The Wavelengths of Helical Bacterial Flagella

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SUMMARY: During normal movement most motile bacteria carry a straight tail, which, when the movement slows, stiffens into helical structures commonly called flagella. The helices of many kinds of bacteria were photographed with a sunlight dark-ground microscope, and their wavelengths measured. Mean values and standard deviations were calculated for each strain and then for the species. 'Biplicity' (two wavelengths per bacterium, one twice the other) was observed frequently. Each strain appears to have its own constant wavelengths. The wavelength differs in different kinds of bacteria from 0.60 to 5.058 μ, the distribution over the various species not revealing a distinct pattern nor any obvious correlation with other characteristics. The wavelength is affected by temperature, pH value, and colloid content of medium. These features, and the effects of drying, make stained preparations useless for measuring.

In sunlight dark-ground microscopy most motile bacteria, except spirilla (Pijper, 1949a, 1955c; Pijper, Crocker, van der Walt & Savage, 1953) and vibrios (Pijper & Nunn, 1949) during normal forward movement show a fuzzy-looking straight tail, as in Pl. 1, fig. 1 (Pijper, 1946, 1949b). As cultures age, and more rapidly in slide-coverslip preparations, these tails stiffen into clear-cut rods, or, more frequently into clear-cut helices (Pijper & Abraham, 1954; Pijper, 1955a; Pijper, Crocker & Savage, 1955). Pl. 1, figs. 2-4, show the shortest and the longest wavelengths so far found. 'Wavelength', also called 'period' or 'pitch', is distance from crest to crest. Helices in one culture may be of two different wavelengths, one twice the other (Pijper & Abraham, 1954; Pijper et al. 1955). For this 'doubling' phenomenon the term 'biplicity' was suggested (Pijper, 1955a). Helices often 'link up' lengthwise and 'pack' crosswise, thus forming 'giant-flagella' (Pl. 1, fig. 5). Slight degrees of packing as in Pl. 1, figs. 3 and 6, do not affect wavelengths.

Reichert (1909), in dark-ground microscopy of colloid-thickened helices, estimated the pitch of these 'screw-like flagella' of Salmonella typhi as 2.5 μ, those of Proteus vulgaris as 2 μ, and those of sarcinas as usually 3 μ, occasionally 2 μ. or even 1.8 μ. Weibull (1949) precipitated shaken-off flagella of one strain each of P. vulgaris and Bacillus subtilis with ammonium sulphate and described the wet helices (really giant-flagella) as having a period of 2 μ. in Proteus vulgaris and 2.5 μ. in Bacillus subtilis, later confirming the value of 2 μ. on ten strains of Proteus vulgaris (Weibull, 1950); he concluded that a definite spiral period is a characteristic feature of bacterial flagella. Leifson (1951), working with bacteria dried and stained on slides, got the impression that perhaps the degree of curvature of flagella might be more important than total length. Recently Leifson, Carhart & Fulton (1955), again using dried flagella-stained bacteria, measured one single wavelength of 10 'flagella' of each one o
75 strains of Proteus and found large differences between otherwise identical strains, in our opinion caused by the drying process. They did not comment on the 'biplicity' discernible even in their dried and distorted preparations. Their suggestion that the proportion of 'curly' and 'normal' flagella (their names for flagella of obviously single and double wavelengths) is affected by pH value is not borne out by their figures. Peluffo (1953), whose technique gave us numbers of giant-flagella just as easily visible to us in ordinary microscopy as in phase-contrast microscopy, measured unknown numbers of these structures by phase-contrast microscopy and found values for Pseudomonadaceae, salmonellas, and P. vulgaris of about the same order as ours.

We have watched the genesis of the helical structures in a number of different bacteria, and, under standardized conditions, which we found essential, examined them for wavelength and presence of biplicity.

METHODS

Organisms. We classified our bacteria after Bergey's Manual (1948), applying the diagnostic methods given there. The salmonellas were also classified serologically.

