J. gen. Microbiol. (1962), **28**, 257–270
Printed in Great Britain

An Environmentally-Induced Transition from the Flagellated to the Non-flagellated State in *Salmonella typhimurium*: the Fate of Parental Flagella at Cell Division

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(*Received* 15 *August* 1961)

SUMMARY

Bacteria in cultures of *Salmonella typhimurium* LT2 were peritrichously flagellated when grown in nutrient broth at 37°; but most were non-flagellated when grown for 6 mean-generation times or more at 44°. When a culture growing exponentially at 37° was transferred to 44°, growth continued at about the same rate; but the synthesis of new flagella was largely curtailed. The fate of the parental flagella was studied by staining and counting flagella on bacteria from samples taken during growth at 44° of cultures first grown at 37°. After 3 mean-generation-times the average number of flagella/flagellated bacterium had fallen from about 8 to about 2 and the proportion of flagellated bacteria from about 100 % to about 60 %. The distribution of numbers of flagella/bacterium was at all times unimodal, with the mode decreasing from about 8 to 0. In non-growing cultures at 44° there was little or no change in the average number of flagella/bacterium, in the proportion of flagellated bacteria, or in the distribution of numbers of flagella/bacterium. It is inferred that parental flagella are neither rapidly shed at 44° nor retained entirely by one daughter cell at each division but are distributed about equally between the two daughter cells.

INTRODUCTION

Observations on the morphology of bacteria by light and electron microscopy have led some workers to infer that in rod-shaped bacteria the growth of the bacterial cell wall occurs mainly or entirely at one pole. Bisset (1951), Bisset & Pease (1957) and Bisset & Hale (1960) examined, by microscopy, preparations of *Salmonella* spp. and of other rod-shaped organisms. They concluded that during growth and division the portion of the parental cell wall which bore flagella passes in its entirety to one daughter cell, and that the other daughter had a new cell wall and grew new flagella. For ease in discussion of the modes of partition of parental flagella, this view will be called the 'all or none' hypothesis. On the other hand, the results of micromanipulation experiments (Stocker, 1956*a*, *b*; Quadling & Stocker, 1957; Quadling, 1958) on the unilinear transmission of motility and, by inference, of flagella, from parent to daughter cells in *Salmonella* spp. are most readily interpreted by assuming that 'parental' flagella are shared about equally

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between the daughter cells produced at each division. This idea will be referred to as the 'sharing' hypothesis.

We report here an attempt to obtain evidence of the fate of bacterial flagella at cell division, by treating growing cultures of flagellated *Salmonella typhimurium* so that growth continues but no new flagella are produced. If, as Bisset suggested, the original cell wall and all the flagella of a peritrichously flagellated bacterium pass to one of its daughters, a bimodal distribution of numbers of flagella/bacterium would be expected to develop after the cessation of synthesis of flagella, with an increasing proportion of the population (all those with 'new' cell walls) having no flagella, and a decreasing proportion (those with the original cell walls and flagella) having several flagella each. Alternatively, if the flagella of a multiflagellate bacterium be shared between its daughters, a unimodal distribution would be expected, with the modal number of flagella/bacterium decreasing as the original flagella are distributed amongst the increasing bacterial population.

It was found that the synthesis of flagella by growing bacteria could be suppressed by transferring a logarithmic-phase broth culture of a wild-type flagellated *Salmonella typhimurium* strain (LT2) from 37° to 44°. Growth continued at about the same rate as before, but the average number of flagella/bacterium fell from about 8·2 to less than 1, and the proportion of non-flagellated bacteria increased from less than 5 % to 70 %, or more. The changes in distributions of number of flagella/bacterium were also investigated. Though the suppression of synthesis of flagella was less complete and abrupt than had been hoped, the results obtained are compatible with the hypothesis that parental flagella are shared at bacterial division; they cannot, we think, be reconciled with the alternative hypothesis. A preliminary report of this work has already been given (Quadling & Stocker, 1956).

METHODS

Salmonella typhimurium strain LT2 of Zinder & Lederberg (1952) was used. The cultural methods were described by Quadling & Stocker (1957). The growth of cultures was followed by turbidity measurements made with a Hilger 'Spekker' photoelectric absorptiometer or by plate counts of viable bacteria made by the method of Miles & Misra (1938). Cultures were grown in nutrient broth made from a papain digest of lean beef (Asheshov, 1941). Changes in temperature of incubation were effected by diluting cultures into broth at 44°. Cultures were incubated in stationary capped, conical flasks held in a water bath at the appropriate temperature (37°, 44°); they were maintained in exponential growth at populations below 10^8 bacteria/ml. by periodic dilution with prewarmed broth.

