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The Unilinear Transmission of Motility and its Material Basis in Salmonella

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SUMMARY: Certain sub-lines of a strain of *Salmonella paratyphi* C contain 10 % or more of motile flagellated bacteria when in exponential growth at 37°. Less than 0.1 % of the organisms are motile after prolonged exponential growth at 20°. Single motile organisms isolated from cultures grown at 37° transmitted motility to only one or a few of their descendants during growth at 20°; they behaved as if their motility were due to non-multiplying motility-conferring (MC) particles. There is a good correlation between the distributions of number of MC particles/motile bacterium as indicated by the numbers of motile descendants at 20°, and the distributions of number of flagella/flagellated bacterium in stained preparations. It is inferred that one MC particle corresponds to one flagellum. The results support the hypotheses that in some cases flagella arise as a consequence of discontinuous intracellular events, each of which leads to the synthesis of a small number of flagella, and that parental flagella are shared amongst progeny bacteria until no descendant has more than one flagellum derived from a parental cell several generations removed. This sharing is not detected unless synthesis of new flagella ceases whilst bacterial growth continues. Single flagella may be unilinearly transmitted for over 20 generations.

During experiments on the transduction of motility in *Salmonella*, Stocker (1956*a, b*) and Lederberg (1956) encountered a class of motile bacterium which divided to produce one non-motile and one motile daughter. The motile daughter in its turn behaved similarly; the character 'motility' was transmitted down a single line of descent to one only of its progeny of any generation. Stocker (1956*a, b*) called this phenomenon the *unilinear transmission* of motility and to account for it he put forward the hypothesis of motility-conferring (MC) particles. The hypothetical MC particles have the following properties:

(1) A single particle enables the bacterium bearing it to swim in broth, but several particles are required to enable the bacterium to swim through a semi-solid medium.

(2) A particle is not replicated, but is retained by one of the daughter cells at division.

(3) A particle, and in consequence motility, can be handed on for many generations, e.g. 20.

(4) When a bacterium bearing several MC particles divides, the particles are shared about equally between the daughters.

The material basis for the hypothetical MC particles is not clear. The unilinear transmission of motility entails the transmission of effective locomotor

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apparatus: possibly a single flagellum or a basal granule which synthesizes it, a whole cell wall bearing one or more flagella, or some 'indivisible' intracellular mechanism which controls the synthesis of flagella. Motile bacteria to which motility has been unilinearly transmitted (and thus by definition having only one MC particle each) are readily isolated by micromanipulation. Unfortunately, it was not possible to test whether each had only one, or several, flagella, since a single unstained flagellum of *Salmonella* is invisible in the light microscope and the staining of selected individual bacteria is not feasible.

Unilinear transmission of motility was also encountered by Lederberg (1956) and by Quadling & Stocker (1957) when investigating the behaviour of rare, non-mutant, motile bacteria which they found occurring spontaneously in some non-motile *Salmonella* strains. Stocker (1956*b*) suggested that unilinear transmission might be detectable whenever motile bacteria multiply to produce predominantly non-motile offspring, i.e. when multiplication continues but inception of *new* flagella ceases. This situation has now been achieved experimentally by transferring exponentially-growing cultures of certain *Salmonella* strains from 37°, at which temperature a high proportion of the bacteria are motile, to 20°, at which temperature bacterial growth continues but synthesis of new flagella ceases. Such experiments have enabled comparisons to be made between distributions of number of motile progeny/motile bacterium (i.e. by definition, of number of MC particles/motile bacterium) and the distributions of number of stained flagella/flagellated bacterium.

There are numerous reports of bacteria which are motile when grown at one temperature and non-motile when grown at other, usually higher, temperatures. Reports of better development of motility at high temperatures of incubation are rare, e.g. Neustadl (1917). The *Salmonella paratyphi* C strains used in the present work (all derived from the same O parent strain) are unusual in that flagellation and motility are best developed at high incubation temperature (37°).

METHODS

Organisms and media. The cultural methods and media used were as described by Quadling & Stocker (1957). Cultures were grown either at 37°, or at laboratory temperature (c. 20°); such cultures are hereafter termed '37° cultures' and '20° cultures'.

