Development of Flagella by *Proteus mirabilis*

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SUMMARY

The sequence of flagellar development accompanying differentiation during multiplication in a plate culture of *Proteus mirabilis* was investigated with the electron microscope and the negative-staining technique; the sequence of development can best be seen from the electron micrographs. The first flagella were produced towards the end of the first hour, and increased to a peak at about 6 hr and then decreased. The bacteria changed from coccoid to rod-shaped to elongated forms; the latter measured up to 80 μ in length and were equipped with several thousand flagella. On the basis of measurements of flagellar complement, the elongated forms (or swimmers) can be regarded as 'flagellin-factories'. The fine structure of both flagella and fimbriae was examined and several new features were seen, in particular certain structures at the bases of both appendages. The diameter of *Proteus* fimbriae was found to be about 40 Å.

INTRODUCTION

This paper deals with the various stages of flagellar development in a plate culture of *Proteus mirabilis* inoculated sparsely all over, a sequence not previously photographed. This paper is one of a series, earlier papers having examined by phase-contrast microscopy the changes in living organisms (Hoeniger, 1964), and by ordinary microscopy the changes manifest in stained preparations (Hoeniger, 1965).

Flagella are now generally regarded to be the locomotor organelles of most eubacteria. In life these organelles are shaped like a helix, but when dried before viewing in the ordinary light microscope or in the electron microscope, their helical structure becomes flattened on the supporting surface and they then assume a sinusoidal shape in the plane, with a wavelength that is fairly constant from one species to another. The chemical and physical properties of bacterial flagella have largely been worked out with *Proteus* flagella (particularly *Proteus vulgaris*). We know that *Proteus* flagella consist almost entirely of protein, called flagellin (Astbury, Beighton & Weibull, 1955), which has a characteristic amino acid composition (Weibull, 1949; Kobayashi, Rinker & Koffler, 1959) and a molecular weight of about 20,000 (Kobayashi *et al.* 1959). It is believed that these protein molecules constitute the subunits of the flagellum proper. According to all recent models, strands or rows of flagellin molecules are arranged helically or lengthwise in the intact flagellum (Kerridge, Horne & Glaupert, 1962; Abram, Vatter & Koffler, 1964*a*; Lowy & Hanson, 1964). In the case of the *Proteus* flagellum, the subunits have a diameter of 45 Å (Rogers & Filshie, 1963) or 50 Å (Lowy & Hanson, 1964), and the flagellum proper a diameter of 120 Å.

More relevant to the present study is the past work on the flagellated *Proteus* bacteria themselves. Leifson and his associates (Leifson, Carhart & Fulton, 1955; Leifson, 1960) studied *Proteus* flagellation under various physiological conditions by staining for light microscopy; Pijper, Nesser & Abraham (1956) made comparable experiments, viewing living bacteria by sunlight dark-ground illumination. Electron micrographs have been published from time to time illustrating flagellated *Proteus* organisms (Houwink & van Iterson, 1950; van Iterson, 1953; 1954; Preusser, 1958; Robinow, 1960; Thornley & Horne, 1962), but these did not follow an orderly sequence of differentiation or flagellar development. The only study thus far of flagellar development in *Proteus* is that by Bisset and his co-worker (Bisset, 1951; Bisset & Hale, 1951).

In the present work, the bacteria were inoculated uniformly but sparsely on a solid medium, and stages of flagellar development followed in the electron microscope by using the negative staining technique. Flagellation was correlated with type of motility in living cultures. Microscopic observations were combined with the determination of the growth-curve in an attempt to correlate morphological with physiological events. This study also afforded an opportunity for re-examining the fimbriae of *Proteus* since the inoculum contained many fimbriate organisms; and for examining the basal appendages of both flagella and fimbriae.