Photomicrography and measurements. When a sufficient number of tails had stiffened into helices as watched under the microscope, they were photographed with the sunlight dark-ground technique described before (Pijper, 1946). Electric lamps were inadequate, at any rate for the finer helices. With a Zeiss apochromat ×60 and a Contax camera the magnification on the film was ×300. For each strain of bacteria at least one full reel of thirty-six pictures was used. As a rule each reel made over 100 photomicrographs of helical structures available for measuring, and as most of them consisted of more than one wavelength, our calculations for each strain usually were based on several hundreds of wavelengths. The exceptions are specially mentioned. For measuring, the negatives were projected downwards in a vertical photographic enlarger with a Zeiss Sonnar 1:2 at a total magnification of ×3000, on to sheets of white paper. The clear-cut helices were traced with a pencil and then measured with vernier calipers allowing 0.1 mm. to be read. Instances of the very regular frequency curves resulting from this technique are shown in Fig. 1. Mean wavelength and standard deviation (s.D.) were calculated from the millimetre values at the magnification of ×3000. To facilitate understanding and comparison we give them here divided by 3 so as to express them in microns (µ). However, we have kept all the decimals so that our original figures can be obtained by multiplying by 3. We do not ascribe accuracy to our figures beyond the second decimal.

RESULTS

Effect of temperature

Appliances for heating and cooling the microscope stage described elsewhere (Pijper, 1955b) were used for measuring helices at wide ranges of temperature. Table 1 shows that wavelengths increased with rises in temperature. The
naturally shorter wavelengths were less affected by heat than the longer ones. The observations were repeated with similar results. As a rule a fresh preparation was made for each temperature observation and each preparation was left on the stage for at least an hour before photomicrographs were made. We once kept a slide of *Bacillus megaterium* 7581 first in a refrigerator at 6° overnight, which shortened the wavelengths. It was then brought to 47° on the microscope stage and showed a wavelength of 3.564 μ (s.D. 0.153) (see Table 1). After this it was cooled on the microscope stage to 3° for 3 hr. and photographed again, it then showed a wavelength of 3.392 μ (s.D. 0.181), corresponding very closely to the value for a fresh preparation at that same low temperature given in Table 1 as 3.389 μ (s.D. 0.111). A preparation of *Salmonella schottmuelleri* 3176 at 15° had a wavelength of 2.245 μ (s.D. 0.044), and this when brought to 25° became 2.299 μ (s.D. 0.048). Because of these, and other similar findings, we made our further measurements within the temperature range of 16–19°. During the colder months the microscope room stayed between 16 and 19°, during the hot weather ice-water flowing through a small tank clamped to the microscope stage maintained this same range of temperature (Pijper, 1955b).

**Effect of pH value**

By adding hydrochloric acid or sodium hydroxide to cultures before measuring, the pH values were adjusted as shown in Table 2. Readings of pH values were made by indicators and checked with a Beckman pH meter. The actual values between slide and coverslip probably deviated somewhat from the intended ones; the effect of the platinum loop, the mica slide (mica was used to get a clearer background), the glass coverslip and further growth during examination was not evaluated. The main finding was that wavelengths were shortest at pH 7-0, increased alkalinity and acidity increasing the wavelengths (Table 2). As with the effect of heat, the naturally shorter wavelengths were least affected. The results were confirmed by measuring wavelengths on *Salmonella typhi* 901 suspended in phosphate buffer solutions of pH 6.05 and