Staining and counting of flagella were carried out by a modification of Leifson's (1951) method as described by Quadling (1958). The flagella were counted on each of the first five organisms seen in each of ten or more successive scans of stained smears, starting at arbitrarily chosen points. In some experiments the slides were coded and the counts repeated by one of us who had no knowledge of the times of sampling. This procedure would be expected to decrease observer bias in the selection of organisms for counting. Possible sources of error in observed distributions of numbers of stained flagella were discussed by Quadling (1958). Broth cultures were examined for motility by low-power dark-ground microscopy as described by Stocker

(1956*b*). To determine the percentage of organisms which were motile, suitably diluted samples were examined in a Helber chamber. Separate counts of motile and of non-motile organisms were made and the figures combined to yield a percentage value.

RESULTS

The effect of various treatments on distribution of numbers of flagella

Typical peritrichous flagellation was shown by cultures of strain LT2 grown exponentially in nutrient broth at 37°; the average number flagella/bacterium was about 8 and the proportion of non-flagellated, non-motile, organisms was less than 5 %. Distribution of numbers of flagella/bacterium in such cultures are given in Table 1 (entries 1 and 4) and in Table 2 (times 15 min. and −15 min.). In preliminary experiments it was found that when exponentially growing cultures of strain LT2 were transferred to 44° growth continued at about the same rate as before but the proportion of non-flagellated bacteria increased as growth continued at 44°. To determine whether this increased proportion of non-flagellated bacteria resulted from the suppression of flagellar synthesis or from the rapid shedding of flagella, distributions of numbers of flagella were determined after treatments involving temperature change, with and without growth. It was found that in the absence of growth there was little change in the number flagella/bacterium during incubation at 44° (Table 1). This was shown for a 37°-grown stationary-phase culture held for 4 hr. at 44°, for a formalin-killed 37°-grown log-phase culture held 4 hr. at 44°, and also for a 37°-grown log-phase culture incubated for 4 hr. at 44° with 100 μg. chloramphenicol/ml. (which arrests growth and prevents synthesis of new flagella). We conclude that flagella grown at 37° are not rapidly shed at 44° and that the change from the flagellated to the non-flagellated condition at 44° is associated with growth of the bacteria rather than with adverse effects on the flagella themselves. When cultures grown at 44°, in which less than 1 % of the bacteria were flagellated, were placed at 37° the bacteria rapidly regained flagella. In one such experiment 20 % of the bacteria had demonstrable flagella after 30 min. growth at 37°, and 48 % had flagella after 60 min. Such results are evidence that the loss of flagella at 44° is phenotypic only, and that new flagella are produced on return to a suitable environment.

Effect of growth at 44° on distribution of numbers of flagella

To investigate the effects of continued growth at 44° on the distribution of numbers of parental flagella, the synthesis of new flagella was curtailed by transferring to 44° an unaerated broth culture which was in logarithmic growth at 37°. Such cultures were maintained in logarithmic growth for over 4 hr. by periodic dilution with fresh broth held at 44° in a water bath. Samples for flagella staining were taken at intervals and fixed with formaldehyde. The growth of cultures was followed by turbidity measurements or by plate counts of viable bacteria: the mean doubling time at both temperatures was about 40 min., but varied from experiment to experiment. The distributions of number of flagella/bacterium were determined by counts of flagella present on 50–200 bacteria from each sample in random fields of smears stained by Leifson's (1951) method. Results are given in Tables 2–4 and are illustrated in Figs. 1–3. In general, little change occurred in the mean number of

Table 1. *Effects of various treatments on flagellation of* Salmonella typhimurium *strain* LT2

Culture samples were fixed by addition of formalin to give a final concentration of 0·1 % (w/v) HCHO and stained by Leifson's (1951) method.