The *Salmonella paratyphi* C O strain of Hayes & Freeman (1946), hereafter termed SL 236, was received from Dr C. C. Spicer (Serological Standards Laboratory, Public Health Laboratory Service, Colindale, London, N.W. 9); this strain was non-motile and non-flagellated when grown at 20° and 37° in any medium tested, and it did not spread in semi-solid agar. A motile mutant, SL 237, was selected from it by picking from a slow-spreading swarm produced in semi-solid medium incubated at 37°. When SL 237 was grown at 20°, in tryptic- or papain-digest broth, less than 0.1 % of the bacteria were motile and less than 0.1 % were flagellated; but when grown at 37° in tryptic-digest broth about 15 %, and in papain-digest broth about 70 %, of the bacteria were flagellated and motile. A further substrain, hereafter referred to as SL 242,

was obtained from SL237 by isolation from a fast-spreading swarm produced in semi-solid medium. SL242 also was non-motile and non-flagellated when grown at 20°; when grown at 37° in tryptic broth about 40 % of the bacteria were flagellated and motile. The proportion of motile bacteria in 37° cultures of SL237 and SL242 varied somewhat from experiment to experiment, presumably as a result of differences between batches of broth. Strains designated by a strain no. followed by a capital letter, e.g. SL237/A, were sublines, each grown from a single bacterium isolated by micro-manipulation from the parent culture.

Staining and counting of flagella. A modification of Leifson's (1951) method was used to stain flagella on bacteria grown in broth. Cultures were fixed with formaldehyde, to a final concentration of 1 % (w/v), then sedimented by centrifugation for 40 min. at 250 g. The bacteria were then washed twice by gentle resuspension in 0.01 % (v/v) Teepol (Shell Chemicals Ltd., 105/109 Strand, London, W.C. 2) in distilled water and recentrifuged. The bacteria were finally resuspended in distilled water. The detergent was used to ensure the uniform distribution of organisms in smears (Snieszko, 1942) and to assist in attaining the cleanliness necessary with this staining technique. Drops of the final distilled-water suspension of organisms were transferred to acid-cleaned slides and stained as described in Leifson (1951).

Bacteria were usually well dispersed in the central part of each smear; counts of number of flagella/bacterium were made on bacteria in this central region. Counts were made at a magnification of $\times 1200$ by using a 2 mm., $\times 95$ oil-immersion objective with phase-contrast illumination and a green filter. The flagella were counted on each of the first five flagellated organisms seen in each of ten or more successive scans started at arbitrarily chosen points. The use of phase-contrast made counter-staining of the bacterial bodies unnecessary.

Micromanipulation. The techniques used followed those of de Fonbrune (1949) as described by Stocker (1956*b*) and Quadling & Stocker (1957).

RESULTS

Demonstration of unilinear transmission of motility

When strain SL237/A was grown at 37° in tryptic-digest broth about 15 % of the bacteria were flagellated and about 15 % were motile; when grown at 20° the proportion of motile bacteria was very low (< 0.1 %) and in stained preparations the proportion of flagellated bacteria was similar. Motile bacteria were isolated by micromanipulation from 37° cultures and were allowed to continue growth at room temperature (*c.* 20°) in microdrops in the oil chamber. Under these conditions each motile bacterium, after seven to eight divisions, gave rise to a predominantly non-motile population containing only one or a few (up to six) motile bacteria (Table 1). Non-motile bacteria when isolated produced only non-motile populations.

Evidence for the unilinear transmission of motility was obtained by making repeated isolations of motile descendants during growth at 20° of populations

each descended from a motile bacterium originally isolated from a 37° culture. A typical experiment is illustrated in Fig. 1. In the lineage illustrated a motile bacterium, isolated from a 37° culture of strain SL237/A, divided at 20° to give three motile bacteria and one non-motile bacterium. Each of these bacteria was isolated to a sterile drop of broth, where growth continued. The populations

Fig. 1. Isolation by micromanipulation of the motile progeny of a motile bacterium from a culture of strain SL237/A grown in tryptic-digest broth at 37°. The progeny were grown at 20° in microcultures. Arrows indicate transfer of a motile bacterium to a sterile microdrop. M=a motile bacterium; S=a non-motile bacterium; +=hundreds; ++=thousands.

were examined at intervals and any motile bacteria present were removed to fresh drops. At no time were there more than three motile bacteria amongst the descendants of the bacterium originally isolated. On the hypothesis that a bacterium which transmits motility unilinearly has one MC particle and that one MC particle corresponds to one flagellum, the original motile bacterium had three flagella; these were shared between the daughters at the first two divisions after isolation and persisted for up to twenty generations amongst the descendants. Similar results were obtained with motile bacteria isolated from 37° cultures of strain SL242/A.

