate was freed from this phage, motility was restored. Therefore, Meynell suggested that the phage φX might affect the energy supply for functional flagella; thus, we have the possibility of a new approach to the active motility mystery.

Apart from the exceptionally thick flagellum (55 μ in diameter) of *Bdellovibrio* (141), and the thicker sheathed flagella of *Vibrio* and some spirilla, the thickness of flagella has not been critically considered or taxonomically exploited. Important and constant differences may well be revealed, as already discussed in the previous section on fine structure. Judging from the appearance of flagella of *Pseudomonas* spp. in silver-plated preparations, differences in thickness seem constant for a given strain, despite the artifacts due to “dipping in honey and rolling in feathers” (136). In electron microscope studies, also, flagella of different thicknesses were apparent (53) in different genera. Artifacts may also occur in electron microscope preparations; present
procedures may well dissolve or distort various components in such hygroscopic structures. On present evidence, however, one cannot agree completely with Murray’s statement that all bacterial flagella are “truly unique structures of great thinness (100 to 200 A).” A diameter of 300 A for V. metchnikovii (44) and of 350 ± 40 A for Vibrio flagella has been reported (35). In this connection, further comparative studies of chemical and physical methods of flagella fixation would be instructive.

Neither has flagellum length been critically analyzed, although this seems to be a constant characteristic of mature flagella of a given isolate. Accurate measurements are tedious, but Leifson (73) found that a physiologically homogeneous group of gram-negative rods inert toward carbohydrate always showed one to six very distinctive short polar flagella. They typically had only one (occasionally two) curve and a characteristic wavelength of 3.1 μ and amplitude of 1.01 μ, which arrangement he called “lophotrichous.” The generic name Lophomonas was also suggested for such organisms (73). Physiologically similar rods with long flagella with more than two curves, occurring in a polar tuft, were also described (73); this flagellar arrangement Leifson called “polar multirichous.” Constant lophotrichous flagellation has been confirmed by many for various “Vibrio” or “Comamonas” strains apparently identical with Leifson’s lophomonads (discussed below). I have noted that many phytopathogenic pseudomonads have flagella of the short lophotrichous type with a longer wavelength, and such organisms are clearly distinguishable from P. aeruginosa or P. fluorescens, with respect to both flagellum length and wavelength.

Kerridge (67) reminded us that flagella do cease to grow, and that no terminal disintegration has ever been suggested in electron microscope pictures. In photosynthetic Chlamydomonas moewusii, however, light conditions affected the length of flagella (83); flagellate cells kept in the dark showed a reduction in flagellum length from 10 to 12 μ within 7 days, but on reillumination the normal length was restored within 1 hr, especially in an aqueous environment. Lewin thought that an osmotic factor was involved. The only light effect recorded, to my knowledge, for bacterial flagellation concerns, hardly surprisingly, photosynthetic bacteria. The phototactic accumulation of purple sulfur bacteria in a “light trap” and their “terror responses” were described by Stanier et al. (136) in connection with energy requirements, but there was no mention of any light effect on flagellar length. I have occasionally seen Pseudomonas cells with extraordinarily long (up to 30.0 μ) flagella in routine silver-plated preparations of young cells. One would certainly like to know what governs their normal cessation of growth.

**Number of flagella per bacterium.** The usefulness for identification purposes of the typically sparse flagellation of Rhizobiaceae and Achromobacteraceae has already been discussed, but the number of flagella per rod in abundantly peritrichate organisms is usually difficult or impossible to determine accurately, due to “in phase” aggregation so that individual fibrils are obscured. Kerridge (67) found that S. typhimurium grown in nutrient broth had an average of six or seven flagella per rod, whereas in a chemically defined glucose-ammonium-salts medium only two or three flagella per organism were produced. Addition of only a complete amino acid mixture to the glucose-salts growth medium restored abundant flagellation. Inhibitor studies with deflagellated cells were instructive. The presence of purine and pyrimidine analogues, such as 5-fluorouracil and fluorodeoxyuridine (FDUR), caused a marked increase in the number of flagella per bacterium, i.e., 7.9 with 5-fluorouracil and 5.2 with FDUR, compared with 3.7 flagella per bacterium in the controls. Kerridge suggested that perhaps cell division was thus inhibited, although flagellum formation was not. Cross secta might be detected if such experiments were repeated and cells were suitably stained. They might well be visible simultaneously with flagella in silver-plated preparations.