Treatment	Numbers of bacteria with flagella numbering																	No. bact. examined	Mean no. flagella/ flagellated bacterium	% bacteria flagellated
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16 and over			
(1) Log-phase culture grown at 37°	1	0	0	3	1	6	2	4	10	8	6	3	3	0	0	2	1	50	8·4	98
(2) As (1) but then grown 4 hr. at 44°	140	42	10	3	3	1	0	1	0	0	0	0	0	0	0	0	0	200	1·6	30
(3) As (1) but fixed with formalin and held 4 hr. at 44°	0	0	0	0	2	5	4	8	11	5	10	0	2	2	0	0	1	50	9·1	100
(4) Log-phase culture grown at 37°	0	0	1	0	1	8	4	3	12	10	5	2	2	1	0	1	0	50	8·0	100
(5) As (4) but then held 4 hr. at 44° in presence of 100 μg. chloramphenicol/ml.	1	0	1	2	1	5	6	7	11	4	5	1	4	0	0	2	0	50	7·8	98
(6) Stationary phase culture grown at 37°	1	1	2	0	1	1	6	6	9	5	8	2	6	1	0	1	0	50	8·4	98
(7) As (6) but then held additional 4 hr. at 44°	0	0	1	1	4	6	5	9	11	3	6	2	2	0	0	0	0	50	6·9	100

Table 2. *Effect of growth at* 44° *on distribution of numbers of stained flagella per bacterium in cultures of* Salmonella typhimurium *strain* LT2

Cultures in logarithmic phase at 37° were transferred to 44° at zero time and maintained in logarithmic growth by periodic dilution. Culture samples were fixed at stated times by addition of formalin and stained by Leifson's (1951) method.

	Time from transfer (min.)	Numbers of bacteria with flagella numbering																	No. of bacteria examined
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16 and over	
Expt. 1*	15	—	0	0	2	1	2	4	3	5	3	8	4	7	3	1	2	5	50
	45	—	1	1	2	3	3	1	3	7	7	7	2	4	4	3	0	0	48
	75	—	2	5	5	8	10	8	6	4	1	0	0	0	0	0	0	0	49
	105	—	19	25	25	12	9	6	1	3	0	0	0	0	0	0	0	0	100
	135	—	42	30	13	13	2	0	0	0	0	0	0	0	0	0	0	0	100
	165	—	49	35	11	3	0	2	0	0	0	0	0	0	0	0	0	0	100
	180	—	62	22	10	2	2	1	1	0	0	0	0	0	0	0	0	0	100
	195	—	63	19	10	5	1	0	0	1	1	0	0	0	0	0	0	0	100
	216	—	73	18	8	1	0	0	0	0	0	0	0	0	0	0	0	0	100
	225	—	37	10	2	1	0	0	0	0	0	0	0	0	0	0	0	0	50
	245	—	80	14	2	3	1	0	0	0	0	0	0	0	0	0	0	0	100
	265	—	80	16	3	1	0	0	0	0	0	0	0	0	0	0	0	0	100
	280	—	86	9	3	2	0	0	0	0	0	0	0	0	0	0	0	0	100
Expt. 2	−15	1	1	0	3	1	6	2	4	10	8	6	3	3	0	0	2	1	50
	+30	0	1	3	0	8	4	0	7	5	3	6	3	5	0	2	2	1	50
	60	2	8	5	8	9	8	12	9	5	2	1	0	1	0	0	0	0	70
	120	18	25	19	24	1	8	1	1	2	0	1	0	0	0	0	0	0	100
	180	42	27	16	7	2	3	1	2	0	0	0	0	0	0	0	0	0	100
	240	140	42	10	3	3	1	0	1	0	0	0	0	0	0	0	0	0	200

* In Expt. 1 the data shown are the distribution of no. of flagella/bacterium amongst 50 or 100 flagellated bacteria; the proportion of non-flagellated bacteria (i.e. the omitted 'zero' class of this table) is shown in Table 3, column (*c*).

flagella/bacterium during the first doubling time at 44°, but after 60 min. this number decreased; it continued to decrease until most bacteria had no flagella and the few remaining flagellated bacteria had only one or a small number of flagella each.