The motile bacteria isolated from 37° cultures of these two strains behave at 20° like one class of motile bacteria produced as a result of abortive transduction of motility (Stocker, 1956*b*; Lederberg, 1956) or like motile bacteria occurring spontaneously in certain non-motile strains (Quadling & Stocker, 1957). That is, they transmit motility only to a few of their descendants, as if their motility were due to the presence of a few non-multiplying MC particles whose further synthesis, in the present case, ceased on transfer to 20°, and as if the MC particles were transmitted to correspondingly few of the progeny of the originally isolated motile bacterium.

Distributions of numbers of motile descendants and of numbers of stained flagella amongst the bacteria

If an MC particle corresponds to a flagellum, then a bacterium (of a 37° culture) with n flagella when allowed to multiply at 20° for several generations would have n motile descendants; and the proportions of bacteria of a 37° culture having 1, 2, 3, etc. flagella should be equal to the proportions having 1, 2, 3, etc. motile descendants at 20°. To determine the frequency distribution of flagella/bacterium, and of motile descendants/bacterium, cultures were first kept in logarithmic growth at 37° for several hours, to enable the population to reach a steady-state composition in respect of motility and flagellation. Culture samples were then placed in the microdissection chamber at 20°; motile bacteria were picked and isolated to individual microdrops of broth. When the populations of the microcultures numbered about 250, the motile bacteria in each drop were counted.

Samples of the same 37° cultures, from which motile bacteria had been isolated, were fixed with formalin, and the distributions of number of flagella/flagellated bacterium determined. Determinations of distributions of number of flagella and of number of motile descendants were made on 37° cultures of SL237 grown in tryptic-digest broth and in papain-digest broth, and on 37° cultures of SL242 grown in tryptic-digest broth (the proportion of flagellated, motile, bacteria in the three cases being respectively *c.* 15 %, *c.* 70 % and *c.* 40 %); results are given in Table 1 and are illustrated by histograms in Fig. 2.

Some possible errors

The observed distributions of numbers of motile descendants and by inference of numbers of MC particles are subject to at least three sources of error. These are: (1) failure to detect some motile bacteria in non-motile microcultures; (2) unconscious selection of more highly motile bacteria for isolation; (3) changes in the composition of cultures (*a propos* of distributions of numbers of MC particles) during the period in which motile bacteria were being isolated.

The efficiency of detection of motile bacteria in micromanipulation experiments was discussed by Stocker (1956*b*) and by Quadling & Stocker (1957). The evidence (e.g. Fig. 1) of repeated detection of motile bacteria over a number of generations indicates that the efficiency of detection was high in the experiments

Table 1. *Distributions* of numbers of flagella/flagellated bacterium and of numbers of motile descendants/motile bacterium in 37° cultures*

Culture	No. of flagella or of motile descendants...	0	1	2	3	4	5	6	7	8	9	10	11	12	No. of bacteria scored	Proportion flagellated and motile in whole culture
SL237/A, tryptic-digest broth	Flagella	.	79	16	4	1	.	.	.	.	.	.	.	.	100	c. 15 %
	Motile descendants	0	56.9	23.2	7.7	7.7	1.5	1.5	0	0	0	1.5	.	.	65	
SL237/A, papain-digest broth	Flagella	.	48	26	14	11	0	1	.	.	.	.	.	.	100	c. 70 %
	Motile descendants	8.2	33.7	20.4	15.3	6.1	3.1	3.1	3.1	2.0	2.0	0	1.0	2.0	98	
SL242/A, tryptic-digest broth	Flagella	.	34	27	22	9	5	2	0	1	.	.	.	.	100	c. 40 %
	Motile descendants	0	24.3	18.6	12.9	10.0	14.3	8.6	2.9	4.3	2.9	0	1.4	.	70	

* Proportions of bacteria with indicated number of flagella or motile descendants are stated, respectively, as % of flagellated or of motile bacteria observed.

described here. Errors of the second category might lead to distortion of the distributions if such highly motile bacteria produced larger numbers of motile descendants than less motile bacteria. However, in the strains used here it was impossible to predict, on the basis of swimming behaviour in broth, whether any given motile bacterium would give rise to one or several motile descendants, i.e. would prove to have had one or several MC particles. Bias of this type is therefore considered improbable.