<table>
<thead>
<tr>
<th>Name of bacterium</th>
<th>Temperatures</th>
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<tr>
<td></td>
<td>3°</td>
<td>16–19°</td>
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<tr>
<td><em>Salmonella typhi</em> 901</td>
<td>2.289</td>
<td>2.294</td>
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<td></td>
<td>(0.059)</td>
<td>(0.063)</td>
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<tr>
<td><em>S. schottmuelleri</em> 3176</td>
<td>2.234</td>
<td>2.267</td>
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<td>(0.053)</td>
<td>(0.075)</td>
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<tr>
<td><em>Bacillus megaterium</em> 7581</td>
<td>3.389</td>
<td>3.432</td>
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<td></td>
<td>(0.111)</td>
<td>(0.130)</td>
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<tr>
<td><em>Serratia marcescens</em> 2302</td>
<td>0.941</td>
<td>0.965</td>
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<tr>
<td></td>
<td>(0.037)</td>
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10.0 (determined with the Beckman pH meter), and finding the wavelengths 2.366 \( \mu \) (s.D. 0.064) and 2.358 \( \mu \) (s.D. 0.061) respectively, both values being higher than the values at pH 7.0. In a similar experiment at 20–25° we found a wavelength of 2.315 \( \mu \) (s.D. 0.059) at pH 6.0, and a wavelength of 2.350 \( \mu \) (s.D. 0.058) at pH 8.0, both values again being higher than the value at pH 7.0. Because of these findings our further measurements were undertaken in media adjusted to pH 7.0.

Table 2. Effect of pH value on helical wavelengths

<table>
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<tr>
<th>pH values</th>
<th>4.0</th>
<th>4.5</th>
<th>5.0</th>
<th>6.0</th>
<th>7.0</th>
<th>8.0</th>
<th>9.0</th>
<th>10.0</th>
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<td>901</td>
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<td>—</td>
<td>2.336</td>
<td>2.312</td>
<td>2.303</td>
<td>2.307</td>
<td>2.362</td>
<td>2.366</td>
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<tr>
<td>2.305</td>
<td>(0-084)</td>
<td>(0-047)</td>
<td>(0-051)</td>
<td>(0-054)</td>
<td>(0-050)</td>
<td>(0-065)</td>
<td></td>
<td></td>
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<tr>
<td><em>S. schottmuelleri</em></td>
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<tr>
<td>3176</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2.267</td>
<td>—</td>
<td>2.294</td>
<td>2.284</td>
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<td>(0-059)</td>
<td>(0-044)</td>
<td>(0-051)</td>
<td>(0-050)</td>
<td>(0-041)</td>
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<tr>
<td><em>Sarcina ureae</em></td>
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<td>—</td>
<td>3.055</td>
<td>—</td>
<td>3.218</td>
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<td>(0-100)</td>
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<td><strong>Proteus mirabilis</strong></td>
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<td>3177</td>
<td>—</td>
<td>1.892</td>
<td>—</td>
<td>1.874</td>
<td>1.872</td>
<td>1.865</td>
<td>1.861</td>
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<tr>
<td>(0-092)</td>
<td>(0-055)</td>
<td>(0-072)</td>
<td>(0-059)</td>
<td>(0-060)</td>
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Effect of colloid substances

Colloid substances such as gelatin, gums, agar and methylcellulose when present in bacterial suspensions precipitate on the bacteria and thus coat bodies, tails and helices, making them more readily visible in dark-ground microscopy (Pijper, 1947). We found that the wavelengths of helices, whilst not markedly affected by methylcellulose, were affected by other colloids. *Salmonella typhi* 901, grown and examined in plain broth, showed a wavelength of 2.289 \( \mu \) (s.D. 0.074) and in 1% (w/v) methylcellulose broth of 2.294 \( \mu \) (s.D. 0.065). In 10 % (w/v) gum-arabic broth the wavelength was 2.423 \( \mu \) (s.D. 0.089), and when grown on nutrient agar and suspended in the water of condensation it was 2.816 \( \mu \) (s.D. 0.070). *S. typhi* 2 in plain broth had a wavelength of 2.289 \( \mu \) (s.D. 0.058) and in 1% methylcellulose broth, 2.274 \( \mu \) (s.D. 0.063). *Bacillus subtilis* 7197 in 1% methylcellulose broth showed a wavelength of 2.157 \( \mu \) (s.D. 0.096) and on agar it was 2.277 \( \mu \) (s.D. 0.042). Because of these findings we subsequently avoided other colloids and used methylcellulose. In a few cases, where no helices appeared in methylcellulose broth, agar was used, and this is specially mentioned.