METHODS

Organism, media and incubation. The strain of *Proteus mirabilis* used in this work was isolated from a stool specimen and maintained on egg slopes (Mackie & McCartney, 1960) at 4°. The inoculum was prepared from cultures grown on slopes of heart infusion agar (Difco) or passed through a Craigie-type motility tube (Tulloch, 1939) of semi-solid agar (heart infusion broth + 0.3% agar). The organisms were suspended in physiological saline to a concentration of about 10^9 bacteria/ml.; 1 ml. of this suspension was spread uniformly over the surface of heart infusion agar in a 15 cm. diameter Petri dish, thus giving an inoculum of approximately one bacterium/20 μ^2 of surface. Incubation was at 30°, this temperature having been found previously (Hoeniger, 1964) to be most suitable for following the morphological changes in living organisms by phase-contrast microscopy.

Measurement of growth. Growth was measured by dry weight and colony count. Bacteria harvested in saline from three plates were pooled and made up to 100 ml. with saline. For colony counts, a 1 ml. sample was diluted, and 0.1 ml. plated on heart infusion medium containing 4% agar to inhibit swarming (Mackie & McCartney, 1960). The remaining 99 ml. of bacterial suspension were centrifuged, and the deposit washed and dried to constant weight at 105°.

Electron microscopy. Negative staining was done with living or fixed bacteria. With living organisms, the bacteria were floated off the agar by adding saline to the plate and rocking to and fro (to avoid breaking off flagella by shearing); the bacteria were then decanted into a centrifuge tube, spun down, and washed with distilled water. With fixed organisms, the bacteria were taken up in 0.5% (w/v) formaldehyde (neutral) and allowed to fix for several hours, then centrifuged down and washed as before. The bacteria were suspended in distilled water at a concentration of about 10^8 organisms/ml. and mixed with an equal volume of 2% (w/v)

potassium phosphotungstate (PTA; Thornley & Horne, 1962) + 0.02–0.04 % bovine serum albumin (BSA; Fraction V from bovine plasma; Armour Pharmaceutical Company, Kanakee, Illinois, U.S.A.); the BSA, which promotes the spreading of the PTA solution by decreasing the surface tension, was added to the PTA solution immediately before staining. The stained bacteria were placed on an electron microscope grid coated with Formvar and carbon by breaking a thin film in a 5 mm. platinum loop on to the surface of the supporting membrane (Murray, 1963). The grid itself rested on a pad of filter paper so that excess fluid drained away as the film of bacteria dried down rapidly. Electron micrographs were taken with a Philips 100B electron microscope, operating at 80 kV with a 25 μ objective aperture, at instrumental magnifications of $\times 2000$ –10,000.

In most experiments with the electron microscope, correlated observations were made on living bacteria. The stage of differentiation in a plate culture could be determined quickly by focusing on the surface of growth derived from the sparsely inoculated plate. Motility, whether rotational, translational or absent, was checked with the phase-contrast microscope in hanging-drop mounts of organisms from the plates suspended in broth (see Stocker & Campbell, 1959).

Measurements of bacteria and flagella. The width and length of bacteria were measured directly from electron micrographs; estimates of volume were based on the assumption that the organisms were cylindrical. The number of flagella/bacterium was likewise determined from micrographs, while the length of each flagellum was measured with a piece of string laid along the curves from free end to point of insertion, whenever possible. In the case of the elongated bacteria seen during later stages of differentiation (which can have hundreds, even thousands of flagella), a limited number (about 50–100) was counted or measured over a bacterial length of several μ .

RESULTS

The choice of inoculum

Previous studies (Hoeniger, 1964, 1965) were made with an inoculum of organisms which had passed through a tube of semi-solid agar, i.e. motility-tube cultures incubated for about 40 hr. When such cultures were now examined in the electron microscope, they were found to consist largely of bacteria which were fully fimbriate (with usually 100–300 fimbriae/bacterium—as in Pl. 1, fig. 1) or had both fimbriae (50–100) and flagella (usually 1–3, but occasionally more as in Pl. 1, fig. 2); the remainder of the bacteria were flagellate but non-fimbriate, or had no appendages. All these organisms were small in size and coccoid to rod-shaped (0.6–0.7 μ wide by 0.7–2.0 μ long).