Fig. 2. Histograms constructed from the data of Table 2. Solid columns record % of flagellated bacteria having the indicated number of stained flagella. Arrows record % of motile bacteria having the indicated number of motile descendants at 20°. (A) SL237/A grown at 37° in tryptic-digest broth. (B) SL242/A in tryptic-digest broth. (C) SL237/A in papain-digest broth.

Errors of the third category are *ex hypothesi* unavoidable: motile bacteria may give rise to small numbers of motile progeny in the course of their first few divisions after isolation (e.g. Fig. 1); thereafter no increase occurs in the number of motile descendants. This is economically interpreted as being due to the sharing of parental MC particles between daughters. Such sharing during the continued growth of 37°-grown cultures at 20° would result in a decrease in average number of MC particles/motile bacterium. To minimize this error a maximum period of 30 min. was allowed between the removal of a culture sample from 37° and completion of isolations of motile bacteria at 20°; this represents about three-eighths of a mean generation-time of these organisms at 20°.

The observed distribution of numbers of stained flagella may be inaccurate for at least two reasons. Organisms may be mechanically deflagellated during preparation for staining and precautions were taken to minimize this error;

control experiments, in which organisms were prepared for staining by dialysis rather than centrifugation, indicated that a decrease not greater than 20 % in mean number of flagella/bacterium might occur during preparation. A second inaccuracy arises from the tendency of flagella to aggregate into bundles (see Stocker 1956*a*); in a heavily stained preparation a bundle of several flagella might appear as one unit. This error was minimized by using lightly stained preparations.

DISCUSSION

When comparing the distributions of number of motile descendants with those of the number of flagella it must be noted that the former refers only to bacteria selected for isolation because of their motility and the latter only to those bacteria which possessed at least one stainable flagellum. The % of motile bacteria did not differ detectably from the proportion of flagellated bacteria; presumably all or nearly all flagellated bacteria were motile and all or nearly all motile bacteria were detected as flagellated in the stained preparations. If these assumptions are justified the two distributions are based on the same population, namely, that fraction of the culture which was flagellated and motile at the time of sampling.

Even if parental flagella are shared amongst progeny and are thereafter transmitted unilinearly with each single flagellum rendering its possessor motile (i.e. even if the single flagellum is the postulated MC particle), exact coincidence of the distributions of number of flagella and of motile descendants/bacterium is not to be expected in a growing culture in a steady-state in respect of synthesis of flagella. Distributions of numbers of stained flagella represent the condition of the culture at the instant of treatment with formaldehyde; presumably developing flagella already in process of extrusion will not be detected if shorter than some critical value. Distributions of numbers of motile descendants comprise bacteria motile by means of flagella which were functional at the time of isolation and also descendant bacteria motile by virtue of flagella which completed their development subsequent to the time of isolation. Presumably flagella which were in course of development when the motile organisms were transferred to 20° will, after further development, confer motility on the progeny which 'inherit' them. Thus, mean numbers of motile descendants/motile organism would be expected to exceed the corresponding mean numbers of stained flagella/flagellated organism. The ratios of these mean numbers (Table 2) for each pair of comparable distributions (average 1.3:1) are in reasonable agreement in the three cases. The significance of the indicated predominance of motile descendants is not entirely clear however. That the inception of new flagella ceases, or proceeds at greatly diminished 'residual' frequency, when populations of these strains are transferred from 37° to 20° is indicated by their gradual transition to almost complete (more than 99.9 %) non-motility during growth at the lower temperature. It is not known whether such inception ceases abruptly or decreases gradually after the temperature change. Increase in numbers of motile descendants due to such residual synthesis is likely to be small compared with that due to completion of

development by flagella already in process of extrusion at the time of temperature change.