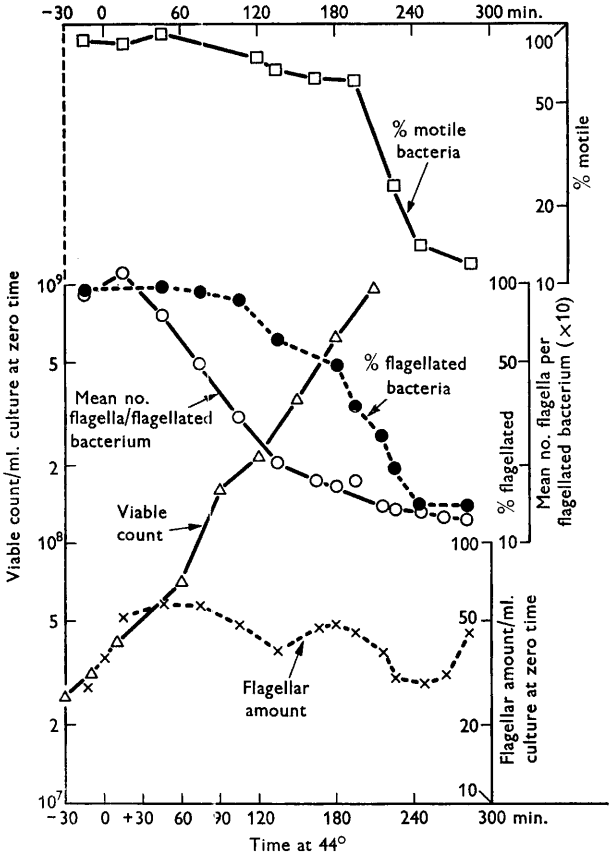

Fig. 1. Effect of growth at 44° on flagellation and motility of a logarithmic phase culture of *Salmonella typhimurium* grown at 37° and transferred to 44° at time zero. Cultures were maintained in logarithmic growth by periodic dilution with pre-warmed broth at 44°. Samples were fixed at stated times by addition of formalin and stained by Leifson's (1951) method. The data are plotted semi-logarithmically and are from Expt. 1, Tables 2 and 3. Viable count as plotted is corrected for dilution during growth.

Data from two experiments are shown in graph form as semi-logarithmic plots against time (Figs. 1, 2). There is an excellent correspondence between estimates of the % motile bacteria and % flagellated bacteria at the times of sampling (Fig. 1). This is evidence for the reliability of the staining technique and for the lack of any significant loss of flagella during preparation and staining, especially at the later times of sampling. For reference, the reciprocal of 'notional' turbidity is plotted in Fig. 2. Notional turbidity was obtained by adjusting observed turbidities to compensate for the periodic dilution of the starting culture which occurred during the experiment. The slope of this line is a measure of the rate at which the culture was diluting its original cell mass by fresh growth. The mean number of flagella/

bacterium decreased from about 45 min. at about the same exponential rate as would be expected if this change were a consequence of dilution by growth. Such a result would be expected if parental flagella were retained only by one daughter bacterium or were shared between both daughters at division. However, the data show that the mean number of flagella *per flagellated bacterium* also decreased steadily towards unity, instead of remaining constant as predicted by the 'all or none'

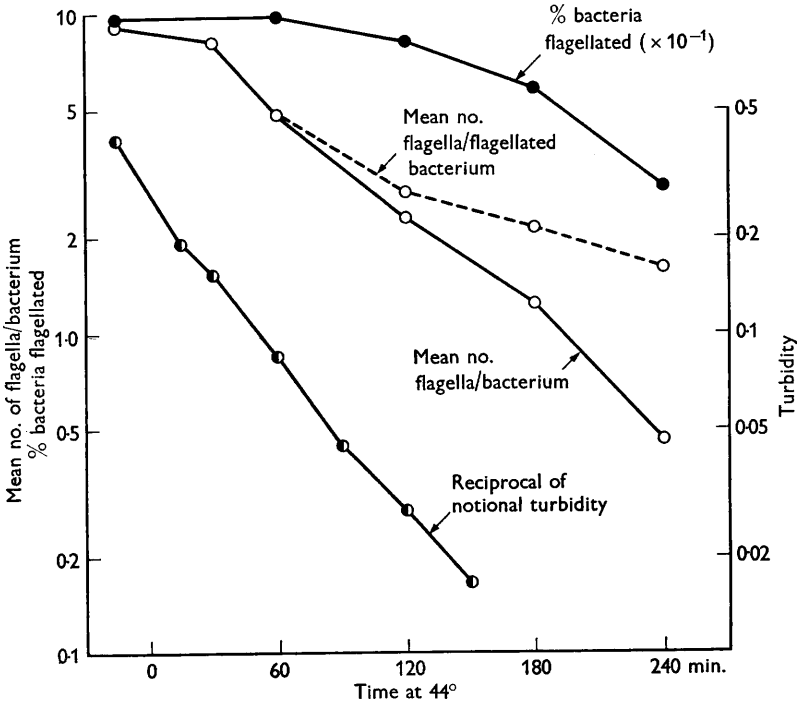

Fig. 2. Effect of growth at 44° on flagellation of a logarithmic phase culture of *Salmonella typhimurium* grown at 37° and transferred to 44° at zero time. Cultures were maintained in logarithmic growth by periodic dilution with pre-warmed broth at 44°. Samples were fixed at stated times by addition of formalin and stained by Leifson's (1951) method. The data are plotted semi-logarithmically and are from Expt. 2, Tables 2 and 4. Notional turbidity is actual turbidity multiplied by a correction factor for dilution during growth.