Effect of going rough

Several of our strains were old and going rough. Prof. Winkler gave us a smooth form of *Salmonella dublin* and its rough variant. The smooth form was more motile and had longer tails and helices than the rough variant. A total of 179 measurements on the smooth form gave 2.355 \( \mu \) (s.D. 0.066) and 115 measurements on the rough variant gave 2.354 \( \mu \) (s.D. 0.096). Obviously going
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rough did not affect wavelength. Because of the better development of tails and helices in smooth forms, wherever possible, we picked the smoothest colonies of our strains. Arena & Schwartz (1937), in stained preparations, saw fewer flagella in rough _S. typhi_ than in smooth, but the difference in shape which they described and depicted (more ‘wider undulations’ in the smooth and more ‘narrower undulations’ in the rough form) evidently were examples of biplicity and not smooth-rough variation, as they thought.

Effect of drying

Flagella staining is preceded by drying which distorts, dislocates and redistributes the helical structures on the slide (Pijper, 1946). The flagella of Pl. 1, fig. 7, were stained by the method of Leifson (1951), and show marked dissimilarity. In such pictures one can pick out some rather regular and matching shapes, and for measuring purposes the temptation to do so is great, but selection is not fair sampling. In our dark-ground photomicrographs all visible helices could be used without selection, as illustrated by Pl. 2, fig. 8. Fig. 1 shows frequency curves of helical wavelengths for four strains of bacteria. A curve was drawn for each one from dark-ground and from stained picture measurements. The former are symmetrical with very little scatter, the latter are so irregular that mathematical analysis does not seem worth while, and the mean values of the latter would obviously differ greatly from those of the dark-ground technique. Apart from changes caused by drying the effects of changes in pH value and in temperature during the staining process must be considerable. Because of all this little value can be attached to measurements of stained flagella.

Helical wavelengths of various bacteria

We give the mean helical wavelength of each strain, and by formal calculation taking into account the number of measurements on each strain, the mean wavelength of the species. There follows a list of the results; the mean wavelengths are given in μ, followed by the standard deviation (s.D.) in brackets.

**Salmonella typhi** (Pl. 2, fig. 8). Mean wavelengths of twelve strains: 2.274 (0.063), 2.276 (0.051), 2.282 (0.060), 2.288 (0.067), 2.289 (0.057), 2.293 (0.061), 2.294 (0.065), 2.295 (0.047), 2.299 (0.059), 2.301 (0.068), 2.304 (0.056), 2.315 (0.050). Overall mean: 2.293 (0.061). Biplicity: in broth culture _S. typhi_ 901 once showed 34 helices of mean wavelength 1.106 (0.039) and in methylcellulose-broth 4 helices averaging 1.2 μ. (Pl. 2, fig. 9.)

**Salmonella paratyphi.** Mean wavelengths of six strains: 2.308 (0.083), 2.340 (0.055), 2.342 (0.054), 2.367 (0.077), 2.371 (0.064), 2.421 (0.058). Overall mean: 2.340 (0.078).

**Salmonella schottmuelleri.** Mean wavelengths of seven strains: 2.252 (0.068), 2.267 (0.075), 2.282 (0.049), 2.298 (0.046), 2.301 (0.053), 2.302 (0.047), 2.385 (0.061). Overall mean: 2.289 (0.064). One strain, 3176, showed abundant biplicity: in one preparation 211 helices of 2.267 (0.075) and 108 helices of 1.131 (0.059). (Pl. 2, fig. 10.)
Salmonella hirschfeldii. Mean wavelengths of four strains: 2.352 (0.084), 2.385 (0.071), 2.409 (0.070), 2.455 (0.063). Overall mean: 2.400 (0.084).

Salmonella typhimurium. Mean wavelengths of ten strains: 2.267 (0.073), 2.287 (0.056), 2.298 (0.054), 2.335 (0.103), 2.344 (0.063), 2.345 (0.070), 2.349 (0.084), 2.384 (0.081), 2.407 (0.068), 2.452 (0.081). Overall mean: 2.350 (0.091). Biplicity: few wavelengths averaging 1.2 μ. in one strain.