The finding of large numbers of fimbriate bacteria from the 40-hr motility tubes was surprising, and it seemed advisable to investigate the types of appendages on organisms from such cultures incubated for various times up to 48 hr, and to compare them with cultures of similar ages grown on solid medium, i.e. agar slopes. The results are given in Table 1; since only a hundred organisms were screened per specimen the data are intended to show trends rather than finite values. The table shows that: in motility-tube cultures the proportion of fimbriate bacteria (i.e. total of bacteria with fimbriae only and those with fimbriae + flagella) increased with incubation-time; the proportion of flagellate bacteria (i.e. total of bacteria with

flagella only and with flagella + fimbriae) decreased; the proportion of non-flagellate non-fimbriate bacteria remained more or less constant. With bacteria grown on solid medium: the slope cultures always contained a large proportion of flagellate bacteria with usually 1-4 flagella/bacterium, less often 5-10. These flagellate organisms closely resembled the 12 hr bacteria whose morphology will be described in detail below (see Pl. 5, fig. 10). The slope cultures contained far fewer fimbriate organisms than the motility-tube cultures, and there were always some without any appendages.

Since 30-36 hr motility tubes and 18 hr slopes had approximately the same proportion of flagellate bacteria, these cultures were used for inoculating plates in the growth experiments which follow.

Table 1. *Types of appendages on bacteria of Proteus mirabilis passed through semi-solid medium (motility-tube culture) or grown out on agar slopes over a period of 18-48 hr*

Type of culture	Percentage of bacteria with			
	Flagella only	Flagella + fimbriae	Fimbriae only	No appendages
Motility-tube, 18 hr*	—	—	—	—
Motility-tube, 24 hr	93	0	0	7
Motility-tube, 30 hr	58	10	20	12
Motility-tube, 36 hr	32	26	28	14
Motility-tube, 42 hr	22	22	46	10
Motility-tube, 48 hr	24	10	56	10
Slope, 18 hr	67	3	8	22
Slope, 24 hr	66	4	4	26
Slope, 30 hr	50	4	24	22
Slope, 36 hr	43	2	19	36
Slope, 42 hr	44	6	26	24
Slope, 48 hr	46	9	15	33

* Sufficient bacteria for electron microscopy had not yet passed through medium.

Sequence of growth in plate cultures

Figure 1 gives the results of an experiment in which growth was measured in terms of viable count (upper curve, open circles) and dry weight (lower curve, closed circles). It will be seen that the dry weight curve has a short lag phase (*c.* 1 hr), an exponential phase of approximately 5 hr, a retardation phase of 4 hr, and a stationary phase beginning at 12 hr. The colony count curve shows an almost imperceptible lag phase, an exponential phase of *c.* 6 hr, a retardation phase of 4 hr, and the stationary phase again begins at 12 hr. There is no true phase of decline during the 48 hr of the experiment. The generation time of *Proteus mirabilis* in this experiment was 40 min., a value which agrees fairly well with phase-contrast observations of division in living organisms (Hoeniger, 1964).

When one correlates the growth sequence in Fig. 1 with morphological changes on the plates and in the organisms themselves, several points seem to emerge: the short lag phase encompasses the time during which the bacteria are known from phase-contrast studies (Hoeniger, 1964) to be enlarging prior to their first division; the latter half of the exponential phase corresponds to the period when most organisms differentiate into long motile filaments commonly called swimmers (see

Discussion); and the retardation and stationary phases to the time during which the elongated organisms break down into progressively shorter rods that eventually become quite small resting organisms.

As for growth starting from a slope culture, i.e. an inoculum of mainly flagellate bacteria, the two curves are similar to those in Fig. 1, and so are the morphological changes. Evidently, resting organisms from slope cultures, with few fimbriate bacteria, behave very much like those from motility-tube cultures, with *c.* 50 % fimbriate bacteria.