hypothesis. Such results, taken in conjunction with: (*a*) an absolute decrease in numbers of bacteria with large numbers of flagella (Table 5); (*b*) the demonstrated maintenance of parental flagella at 44° in the absence of growth (Table 1), are difficult to reconcile with the simple 'all or none' hypothesis that parental flagella are retained entirely by one daughter cell at each division. The initial low rate of decline in the proportion of bacteria with flagella also suggests that such 'all or none' partitioning of peritrichously-inserted flagella did not occur. The distributions of number of flagella/bacterium during incubation at 44° are illustrated in histogram form in Fig. 3. These distributions were unimodal and did not become bimodal as predicted by the 'all or none' hypothesis discussed in the Introduction and below.

Residual synthesis of flagella

The experiments mentioned above were designed to distinguish between the hypotheses: (1) 'all or none' partition; (2) 'sharing' of flagella at cell division. It was thought that a decisive test would be to follow the parameter 'mean number flagella/flagellated bacterium' during the transition from almost 100 % of flagellated organisms to the state in which nearly all were non-flagellated. The 'all or none' hypothesis in its simplest form predicts a decrease in this parameter of, at most, a

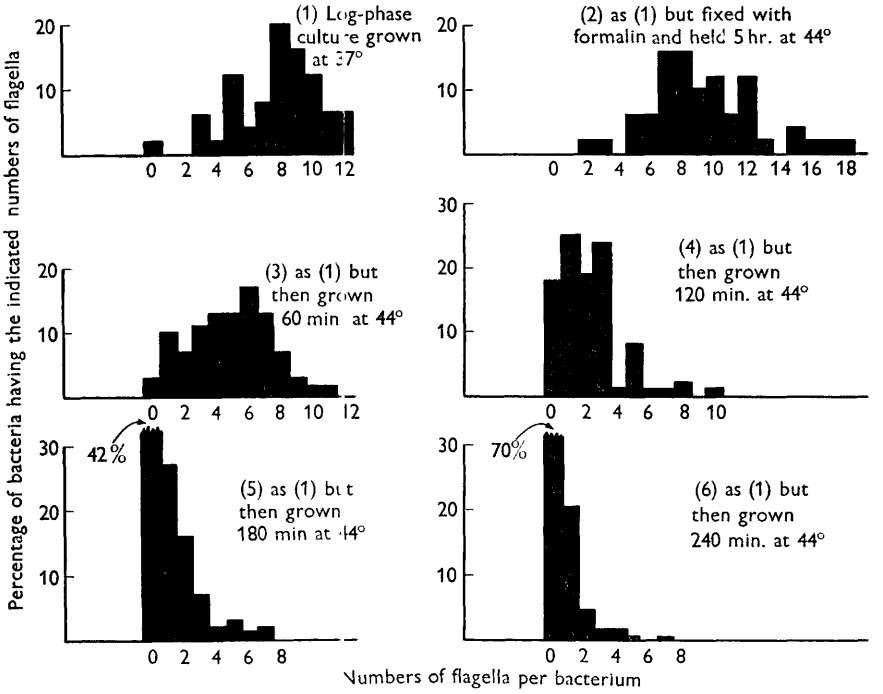

Fig. 3. Effect of incubation at 44°, with and without growth, on the distribution of numbers of flagella per bacterium in *Salmonella typhimurium*. Data of distributions (3) to (6) inclusive are from Expt. 2, Table 2. Cultures in logarithmic growth at 37° were transferred to 44° at zero time and maintained in logarithmic growth by periodic dilution with prewarmed broth at 44°. Samples were fixed at stated times by addition of formalin and stained by Leifson's (1951) method.

half (attributable to completion of bacterial divisions in progress at the time of cessation of flagellar synthesis) and then no further decrease, whilst the 'sharing' hypothesis predicts a steady decline towards unity. Although the changes observed in the mean number flagella/flagellated bacterium clearly favoured the sharing hypothesis, this result was not in itself sufficient to determine the way in which parental flagella were partitioned at cell division, since it was possible to envisage situations in which a slow decline in rate of synthesis of new flagella might lead to a slow decline in the mean number flagella/flagellated bacterium. Accordingly, the extent of the synthesis of new flagella which occurred after the temperature change

from the changes in total 'flagellar amount' during experiments. This parameter is estimated by multiplying the mean number flagella/bacterium by the notional turbidity calculated for the culture at the time of sampling or by the calculated notional viable count at the time of sampling (Tables 3, 4). Notional turbidity or