It is concluded from the general similarities of comparable distributions that a correlation exists in these strains between the number of flagella borne by an organism and the number of motile descendants to which it can give rise under conditions in which flagella inception, but not growth, has ceased. The simplest explanation for this correlation is that each motile descendant receives one of the flagella of the originally-isolated bacterium, so that a bacterium with,

Table 2. *Summary of results on the correspondence between stainable flagella and numbers of motile descendants*

All cultures were initially grown at 37°; motile descendants were counted when populations derived from single motile bacteria had grown to 100–500 at 20°. The flagella in samples of 37°-grown cultures were stained by Leifson's (1951) method.

Strain	Bacteria flagellated and motile (%)	Mean no. motile descendants/motile bacterium		Mean no. flagella/flagellated bacterium	Ratio: mean no. motile descendants:mean no. flagella	
		Observed	Adjusted $\times 1.2^*$		Observed	Adjusted $\times 1.2^*$
SL237/A†	c. 15	1.59	1.91	1.27	1.25	1.50
SL237/A‡	c. 70	2.58	3.10	1.91	1.35	1.62
SL242/A†	c. 40	3.30	3.96	2.37	1.39	1.67
Pooled data	—	—	—	—	1.33	1.60

* Observed mean adjusted by factor of $\times 1.2$ to allow for estimated extent of 'dilution-out' of flagella during the period 0–30 min. in which isolations were made.

† Culture grown in tryptic-digest broth.

‡ Culture grown in papain-digest broth.

for example, three flagella gives rise to three motile descendants after several generations of growth. This implies that parental flagella are shared among progeny bacteria in such a way that after several generations of fission, in the absence of inception of new flagella, each motile descendant will have no more than one flagellum. Further evidence that parental flagella are shared amongst progeny bacteria, in the manner indicated above, was reported by Quadling & Stocker (1956).

The conclusions detailed above conflict with those reached by Bisset (1951) and by Bisset & Pease (1957). These authors inferred from electron micrographs and stained preparations that in dividing Gram-negative bacteria the portion of the cell wall bearing the parental flagella was retained in its entirety by one daughter, the other daughter growing new flagella. If parental flagella are partitioned in this 'all or nothing' manner, in order to account for the results presented here it is necessary to postulate that there is a gradual loss of ability to synthesize new flagella amongst the immediate progeny of the originally isolated motile bacteria, so that, for example, in the case of a bacterium which gives rise to four motile descendants synthesis of new flagella continues for two generations after transfer to 20°. Furthermore, the length of time at 20° taken for this loss to occur in a particular strain grown in a particular medium would have to be correlated with the numbers of flagella

originally borne by the organisms. Whilst this interpretation cannot be excluded, the conclusions in the preceding paragraph are preferable on the grounds of economy of hypothesis.

If these conclusions are correct, the observed distributions of number of flagella/bacterium can be regarded as a product of (1) growth, and division, which result in the sharing-out of already-present flagella, and (2) the frequency and characteristics of intracellular 'events' which lead to the synthesis of new flagella. The event hypothesis was advanced by Quadling & Stocker (1957) to describe the infrequent synthesis of MC particles in 'bursts' of small numbers during growth of bacteria of some non-flagellated *Salmonella* strains; an event was regarded as a transient change in cell phenotype to the flagella-synthesizing state. The large proportion of non-flagellated bacteria in the cultures used in the present work indicated that, even at 37°, only a proportion of the bacteria synthesize flagella at any one time. Presumably events analogous to those described by Quadling & Stocker (1957) occur also in these strains. When events occur with low frequency the flagella generated during an event will be shared amongst the progeny so that each will tend to have no more than one flagellum by the time a fresh event supervenes. The condition will then be reached in which bacteria with 0, 1, 2, . . . , n (where n is the maximum observed number of flagella produced per event) are characteristically present in the culture, as described for the strains studied here. Cultures presenting this appearance have been said to show 'degenerate peritrichous flagellation' (Conn & Wolfe, 1938).

In general, the results reported here support the hypothesis that an MC particle is equivalent to a flagellum and that MC particles (flagella) are produced in bursts of small numbers. They also provide further evidence of non-genetic heterogeneity *à propos* of flagellation and motility which occurs in clonal bacterial populations growing in a common (liquid) environment; such overt heterogeneity is presumably indicative of underlying biochemical heterogeneity.

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