Fig. 1. Growth of *Proteus mirabilis* on heart infusion agar starting from an inoculum of partly fimbriate, partly flagellate bacteria (motility-tube culture). Calculated as colony count (open circles) and dry weight (closed circles), both parameters being derived per crop from three 15 cm. plates.

Electron microscopy of negatively stained bacteria

To turn now to the major part of this paper: the morphological sequence, particularly as regards flagellar development, which takes place during 48 hr of incubation. The first observable change occurs during the first hour: the bacteria enlarge significantly, their diameter increasing from $0.6\text{--}0.7\ \mu$ to between 0.9 and $1.2\ \mu$; new flagella grow out and appear as short curved spikes (see Pl. 2, figs. 3, 4). The bare bacterium in Pl. 2, fig. 3 has 6 apparently new flagella, and the fimbriate bacterium in fig. 4 has 9. (Of course, in this type of experiment it is impossible to distinguish new from old flagella with certainty.) The mean flagellar lengths on these two bacteria are $1.03\ \mu$ and $1.09\ \mu$, respectively; the distribution of lengths is plotted as a histogram in Fig. 2 parts (a) and (b). When organisms from 1 hr plates were observed in the living condition using hanging-drop mounts, many showed a rotating motility. In this and other observations of motility, it was necessary to allow for that proportion of the inoculum, i.e. approximately 50 %, which already possessed long flagella and so moved in quite a rapid, translatory fashion (see Table 1).

That the bacteria continue to grow and synthesize flagella during the next hour can be seen from Pl. 2, fig. 5. This rod has 36 flagella with a mean length of $1.98\ \mu$;

the distribution of the lengths of its flagella is shown in Fig. 2 (c). Hanging-drops of such 2 hr cultures showed that the proportion of bacteria with rotating motility had decreased, many now moving in a translational manner.

The end of another bacterium from a 2 hr culture is to be seen in Pl. 6, fig. 11: this bacterium bore a cluster of some hundred fimbriae at either end. In fact, fimbriae which were present on about 50 % of the inoculated organisms were

Fig. 2. Distribution of lengths of flagella on *Proteus mirabilis* bacteria shown in electron micrographs. Age of culture is indicated in hr; N = no. of flagella/bacterium.

progressively segregated to the tips of the bacteria in succeeding generations and were not seen beyond 2½ hr of incubation. We shall have more to say about Pl. 6, fig. 11, later.

By the time 3½ hr have elapsed, elongated forms are to be found: a pair of these which had not yet separated is shown in Pl. 3, fig. 6. Both organisms have 116 flagella and many of these have not yet attained their full length, the mean lengths being 2.4 and 2.7 μ. The range of flagellar lengths has been plotted in the histograms of Fig. 2 (d). It was further observed that by this time most bacteria in living preparations moved in a translational manner.

During the next 2-3 hr progressively longer and significantly thinner bacteria are to be seen, the well-known *Proteus* swimmers. These can range anywhere from 6 to 80 μ long, are 0.65-0.75 μ wide, and are equipped with hundreds, even thousands of flagella. A relatively short swimmer from a 4½ hr culture is shown in Pl. 4, fig. 7;

its complement of 176 flagella had a mean length of $4.0\ \mu$; the actual distribution of lengths is plotted in Fig. 2 (*e*). Plate 4, fig. 8, presents the tip of a longer (i.e. $22\ \mu$) swarmer from a $5\frac{1}{2}$ hr culture; by this time more than 60 % of the bacteria on the agar plate had differentiated into such long motile filaments. Approximately 300 flagella could be counted on a $5\ \mu$ length at the tip of the bacterium in Pl. 4, fig. 8; thus, by inference, the whole organism had over a thousand (*c.* 1300). The flagella range from 2 to $10\ \mu$ long, the mean from a measurable sampling of 50 being $4.9\ \mu$; as it obviously is not possible to plot all the lengths of flagella on this organism, no histogram has been prepared. Further it is to be noted that these flagella are produced all over the surface of the organism, a feature which could not be distinguished in most shadow-cast specimens (Houwink & van Iterson, 1950; Preusser, 1958; Robinow, 1960). When one examines such bacteria in the living condition they are seen to move in a sinuous manner which is doubtless responsible for their synonym of 'snakes'.