Table 3. *Summary of data* on the effect of growth at 44° on the motility and flagellation of* Salmonella typhimurium *strain* LT2

Cultures in logarithmic growth at 37° were transferred to 44° at zero time and maintained in logarithmic growth by periodic dilution. Entries in columns (*f*), (*g*) and (*h*) are expressed as per ml. culture at zero time, to allow for dilution.

Time from transfer (min.)	Mean no. of flagella/ flagellated bacterium	% bacteria flagellated	% bacteria motile	Mean no. of flagella/ bacterium $\left(=\frac{b \times c}{100}\right)$	Notional viable count ($\times 10^7$)	Smoothed viable count† ($\times 10^7$)	Flagellar amount ($= e \times g$)
(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)	(*h*)
−30	—	—	—	—	2·62	2·6	—
−15	9·1	97 (116/120)	88	8·8	—	3·2	28
−10	—	—	—	—	3·4	3·4	—
0	—	—	—	—	—	4·0	36‡
10	—	—	—	—	4·22	4·6	—
15	10·1	—	85	10·1‡	—	5·0	51‡
45	7·63	98 (118/120)	93	7·5	—	7·9	58
60	—	—	—	—	7·4	9·8	—
75	4·9	93 (130/140)	95	4·6	—	12	55
90	—	—	—	—	16·1	—	—
105	3·04	88 (70/80)	—	2·7	—	18	49
120	—	—	76	—	21·8	24	—
135	2·03	61 (82/134)	67	1·2	—	30	36
150	—	—	—	—	36·3	38	—
165	1·76	—	62	0·97‡	—	48	47‡
180	1·67	49 (90/195)	—	0·82	63·4	60	49
195	1·73	34 (21/62)	51	0·59	—	76	45
210	—	—	—	—	96·6	94	—
216	1·37	25 (32/126)	—	0·34	—	104	35
225	1·34	19 (19/100)	24	0·25	—	118	30
245	1·31	14 (21/150)	14	0·18	—	158	28
265	1·25	10 (16/158)	12	0·13	—	213	28
280	1·21	14 (20/142)	—	0·17	—	265	45

* Expt. 1, Table 2.

† Entries for times −30 min. to 216 min. inclusive are interpolated; the remainder are extrapolated.

‡ In columns *e* and *h* indicates amount calculated from an interpolated value in columns (*b*) and/or (*c*).

( ) In column (*c*) indicates actual figures used in calculation of percentage.

— No observation.

viable count was estimated by taking account of the dilutions which had been made during the experiment and calculating back to obtain the hypothetical value which would have been found had the original culture, undiluted, remained in exponential growth at 44°. Flagellar amount is thus hypothetical and proportional to the total number of flagella/bacterium which would have been present if the whole culture had been maintained in exponential growth. The data in Table 4 (from Exp. 2, Table 2) indicate that total flagellar amount increased slowly throughout 240 min.

of growth at 44°, with the greatest increase occurring within the first 30 min. (about 1 mean generation time) after the temperature change. In contrast, the data in Table 3 (from Exp. 1, Table 2) show an increase in the first 30–60 min. at 44°, followed by a slow decrease in flagellar amount during the next 180 min. of incuba-

Table 4. *Summary of data* on the effects of growth at 44° on flagellation of* Salmonella typhimurium *strain* LT2

Culture in logarithmic growth at 37° transferred to 44° at zero time and maintained in logarithmic growth by periodic dilution.

Time from transfer (min.) (*a*)	No. of bacteria examined (*b*)	Mean no. of flagella/ flagellated bacterium (*c*)	% bacteria flagellated (*d*)	Mean no. of flagella/ bacterium $\left(=\frac{c \times d}{100}\right)$ (*e*)	Turbidity† (*f*)	Flagellar† amount (*e* × *f*) (*g*)
−15	50	8·4	98	8·2	0·25	2·05
0	—	—	—	9·0‡	0·36	3·24
30	50	8·0	100	8·0	0·65	5·20
60	70	4·9	97	4·8	1·18	5·66
120	100	2·7	82	2·2	3·6	7·92
180	100	2·1	58	1·2	11·1	13·3
240	200	1·6	30	0·48	37‡	18·0

* Expt. 2, Table 1.