Salmonella enteritidis. Mean wavelengths of three strains: 2.311 (0.109), 2.328 (0.070), 2.370 (0.064). Overall mean: 2.335 (0.088).
Salmonella dublin. Mean wavelengths of two local strains from Prof. Henning: 2.279 (0.087) and 2.280 (0.087). The German strain mentioned above gave 2.355 (0.066). Fermentatively the three strains were identical, including action on rhamnose (Kauffmann, 1951).

Proteus mirabilis. Mean wavelengths of nine strains: 1.919 (0.066), 1.928 (0.068), 1.929 (0.074), 1.933 (0.054), 1.945 (0.071), 1.974 (0.060), 2.007 (0.064), 2.031 (0.076), 2.031 (0.086). Overall mean: 1.962 (0.081).

Proteus vulgaris. Mean wavelengths of two strains: 2.049 (0.093), 2.143 (0.063). Overall mean: 2.098 (0.092). Biplicity: a few helices of average wavelength of about 1.μ. (Pl. 2, fig. 11.)

Bacillus megaterium. Mean wavelengths of four strains: 3.298 (0.205), 3.362 (0.101), 3.432 (0.130), 3.451 (0.145). Overall mean: 3.389 (0.166). No biplicity but one strain showed a number of shorter wavelengths averaging 1.1.μ. (Pl. 2, fig. 12, long wavelength.)

Bacillus cereus. Mean wavelengths of eight strains: 2.169 (0.088), 2.204 (0.081), 2.255 (0.075), 2.329 (0.066), 2.392 (0.060), 2.479 (0.095), 2.516 (0.104), 2.567 (0.066). Overall mean: 2.356 (0.164). Biplicity: the strain of 2.169 showed some wavelengths of about 1.2.μ. (Pl. 2, fig. 13), and the strains of 2.516 and 2.567 had a number of wavelengths averaging 1.3.μ.

Bacillus subtilis. Mean wavelengths of eight strains: 2.138 (0.099), 2.144 (0.104), 2.154 (0.066), 2.157 (0.096), 2.158 (0.082), 2.166 (0.100), 2.229 (0.060), 2.295 (0.080). Overall mean: 2.186 (0.108). Biplicity: four strains had some wavelengths of about 1.1.μ.

Bacillus pumilus. Mean wavelengths of six strains: 2.333 (0.122), 2.335 (0.096), 2.348 (0.112), 2.349 (0.123), 2.374 (0.094), 2.378 (0.128). Overall mean: 2.353 (0.112). Biplicity: one strain had some wavelengths of about 1.2.μ.

Pseudomonas aeruginosa. Mean wavelengths of three strains: 1.477 (0.048), 1.502 (0.076), 1.552 (0.086). Overall mean: 1.530 (0.086). Helices were scanty and the number measured in two strains less than 100.

Pseudomonas diminuta. Leifson & Hugh (1954) made this a new species from the very short wavelength of its stained flagella (0.62μ.). We found scanty helices and measured 17 with an average wavelength of 0.60μ. (Short wavelength helices are always less affected by outward circumstances) (Pl. 1, figs. 2, 3).

Pseudomonas of undetermined nature, from retting tank. Mean wavelength of 121 measurements: 1.342 (0.075).

Azotobacter agile, from local soil. Mean wavelength of 86 measurements: 2.060 (0.074).

Azotobacter insigne. Mean wavelength of 164 measurements on the strain from Derx (1951) 5.058 (0.157) (Pl. 1, fig. 4).

Aerobacter aerogenes. Mean wavelengths of five strains: 2.247 (0.067), 2.283 (0.086), 2.352 (0.085), 2.365 (0.110), 2.419 (0.107). Overall mean: 2.336 (0.114).