After 6 hr of incubation the elongated forms start breaking down into progressively shorter units. Plate 5, fig. 9, shows a fairly short swarmer which had divided quite symmetrically to give two rods still attached together at the time of fixation. The average length of flagella on these rods is 4.9 and $6.2\ \mu$ respectively; the range of lengths in each case is to be seen in Fig. 2 (*f*). Living bacteria at this stage (i.e. in a $6\frac{1}{2}$ hr culture) were all still moving in a translatory fashion.

The process of subdivision continues until the culture consists of coccoid bacteria or short rods like those (from a 12 hr plate) in Pl. 5, fig. 10, which move either rotationally or translationally, depending on the length of the flagella. It will be noted that some of the flagella on the bacteria in Pl. 5, fig. 10, are shorter than during previous stages (their average lengths are 3.7 and $4.1\ \mu$); the distribution of length is plotted in Fig. 2 (*g*).

The sequence of cellular differentiation and flagellar development described in the preceding paragraphs is summarized in Table 2. The data for each age of culture in regard to shape and size-range of organisms, range of number of flagella/bacterium, and average flagellar length (mean $\pm$ s.d.) have been determined from at least 10 micrographs; the one exception is the $5\frac{1}{2}$ hr measurement of average flagellar length which comes from a single specimen (that in Pl. 4, fig. 8). The heading 'Developing flagella' is based on the assumption that the shorter flagella seen during early stages of differentiation are newly developed ones (see above, p. 33). Further, in determining the type of motility in hanging-drop mounts, allowance was always made for the inoculated bacteria (about 50 %) known to possess long flagella and hence moving in a translatory fashion.

As Table 2 shows, the average flagellar length increases from 1 to $5\frac{1}{2}$ hr, then falls. An even more informative measure of flagellar synthesis is obtained by calculating the number of flagella/unit volume of bacterium and the total flagellar length/unit volume, as presented in Table 3. It will be seen that there is a gradual increase in both number and total length of flagella to a maximum of about $5\frac{1}{2}$ hr, and then a decline. That the average flagellar length increases with time to a peak, and then declines can also be seen from the histograms of Fig. 2. The rise naturally reflects an increase in the production of flagellin, so that we can really regard the swarmers as 'flagellin-factories'. The decline suggests that in later stages the bacteria are synthesizing flagella at a lower rate.

Table 2. *Characteristics of bacteria and flagella of Proteus mirabilis growing out on agar over 48 hr at 30°, inoculated from motility-tube culture*

Age of culture	Bacteria			Developing flagella		
	Shape or type	Range of diameter (μ)	Range of length (μ)	Range of no./bacterium	Average length (μ)*	Type of Motility
0 hr (inoculum)	Coccoid to rod-shaped	0.6-0.7	0.7-2.0	—	—	—
1 hr	Coccoid to rod-shaped	0.9-1.2	1.1-2.4	1-10	0.75 $\pm$ 0.29	Rotating
1½ hr	Rods	0.9-1.2	1.7-2.6	5-30	1.54 $\pm$ 0.22	Rotating
2 hr	Rods	0.8-1.0	2.0-3.7	15-60	2.02 $\pm$ 0.32	Rotating and translational
2½ hr	Rods	0.8-0.9	3.0-4.2	65-80	2.38 $\pm$ 0.04	Translational
3½ hr	Rods and short swimmers	0.7-0.8	3.8-6.0	90-140	2.61 $\pm$ 0.11	Translational
4½ hr	Swimmers	0.65-0.75	6.0-c. 20.0	c. 150-450	3.84 $\pm$ 0.32	Translational
5½ hr	Swimmers	0.65-0.75	c. 16-80	c. 500-5000	5.25	Translational
6½ hr	Rods and short swimmers	0.65-0.75	3.0-8.0	40-120	4.85 $\pm$ 1.0	Translational
8 and 12 hr	Coccoid to rod-shaped	0.65-0.8	1.0-2.0	5-30	3.69 $\pm$ 0.41	Translational
24 and 48 hr	Coccoid to rod-shaped	0.55-0.65	0.6-1.4	1-10	2.46 $\pm$ 1.70	Rotating and translational

* Mean $\pm$ s.d.