Among polarly flagellate bacteria, an early interest in this detail is detectable in Migula’s description of Pseudomonas aeruginosa as monotrichous and P. fluorescens cells with one, two, three, or more flagella. Since then, several workers (perhaps in recognition of the usefulness of this character in the algae and fungi for identification purposes) have attempted to determine whether the number of flagella per rod was a useful character to differentiate species of Pseudomonas. Rhodes (121), Lyssenko (93), Colwell and Liston (20), Butiaux and Gagnon (14), and Park (106) all concluded that the value of this character was debatable. It certainly did not appear to correlate with any other known feature. Rhodes concluded (121), from fairly extensive investigations of ca. 250 isolates of Pseudomonas, that soil and water pseudomonads typically had 1, 2, 3, or up to 10 flagella per rod (at one pole), and that this variety of numbers could often be seen in one microscope field (see illustrations in 122). Park (106), too, found that 22 of 30 P. fluorescens isolates had 1 to 10 flagella per rod. Very few (eight) isolates of P. aeruginosa were examined by Rhodes, but she commented that there was a definite tendency to monotrichy; Park reported
9 of 10 P. aeruginosa isolates with mainly one polar flagellum. More than 6,000 flagellate cells of Pseudomonas were examined by Lautrop and Jessen (72), and they found that 97.0% of P. aeruginosa rods were monotrichate. Their careful counting disclosed that P. fluorescens rods had one, two, or three flagella with approximately equal frequency, but that more than three were also common. All this information they expressed quantitatively as "high and low flagella index" groups, and their scoring method minimizes the occasional aberrancy, such as the occasional cell of P. aeruginosa with two, and the very rare cell with three, polar flagella. Occasional biflagellation in typically monotrichate species has also been recorded in V. metchnikovii (53), Aeromonas (106), Vibrio 01 (106), V. alcaligenes strain 9239 (which is possibly synonymous with Comamonas perolans) (106), and the C27 Aeromonas (33); typical monotrichy is taxonomically useful in these genera too. Interesting parallels with evolutionary significance occur in the algae, with the stabilization of the nonmotrichate forms (38). As Lautrop and Jessen remarked (72), it is certainly unfortunate that Leifson's Atlas shows a monotrichate P. fluorescens, and refers to the "normal polar monotrichous flagellation" of this species (75, p. 26 and 27). The Danish authors also observed that the number of polar flagella increased when the temperature of incubation was lowered to 10°C. This was not mentioned by Frank (36) in his studies of the flagellation of Pseudomonas geniculata at 8 and 27°C. His conclusion reads, "polar flagella, 1 to 12, at both temperatures." Such a statement reinforces the plea of Lautrop and Jessen for quantitative observations and meaningful descriptions. I concur with these authors in their disagreement with Krassilnikov's statement (70) that gelatin-liquefying strains have three or four flagella, whereas the nonliquefying strains have only one or two. The reasons for the reported more abundant polar flagellation at lower temperatures certainly merits further study. Perhaps the older flagella are not shed so rapidly because the logarithmic phase is prolonged; it is known that an individual flagellum has a fixed life span in the logarithmic phase, and grows more rapidly at 37 than at 25°C (0.1 and 0.085 μ per min, respectively) in Salmonella (139).

Haynes and Rhodes (46) claimed that they could distinguish P. aeruginosa and P. chlororaphis on flagella number; of 47 isolates of P. aeruginosa, 21 had cells with only one polar flagellum, and 24 isolates showed cells with either one or two polar flagella (no quantitative estimates described, nor was the incubation temperature, age, or medium stated), 17 of 18 of their isolates of P. chlororaphis apparently contained cells with at least 4, and sometimes 8, flagella. This difference was claimed to be highly correlated with other differences, namely, growth temperature range, color of growth, pyocyanin production, and levan production. Hybridization of these two species was reported by Marmur et al. (98), suggesting a very similar DNA composition.

**General Comments on the Taxonomic Value of Flagellation Characters**

The criterion of flagellation (or nonflagellation) is mentioned on 36 of the 46 pages of Skerman's key to the genera of bacteria in his Guide (131), and occasionally it is the only feature used for key purposes. The Guide is a masterly compression of, presumably, the most important and reliable information about bacterial genera. Skerman also considered that the feature was worth recording in almost every genus description, and commented on, for example, the relative ease of distinction of (i) the sulfur-containing filaments of Sphaerotilus, which produce motile swarm cells with subpolar flagella, from the nonflagellate Thioliria or Beggiaoa, (ii) motile Listeria and nonmotile Mycobacterium, (iii) sporangium-forming Actinoplanes with motile sporangiospores and Streptosporangium with nonmotile sporangiospores, together with many examples of consistently nonmotile genera. One perhaps tends to overlook the diagnostic usefulness of nonflagellation in almost all cocci, Lactobacillaceae, and Actinomycetales, although one admits the lengthening list of exceptions, such as peritrichous Cillobacterium (9), some streptococci, [motile streptococci reported from grass slilage, all group D, claimed "not uncommon," three references given by Steel, (137)], and Sarcina ureae (9), which, incidentally, is now reported to have a DNA base composition similar to that of flagellate, sporeforming Bacillaceae (98). Shigella and Klebsiella are still described as nonmotile; so are Bacillus anthracis, Aeromonas salmonicida (32, 133), and the melanin-producing nonmotile variant of Pseudomonas aeruginosa which causes pylonephritis (87).