† Entries in columns (*f*) and (*g*) are expressed as per ml. of culture at −15 min., to allow for subsequent dilution.

‡ Interpolated or extrapolated.

Table 5. *Decrease in absolute numbers of bacteria with higher numbers of flagella during growth of* Salmonella typhimurium *strain* LT2 *at* 44°*

Time after transfer to 44° (min.)	Factor of increase in viable count	No. of organisms in sample with 5 or more flagella		No. of organisms in sample with 8 or more flagella		Sample size (no. of bacteria)
		Present	Expected†	Present	Expected†	
+45	1	41	—	34	—	48
+75	1·57	29	26	5	23	49
+105	2·32	19	35	3	30	100
+135	3·85	2	21	0	18	100

* Data of Expt. 1, Tables 2 and 3.

† Entries are numbers expected on the assumptions that 'all or none' partition of parental flagella occurred at cell division, synthesis of new flagella had ceased, and that parental flagella were not shed during the period of the experiment.

tion at 44°, with some slight increase later. Presumably this decrease was attributable to some shedding of flagella or possibly to lysis of part of the population. It seems that the completeness of the hoped-for cessation of flagella synthesis varied from experiment to experiment. However, in an experiment in which measurable residual synthesis occurred and in experiments in which it did not occur, the mean number flagella/flagellated bacterium decreased steadily, as called for by the 'sharing' hypothesis.

DISCUSSION

It should be noted that the simple hypothesis of 'all or none' partition of parental flagella requires modification if it is to be applied to our material. Bisset (1951) envisaged a situation in which a peritrichously flagellated organism of *Salmonella typhi* reproduced by 'budding'; the parent bacterium retained all the old flagella and the daughter 'bud' grew new flagella. To cover the common case in which nearly 100 % of bacteria are motile (as in strain LT2) one must assume that the new flagella on the daughter are sufficiently long, at the moment of fission, to confer motility and also to be stained and counted. In a culture which is in a steady state with respect to average number flagella/bacterium, the observed mean number flagella/bacterium would result from an averaging of those on bacteria which have just completed fission, of those on bacteria which are just about to divide (and have about twice as many flagella) and of all intermediate classes. On the assumptions that the 'all or none' hypothesis is correct and that synthesis of new flagella ceases abruptly after one mean generation time at 44°, one would expect the mean number flagella/bacterium to decrease to a value between 1 and 0·5 of the original steady-state mean. This decrease would occur as a consequence of divisions initiated before suppression of flagellar synthesis. If a residual synthesis of flagella were to occur in such a system, on the basis of our observations (Table 3) one would expect it to take the form of synthesis of one or a small number of flagella by a minority of the population. Thus the modified 'all or none' hypothesis predicts that at two or three generations after transfer to 44° a bimodal distribution of number flagella/flagellated organism will occur with one mode between 0·5 and 1·0 of the original mean, and on the other, due to residual synthesis, at about unity. No such bimodal distribution was found at appropriate times (i.e. Table 2, Exp. 1, 75 and 135 min.; Exp. 2, 60 and 120 min.).

The conclusion that parental flagella are shared between the daughters is well supported by our quantitative evidence. We have shown that parental flagella are not rapidly shed under the physical conditions of our experiments. We have also shown that during growth at 44° the synthesis of new flagella is curtailed, the mean number flagella/flagellated bacterium decreases steadily towards unity and the absolute numbers of bacteria with higher numbers of flagella decrease. Bisset and co-workers (1951, 1957, 1960) examined rod-shaped and coccoid organisms and concluded that flagella are retained upon 'mother cells' and that 'daughter cells' or 'buds' are devoid of flagella or have them in an early stage of development. This conflict of views remains unresolved; further work may show that either or both view points are oversimplified. Our conclusion is to some extent an oversimplification; clearly one would not expect two flagella inserted close together near one pole of a cell to become separated to different daughters at the succeeding division. We do, however, suggest that flagella located near opposite poles would pass to different daughters and that the greater the proximity of those flagella to opposite poles the greater the probability that they will pass to different daughters at the next division.

Our results could be accounted for under the 'all or none' hypothesis by assuming that the decrease in mean number of flagella/flagellated bacterium is due to a shedding of flagella which occurs during growth but not under other conditions.