Characteristics of Proteus flagella and fimbriae

Apparently fully grown flagella, 6–11 μ in length, are to be seen on a proportion of the inoculated bacteria (as in Pl. 1, fig. 2), on the swimmers (Pl. 4, figs. 7, 8) and on short rods derived by the breakdown of the latter (see Pl. 5, fig. 9). These organelles have a mean wavelength or pitch (Leifson, 1960) of $2.13 \pm 0.14 \mu$ (s.d.), and are 120 Å wide. In a number of preparations where the flagella had been pulled off as the PTA dried down, hook-like endings were observed. Plate 6, fig. 11, shows the end of a rod from a 2 hr culture with one free flagellum just above the cell and another, indicated by arrows, on the lower surface, evidently still attached and penetrating the wall. Both flagella terminate in hooks. These basal hooks measure 300–400 Å in length, and have the same diameter as the rest of the flagellum. Sometimes the hooks were found attached to rounded cytoplasmic structures about

Table 3. *Development of flagella by Proteus mirabilis in plate culture at 30°; data calculated from electron micrographs*

Age of culture	No. flagella/unit vol. bacterium (μ^3)		Total flagellar length (μ)/ unit vol. bacterium (μ^3)	
	Mean	s.d.	Mean	s.d.
1 hr	3.15	1.82	2.64	1.60
1½ hr	9.91	5.64	16.36	9.65
2 hr	18.27	7.63	37.38	14.08
2½ hr	37.43	11.51	74.07	15.79
3½ hr	45.58	6.17	116.55	14.54
4½ hr	80.90	7.67	305.33	32.56
5½ hr	150.00	—	603.00	—
6½ hr	44.36	8.55	214.05	28.96
8 and 12 hr	16.84	5.23	60.37	14.86
24 and 48 hr	18.10	9.61	61.02	44.82

200 Å wide (i.e. between 150 and 250 Å); a cluster of flagella ending in such structures can be observed in the partially lysed swimmer of Pl. 6, Fig. 12. The possible significance of these rounded structures as 'basal bodies' for the flagella will be discussed below. The free ends of the flagella always appear squared off. The presence of an axial core of phosphotungstate in a number of short, developing flagella suggested that the flagella themselves are hollow.

The fimbriae of *Proteus mirabilis* are to be found chiefly on inoculum bacteria taken from motility-tube cultures (see Pl. 1, figs. 1, 2) and in plate cultures derived from them (see Pl. 2, fig. 4 and Pl. 6, fig. 11). On the inoculated bacteria they numbered between 50 and 300 and were peritrichously arranged. In plate cultures, the fimbriae were gradually 'diluted out' as the population multiplied, being segregated towards the poles of the bacteria. A particularly good example of polar fimbriae is to be found in Pl. 6, fig. 11. *Proteus* fimbriae are straight filaments, in contrast to the wavy flagella, and usually project singly from the periphery of the organism though occasionally they form small bundles with the individual fibres twisted around one another. The fimbriae are 0.2–1.3 μ in length and have a diameter close to 40 Å; i.e. they are one-third as wide as the flagella. In a few instances, evidence was obtained of an attachment organelle on *Proteus* fimbriae, as can be seen in Pl. 6, fig. 13. These polyhedral structures are *c.* 250 Å wide, and closely

resemble the protusions from which fimbriae have been found projecting on the surface of lysed organisms of a *Pseudomonas* species by Dr T. Yamamoto (personal communication).