A few examples in which studies of flagellation have effected taxonomic clarifications in specific groups of bacteria may be cited as follows:

**Acetic acid bacteria.** These are now known to be, when motile, either peritrichously flagellate with a flagellar wavelength of 2.9 μ, or polarly multilirichate with a flagellar wavelength of 1.4 μ. This grouping correlates with the physiological ability to oxidize acetic acid to CO₂ and water (such organisms being known as the peritrichous, lactaphilic Acetobacter spp.), or the in-
ability to oxidize acetic acid (the polarly flagellate, glycoliphic *Acetomonas* spp.) (16, 50, 74, 126, 127). According to more recent data (26, 157), other metabolic characters and DNA base composition values also correlate well with the above two generic recommendations originally based upon different flagellation (74).

*Azotobacter*. It is now recognized that the gram-negative nitrogen-fixing bacteria with *Azotobacter*-like characteristics may be peritrichously flagellate. *A. vinelandii* and *A. agilis* typically possess 30 to 50 slender flagella, 30 to 40 μ thick (3, 51); *A. chroococcum*, also peritrichate, is reported to have fewer flagella (3). Monopolarly flagellate nitrogen-fixing rods, which were culturally and serologically distinguishable from the peritrichate forms, have also been described (3, 60). Opinions are divided, but Jensen et al. proposed a new genus, *Derxia*, for the monotrichate polar type; others recognize it as *A. macrocyclogenes* (3, 75). Further investigations are desirable.

*Rhizobium*. Leifson and Erdman (79) found that 82 isolates of *Rhizobium* could be differentiated into two subgroups on the basis of flagellation. In one group, the rods were sparsely peritrichate, with a flagellar wavelength of 1.3 to 1.6 μ, and these organisms grew faster on a manitol-salts medium than did a second group of rhizobia, which were found to consist of rods with one subpolar flagellum, with a flagellar wavelength of 1.9 to 2.2 μ.

Sulfate-reducing bacteria. Determination of the flagellation of several isolates of sulfate-reducing bacteria (129) revealed some polarly flagellate organisms, which possessed cytochrome c₃ and also desulfoviridin. These were clearly different from the peritrichately flagellate sulfate-reducers, both with regard to the flagellation, and also the absence of cytochrome c₃ and desulfoviridin; sometimes the peritrichous forms produced spores, but possible affinities with *Clostridium* are not supported by the guanine plus cytosine (G + C) base ratio values obtained (129).

*Aeromonas*. An examination of the flagellation characters of 130 cultures, representing many named genera and species, revealed three distinct groups (33): (i) organisms with one polar flagellum, wavelength 1.7 μ; (ii) isolates with one to five undulant flagella, wavelength 3.5 to 4.0 μ; and (iii) nonflagellate isolates. For these three groups, the species names *A. hydrophila*, *A. shigelloides*, and *A. salmonicida*, respectively, were suggested (33). These suggestions were reinforced by an admirable table of quantitative data concerning physiological and biochemical characteristics, which correlated well with the three divisions proposed.

Two cultures of *A. hydrophila* were studied by Clement and Gibbons (19); all the properties of these isolates, including the recognition of permanent peritrichous flagellation, led these investigators to recommend relabeling, i.e., as *Aerobacter cloacae*.

Leech (*Hirudo medicinalis*) pathogens. An organism known as *Pseudomonas hirudinis* was found to have the typical "mixed" flagellation of *Aeromonas* when young, agar-grown cultures were flagella-stained (33). The ability to metabolize carbohydrates anaerobically confirmed the aeronomad nature of this pathogen. Another leech pathogen named *Corynebacterium vesiculare*, was found to be a monotrichate gram-negative rod, and therefore opinions were invited for the support of the more logical name of *Pseudomonas vesiculare* for this organism (40).