Escherichia coli. Mean wavelength of four strains: 2.278 (0.108), 2.315 (0.101), 2.379 (0.103), 2.497 (0.095). Overall mean: 2.366 (0.121). Biplicity: two strains had some wavelengths of about 1.15μ.
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*Serratia marcescens.* Mean wavelengths of three strains: 0.965 (0.037), 2.346 (0.080), 2.591 (0.108). The first one produced a variant with a mean wavelength of 0.979 (0.050). The values are too divergent for an overall mean.

*Serratia indica* and *S. kilensis.* One strain each with practically identical wavelengths: 2.651 (0.117) and 2.649 (0.089).

*Chromobacterium ianthinum.* One strain, producing helices on agar only. Mean wavelength: 1.115 (0.085).

*Caryophanon latum.* One strain, identified by Pringsheim & Robinow (1947), showed helices on acetate yeast extract medium only. Measurements once done on 84 helices gave a mean of 1.821 (0.081) and on another occasion on 185 helices 1.831 (0.054). Biplicity: some wavelengths averaging 0.9 μ.

*Nocardia turbata.* Strain received from Dr Dagny Erikson and described by her (1954) produced a number of motile organisms on agar, sometimes branching, often detached. Mean wavelength of 52 helices (Pl. 2, fig. 14): 2.500 (0.125).

*Sarcina ureae.* The mean value previously given (Pijper & Abraham, 1954) as 3.193 (0.0048) was determined in an alkaline medium without regard to temperature; at pH 7.0 and temperatures between 16 and 19° it was 3.055 (0.100). Biplicity again was abundant.

**DISCUSSION**

We can see no 'pattern' in the distribution of the various wavelengths over the different species, nor is there a correlation between wavelength and other attributes. Three wavelengths were observed in *Serratia marcescens* and at least two in *Pseudomonas* spp. *Bacillus megaterium* and *B. cereus* both have large diameters, but their wavelengths are very different. *Caryophanon latum* with its large body has a relatively short wavelength. The salmonellas tended to have wavelengths of about 2.3 μ, but there seem to be distinct differences between the members of this group, and not all strains of *Salmonella dublin* had the same wavelength. Biplicity was a varying phenomenon, frequent in some strains and rare or absent in closely allied ones.

Helical wavelengths, however typical in some strains (*Pseudomonas aeruginosa* and *P. diminuta*, salmonellas in general, *Bacillus megaterium*, *Sarcina ureae*, *Azotobacter insigne*), will be of limited use for classification. There is too much overlapping, the differences are not very great, and there always is a possible latent biplicity. As temperature, pH value and colloid substances affect wavelengths, accurate measurement is an involved process.

We have witnessed the transition from straight tail to helix in nearly all our bacteria. Exceptions were the pseudomonads, the azotobacters and the nocardia, where we saw no straight tails; perhaps these were too thin. When preparations of these bacteria were left on the stage for some hours, until motility had ceased, helices became visible, though usually in small numbers. Finally, we emphasize that in spirilla there is no transition from straight tail to helix; flagella with a typical twist, which is not helical, are present all the time in spirilla (Pijper, 1955c).
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REFERENCES


EXPLANATION OF PLATES

All magnifications are \( \times 2000 \), except Pl. 2, fig. 8, which is \( \times 1000 \).

**PLATE 1**

Fig. 1. *Salmonella schottmuelleri* 3176.
Figs. 2, 3. *Pseudomonas diminuta*.
Fig. 4. *Azotobacter insigne*.
Fig. 5. Giant-flagellum of *Salmonella schottmuelleri* 3176.
Fig. 6. Giant-flagellum of *Bacillus cereus* 7464.
Fig. 7. *Sarcina ureae* stained with Leifson’s method for flagella.

**PLATE 2**

Fig. 8. *Salmonella typhi* 901.
Fig. 9. *S. typhi* 901, showing biplicity.
Fig. 10. *S. schottmuelleri* 3176, showing biplicity.
Fig. 11. *Proteus vulgaris* 4175, showing biplicity.
Fig. 12. *Bacillus megaterium* 7581.
Fig. 13. *B. cereus* 7464, showing biplicity.
Fig. 14. *Nocardia turbata*.

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Plate 1

(Facing p. 380)