DISCUSSION

The sequence of bacterial differentiation described, which started from a uniformly distributed inoculum, corresponds precisely to that observed by phase-contrast microscopy in plate cultures of *Proteus mirabilis* inoculated at a single point (Hoeniger, 1964—see Figs. 10–19). It seemed, therefore, justified to apply the term ‘swarmer’ to the elongated forms observed at about 4–6 hr in the type of experiment described in this paper. Swarmers removed from either of these two types of cultures all move in the same sinuous translatory manner when viewed in hanging-drop mounts. Further, the sequence here described of the breakdown of swarmers into progressively shorter organisms was exactly the same as that observed in my earlier work (Hoeniger, 1964; Figs. 25–27). However, one is probably not justified in using the term ‘swarming’ to refer to the period of churning, swirling activity that takes place on the uniformly inoculated plate between 4 and 6 hr. ‘Swarming’ should be restricted to the spreading of growth over the uninoculated area of the medium. It is indeed to this latter type of migration by *Proteus* swarmers that the term was applied in the past (Russ-Münzer, 1935; Lominski & Lendrum, 1947; Kvittingen, 1949; Hughes, 1957).

Several aspects of the sequence by which the small *Proteus* bacteria of the inoculum are differentiated, via rod-shaped organisms, into elongated swarmers merit comment. The fact that the peritrichous fimbriae present on about 50 % of the inoculum are progressively segregated during the first 2½ hr to the poles of derivative bacteria suggests that new cell-wall material is being synthesized in the equatorial region rather than by diffuse intercalation of new material with old. Such an equatorial manner of cell-wall replication has already been observed in streptococci (Cole & Hahn, 1962; Chung, Hawirko & Isaac, 1964); *Salmonella typhi*, on the other hand, synthesizes new wall by intercalation (Cole, 1964). The application of fluorescent antibody staining to cell-wall replication during the differentiation of *Proteus* swarmers will throw further light on this problem. The synthesis of flagella during bacterial differentiation appears to be a random process. The longest, and presumably the first, flagella (which develop during the 1–2 hr after inoculation) are located either at the equator (as in Pl. 2, fig. 3), or towards a pole (Pl. 2, fig. 4), or in both regions (Pl. 2, fig. 5). There is no evidence to support the view that the first flagellum is invariably produced by *Proteus* at a single pole (Bisset & Hale, 1951). Nor were the flagella shed during later stages as earlier observed by Bisset & Hale.

During the developmental sequence leading to swarmers, the whole physiological organization of the bacterium changes. There is a switch from the production of vegetative rods to that of elongated forms. This switch is presumably mediated through an inhibition of the normal mechanism of cell division. The bacteria thus produced are quite different from their progenitors: not only are they much longer and significantly thinner, but they have many more flagella/unit volume (see Table 3). In fact, the swarmers have already been called ‘flagellin-factories’. This suggests that there is a partial curtailment in the production of cellular protein which results in the greatly increased production of flagellar protein.

To discuss now the characteristics of *Proteus* flagella and fimbriae described in this paper. The flagellar width of 120 Å agrees well with that reported for shadowed preparations of *Proteus vulgaris* by Astbury & Weibull (1949); the wavelength of $2.13 \pm 0.14 \mu$ is likewise close to previous determinations (Reichert, 1909; Weibull, 1950; Leifson *et al.* 1955; Pijper *et al.* 1956). The hook-shaped endings seen in Pl. 6, figs. 11 and 12, are reminiscent of the little hooks or 'rootlets' observed on isolated flagella of *Agrobacterium radiobacter* (Houwink & van Iterson, 1950), *P. vulgaris* (Rogers & Filshie, 1963) and *Vibrio metchnikovii* (Glauert, Kerridge & Horne, 1963), and of the hockey-stick shaped endings seen on intact flagella of *Spirillum* spp. (Houwink, 1953; Murray & Birch-Andersen, 1963) and *V. metchnikovii* (Glauert *et al.* 1963). The observation that the flagellar hooks are attached within the cell to rounded structures about 200 Å wide (see Pl. 6, Fig. 12) is indeed interesting. Similar basal discs were recently observed in lysed bacteria of *V. metchnikovii* (Glauert *et al.* 1963), *P. vulgaris* (Abram, Vatter & Koffler, 1964*b*) and some *Bacillus* species (Abram *et al.* 1964*b*). Van Iterson & Leene (1964) have found the basal regions of the flagella in *P. vulgaris* to be sites for binding reduced tellurite, which suggests the presence there of reductive enzymes. It is the opinion of the present writer that these 'basal bodies' are the regions in which the flagellin molecules are aggregated to form flagella, there being anywhere between 3 and 10 strands or rows of such flagellin molecules/flagellum according to the various models proposed to date (Kerridge *et al.* 1962; Abram *et al.* 1964*a*; Lowy & Hanson, 1964).