*Pseudomonas maltophilia*. Twelve cultures of Gram-negative, rod-shaped bacteria, variously named *Pseudomonas* sp., *P. alcaligenes*, *Alcaligenes faecalis*, *Bacterium bookeri*, *A. bookeri*, and *Bordetella bronchiseptica*, were examined (55, 56), and all were found to possess one to six formalin-sensitive, polar flagella. They also produced a yellow, water-insoluble pigment, and oxidatively metabolized fructose, mannose, and, especially avidly, maltose. Hence, after a careful study of many properties and the confusing literature, the name *Pseudomonas maltophilia* was suggested to clarify the chaotic nomenclatural situation. Conversely, *Pseudomonas odorans* was found to be peritrichously flagellate, and in all respects more clearly allied to *Alcaligenes* (96); *Alcaligenes odorans* was therefore proposed as a more appropriate name (96).

*Agar-digesting bacteria*. Eighteen isolates of marine agar-digesting bacteria were found to have one polar flagellum (43, 78), and the similarity between some of these and *Pseudomonas gelatica* was indicated (43). The recognition of *Alginomonas* for the polarly flagellate, and *Agarbacterium* for the peritrichate and nonmotile agar-digesting isolates, was suggested as a temporary recommendation pending further study (9).

Yellow-pigmented bacteria. The realization that the rods of Whitmore's bacillus, *Loefflerella pseudomallei*, which often formed yellow colonies on solid media, had one to four polar flagella caused Brindle and Cowan (10) to propose the name *Pseudomonas pseudomallei* for this organism, although possible xanthomonad affinities were also suggested. Others found that ganisms from the yellow mucoid colonies of *Xanthomonas vredovoros* had one to two lateral flagella (47); their ability to ferment carbohydrates and other properties indicated further
closer affinities with *Erwinia lathyri* rather than xanthomonads (47). Then, among the flavobacteria, *F. piscicida* was found to be a monotrichate-oxidizing organism, and so more logically *Pseudomonas piscicida* (19), whereas the motility of other flavobacteria was found, in fact, not to be due to flagella; these gliding creatures were thought to be *Cytophaga* spp. by Floodgate and Hayes (34).

**Vibriolike organisms.** Many serological types of pathogenic and nonpathogenic vibriolike organisms were studied by Davis and Park (25). The monotrichate organisms were found to possess many of the properties associated with the classical fermentative *Vibrio comma* and El Tor strains, although the comment was made that on an overall similarity basis these organisms were related to *Aeromonas*. Other strains however, were multitrichous, with two to six polar flagella; they oxidized carbohydrates, and a consideration of all their properties led these authors (25) to conclude that, despite labels of *V. cuneatus*, *V. cyclosites*, and *V. neocystis*, they were typical *Pseudomonas fluorescens*. A further group of Davis and Park's strains had cells with 2 to 14 strikingly short polar flagella, with usually only one curve. Such organisms were inert toward carbohydrates, and were considered identical with the "*Lophomonas*" alcaligenes described by Leifson and Galarneau (39, 73). Davis and Park (25) proposed the new generic name *Comamonas* (protozoan name *Lophomonas* invalid) and *C. percolans* (synonymous with *V. percolans* and *V. alcaligenes*), as the new type species. The very close similarity, or identity, between *V. percolans* and *Comamonas* was further supported by sex-duction experiments (98). To add to the monenclatural confusion, Hugh (54) pointed out that both *Lophomonas alcaligenes* and *Comamonas percolans* are names which postdate Guenter's description of *Vibrio terrigenus* 1894, which seems to be the same organism. Therefore, the combination *Comamonas terrigena* was proposed by Hugh (54) to credit Guenter with the first isolation and description of this species, which is readily identified by its distinctive tuft of polar flagella with a wavelength of 3.1 μ and amplitude of 1.01 μ.

Gram-negative rods or vibrios which are metabolically inactive are still not classified satisfactorily. New criteria, and more information of all kinds, are needed. Serological relationships suggesting shared antigenic groupings, in named cultures of *Achromobacter*, *Alcaligenes*, *Pseudomonas*, *Aeromonas*, *Vibrio*, and *Comamonas*, have been demonstrated by various workers (48, 69), by use of somatic, flagellar, and soluble polysaccharide antigens as diagnostic tools.

**FURTHER SUGGESTIONS FOR FUTURE INVESTIGATIONS WITH THE USE OF MODERN TECHNIQUES**

One now anticipates clarifications if genetic studies involving the transduction or transformation of flagellar characters are attempted, such as have been used to reveal relationships among very complex peritrichously flagellate enteric bacteria (already discussed). Affinities of nonmotile organisms might also thus be revealed.