Although this possibility cannot be eliminated there is no evidence in its favour. Indeed the stability of the unilinear transmission of motility (Stocker, 1956*a*, *b*; Quadling & Stocker, 1957) provides strong evidence that such shedding is infrequent. It is also conceivable that incubation at 44° may introduce aberrant modes of division. However, studies on the growth of cultures by plate counts, turbidity measurements and by microscopic examination, revealed no abnormality. Our conclusion that flagella are shared out is also supported by indirect evidence obtained during micromanipulation studies on the unilinear transmission of motility from parent to progeny (Stocker, 1956*a*, *b*; Quadling & Stocker, 1957; Quadling, 1958). It was found that in certain situations motile salmonellas transmitted motility to only one or a few descendant bacteria. Evidence was obtained (Quadling, 1958) that such motile descendant bacteria had only one or a small number of flagella each; it was inferred that these flagella were distributed to the daughters at fission until these daughters received one or no flagella each.

Duguid & Wilkinson (1961) examined, by electron microscopy, the distribution of fimbriae amongst dividing enterobacteria growing under cultural conditions in which fimbriation was diminishing. Most dividing organisms were equally fimbriated at each end, were scantly fimbriated at each end, or were completely non-fimbriated. Bacteria which were fimbriated at one end but not at the other were very rare (less than 1 %). Duguid's observations are therefore concordant with our results. Results similar to ours were obtained with salmonellas by Kerridge (1960, 1961) in experiments in which he increased the incubation temperature from 37° to 44°. His work provides evidence for the reproducibility of our results.

Evidence for the sharing of parental cytoplasmic materials between daughters has been obtained by a number of workers. Benzer (1953) showed that the adaptive β-galactosidase of *Escherichia coli* was uniformly distributed, in the absence of inducer, amongst progeny of induced bacteria. Novick & Weiner (1957) working with the same system obtained results which could be interpreted by assuming that permease enzyme molecules were randomly distributed to daughter bacteria. Van Tubergen (1959) and Van Tubergen & Setlow (1961) investigated the distribution of various parental components amongst the progeny during exponential growth of *E. coli*. Specific cell components were labelled by uptake of appropriate tritium-containing nutrient, and the distribution of the labelled parental material amongst the progeny bacteria was determined by microradioautography at intervals after transfer of the bacteria to unlabelled medium. Parental protein (labelled with ^{3}H-proline), ribonucleic acid (labelled with ^{3}H-uridine) and cell-wall material (labelled with ^{3}H-diaminopimelic acid) were distributed randomly amongst the progeny bacteria, whereas parental deoxyribonucleic acid (labelled with ^{3}H-thymidine) was distributed non-randomly in large structures, stable for at least 5 generations. Cell-wall synthesis appeared to take place along the whole length of the cell wall.

The observations of Bergerser (1953) on *Escherichia coli* grown in the presence of chloramphenicol suggest that the mature cell wall is not necessarily a 'dead' structure; he showed that under these conditions additional 'growing points' appeared at the sides of some bacteria. Our observations on the behaviour of Salmonella at the conclusion of the logarithmic phase of growth also bear on this point. The mean size of the organism was observed under the microscope to decrease to about one quarter that of logarithmic-phase bacteria by direct division of the

pre-existing soma. Such observations implied that the walls of the bacterial cells were capable of morphological adjustment and were not 'dead' in the sense of being permanently differentiated.

The relevance of our findings on the fate of parental flagella at cell division to the mode of growth of the cell wall of rod-shaped bacteria is not clear. Assuming that bacterial flagella or their hypothetical basal granules are not able to move independently of the cell wall, our results provide evidence for the eventual dispersion of parental cell walls into discrete fragments, at least as many in number as the mean number of flagella per cell when fully flagellated. Van Tubergen (1959) and Van Tubergen & Setlow (1961) inferred from observations on the distribution amongst the progeny of parental cell-wall material that in *Escherichia coli* the number of 'intact structures' comprising the cell wall of one bacterium was at least 200. There is evidence that some areas of the bacterial cell wall (the sites of future cell division) are more active in synthesis than others (Lederberg, 1957; Mitchell & Moyle, 1957; Murray, Francombe & Mayall, 1959). Our results do not necessarily conflict with such a concept.

We thank Dr J. P. Duguid for helpful discussion and for reading the manuscript. The technical assistance of Mr H. A. Milne is gratefully acknowledged. This paper is based on part of a Ph.D. thesis submitted by one of us (C. Q.) to the University of London, 1956.

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