The fimbriae of *Proteus mirabilis* are thinner than those of other enterobacteria (Duguid & Gillies, 1958; Duguid, 1959; Brinton, 1959; Thornley & Horne, 1962). In fact, the dimension given here of *c.* 40 Å is significantly lower than previously reported from shadowed preparations of intact fimbriae (Coetzee, Pernet & Theron, 1962; Shedden, 1962), and slightly lower than that from negatively stained preparations of isolated fimbriae (Rogers & Filshie, 1963). Of particular note is the presence of a 'basal body' also on these organelles, as can be seen in Pl. 6, fig. 13. This body may arise either within the cytoplasm proper or in the cytoplasmic membrane. Thus fimbriae and flagella in general appear to originate inside of the bacterial cell wall. Indeed, both structures are retained when appropriate strains of *Escherichia coli* or *P. mirabilis* are converted to sphaeroplasts by treatment with penicillin (Lederberg & St. Clair, 1958; Maccacaro & Turri, 1959; Martin, 1963). The basal structures on the fimbriae, like those on the flagella, are probably the sites at which the protein subunits (Brinton & Stone, 1961; Brinton, 1965) are assembled into the fimbriae proper.

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EXPLANATION OF PLATES

In figs. 1–10, the marker represents 1 μ ; in figs. 11–13, it represents 0.1 μ .

PLATE I

Electron micrographs of inoculum organisms which had passed through a tube of semi-solid agar, i.e. from a motility-tube culture.

Fig. 1. Pair of fimbriate coccobacilli.

Fig. 2. Fimbriate rod with 6 long flagella.

PLATE 2

Electron micrographs of bacteria showing early stages of flagellar development.

Fig. 3. Bare rod with 6 young flagella; from 1 hr plate.

Fig. 4. Fimbriate rod with 9 young flagella; from 1 hr plate.

Fig. 5. Rod with 36 flagella of various lengths; from 2 hr plate.

PLATE 3

Fig. 6. Electron micrograph of a pair of young swimmers which have not yet separated; from a $3\frac{1}{2}$ hr plate.

PLATE 4

Electron micrographs of *Proteus* swimmers.

Fig. 7. Short swimmer from a $4\frac{1}{2}$ hr plate.

Fig. 8. $6\ \mu$ tip of a much longer (i.e. $22\ \mu$) swimmer taken from a $5\frac{1}{2}$ hr plate.

PLATE 5

Electron micrographs of bacteria derived by the breakdown of swimmers.

Fig. 9. Pair of rods from $6\frac{1}{2}$ hr plate culture, with many long flagella.

Fig. 10. Pair of rods from 12 hr plate, with flagella of various lengths.

PLATE 6

Electron micrographs of *Proteus* flagella and fimbriae showing details of basal structure.

Fig. 11. Tip of rod from a 2 hr plate bearing flagella and fimbriae; terminal hooks on 2 flagella are indicated by arrows.

Fig. 12. Flagella penetrating the cellular envelope of a partially lysed swimmer; each flagellum ends in a hook which is attached to a spherical mass of cytoplasm.

Fig. 13. Three fimbriae with polyhedral appendages presumed to be cytoplasmic in origin.