Data is accumulating about the G + C base ratio composition of purified DNA from some of the taxonomically difficult organisms. Marmur et al. (98) and Colwell and Mandel (21, 22) referred to many studies of both freshwater and marine vibrios, pseudomonads and related organisms, and De Ley and Schell (26) discussed conclusions about acetic acid bacteria. The results are still preliminary, and pitfalls such as two "species" of DNA in some *Halobacterium* species, probably due to the presence of lysogenic bacteriophage (63), must be recognized. Nevertheless, the data so far are most encouraging. As expected, there are confirmations that some of our taxonomy is very illogical and nonbiological or "artificial": for example, no differences in the G + C values of the DNA of marine and nonmarine vibrios, which would effect their separation on this basis, have been detected (22); nor do physiological groups based on nonessential characters, such as agar decomposition or chitin utilization, appear distinct (22, 43, 78).

Despite such fashionable and refined analyses, the mere differentiation of polar and peritrichous flagellation is proving of value. One hopes that these different flagellations may eventually be explicable in biochemical, biophysical, and genetic terms. Eventually, perhaps, we shall be able to match amino acid sequences and arrangements in flagellins with gene maps and base arrangements in the cell's DNA molecules. Meanwhile, it is surely profitable to recognize motility and to appreciate as much flagella detail as possible. Characteristics, such as number of flagella, their length, position, thickness, wavelength, and amplitude, ultramicroscopic structure, specific serological groupings, amino acid composition and sequence, and also the structures of the DNA and the base sequences in the genes controlling flagella synthesis and functioning, may well be of great significance for both natural and artificial classification purposes, or for rapid practical diagnosis. New ideas should abound. For example, amino acid analysis of flagellins isolated from 10 strains of both mesophilic and thermo-
philic *Bacillus* (1) revealed no constant differences in these two ecological groups, despite the greater stability to heat, acid, urea, etc., of the thermophilic flagellins. This apparent discouragement may of course merely reflect the artificiality of the current taxonomy. It seems possible to me that the more specialized thermophiles may have arisen from several types of less fastidious mesophilic *Bacillus* spp., and that these adaptations perhaps involved only physical changes in their flagellins.

With rapid increase of the type of information suggested above, we can be sure that every appropriate mechanical device which will enable us to catalogue and compare this possible avalanche of data will be utilized gratefully by future systematists. Already we possess a considerable body of information about the flagellar antigens of at least 700 serotypes of *Salmonella*; this, together with other serological information, (somatic antigens), and also 14 biochemical characteristics, was computed electronically by Lockhart and Holt (89) to obtain an objective arrangement of the Kauffmann data. The results were extremely interesting, especially the main finding that the primary basis for group formation was the possession of the 1,2; 1,5; 1,6 or 1,7 flagellar antigens in the second phase. The next (secondary) grouping was determined by the somatic antigens. Thus, it seemed that the first-phase flagellar antigens and the biochemical properties did not influence that particular sorting procedure, which was a new computer technique using the monothetic criterion of Lockhart and Hartman (88). It involved the selection of, for example, *Salmonella cairo* as a typical member of Kauffmann group B, and the compounding of organisms around it in an order of decreasing similarity. On the basis of this scheme, the rest of the Kauffmann group B (a monothetic classification in fact, based on somatic antigens) organisms did not group around *S. cairo*, nor was it a biochemical grouping evident. Instead, a grouping based on second-phase flagella antigens emerged. These authors emphasized an important point, namely, that the computation was an attempt to rearrange the Kauffmann data, and it did not result in a “classification.” In this instance, it was “better” in that it was more practical, but it showed interesting differences from the Kauffmann-White schema based essentially upon somatic antigens. This “hint” from the computer concerning useful criteria for rapid identification or group formation may, perhaps, further serve to persuade the most antiflagella bacteriologists that bacterial flagella should not be relegated as “mere appendages.”

Our knowledge about bacterial flagella has recently been most profitably stimulated by the legion of biochemists and biophysicists, aided by computers, who are interested in protein synthesis, and in the molecular changes occurring in kmef protein fibrils during muscle and flagellum contractions associated with movement (42, 85, 130). The discovery of an easily-separable cellular protein of great purity, namely, bacterial flagellin, has resulted in new data of marked relevance. Flagella may indeed be significant “informational macromolecules,” together with the (presumed) DNA which governs their synthesis and functioning. Systematists should perhaps recognize the widespread aversion of many colleagues to “an over-obsession with nomenclatural wrangles,” and try to direct the attention of biochemists, biophysicists, and geneticists to those flagellate bacteria whose present systematic relationships are so controversial. “A prudent man enticeth his neighbour and leadeth him into the way that is good.”

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