Flagella-Shape Mutants in *Salmonella*

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SUMMARY

Seven flagella-shape mutants were isolated from a curly-flagella strain of *Salmonella abortusequi*. The motility in broth and spreading ability on semi-solid medium of these mutants as well as flagellar morphology were examined by dark field and electron microscopy. They were classified into the following five mutant types, heteromorphous, small-amplitude, para-curly, short and hooked-curly.

Each of these mutants manifests its characteristic flagellar shape and motility, and the spreading ability of these mutants on semi-solid medium decreases in the order: normal, heteromorphous, small-amplitude, para-curly, short, hooked-curly, curly.

Except in the small-amplitude and short mutants, the shape of a flagellar bundle of living bacteria under the dark-field microscope corresponded in each mutant to a spiral of the flagella observed under the electron microscope. In the small-amplitude mutant, transconformation of the flagellar bundle from small-amplitude to curly was observed in organisms suspended in 0.5% (w/v) methylcellulose solution. In the short mutant, the flagellar bundle was not seen in the dark-field microscope.

Difference of antigenic specificities between the flagella of the parental curly and each of the mutants were not detected when examined by absorption-agglutination tests.

From transduction analyses with P 22 phage, it was found that the traits of each of the four mutants heteromorphous, small-amplitude, para-curly, short were transferred with the structural gene of phase-2 flagellin and had no effect on the flagellar shape and motility in phase-1. From this, it is inferred that the characteristic flagellar shape and motility of these mutants are primarily to be attributed to the conformation of the flagellin which composes the mutant flagella.

INTRODUCTION

Bacterial flagella are becoming recognized as unique material for the study of cytomorphogenesis. It has been occasionally noticed that more than two different shapes of flagella appear in a bacterial strain. The presence of both normal and curly are the most common. The phenomenon was called flagellar 'biplicity' (Pijper, 1957; Leifson, 1960). In *salmonella*, curly flagella were found to appear by mutation of the normal (Iino, 1962*a*). A typical curly mutant does not have the ability to spread in semi-solid medium and move rotationally in broth and its wavelength is about one-half the normal. Curly character of the mutant is flagellar phase-specific, and genetical evidence showed that the site of mutation maps in the structural gene of the flagellin concerned. By the fingerprinting analysis, it was demonstrated that the tryptic peptides of the curly flagella were different from the normal in one

peptide (Enomoto & Iino, 1963). Thus genetic analyses of curly mutants are disclosing the correlation between flagellar shape and primary structure of the component protein flagellin. On the other hand, the change from normal to curly was found to occur in a single organism under the dark-field microscope (Pijper, 1957). From this observation it was suggested that the biplicity could occur by transconformation of already formed flagella even if their chemical composition was the same. *In vitro* reconstitution experiments have examined the process of polymerization of flagellin into flagella (Abram & Koffler, 1964; Asakura, Eguchi & Iino, 1964; Lowy & McDonough, 1964), and flagellar regeneration experiments have been concerned with the chemical nature of flagellum-forming apparatus (Kerridge, 1960). The present paper reports a part of an investigation into the genetic system which controls 'flagellar morphogenesis'. It deals with the characterization and preliminary genetic analysis of five types of flagella-shape mutants isolated from a strain of *Salmonella abortusequi*.

METHODS

The curly strain SJ 30 used in the present experiments was a mutant of *Salmonella abortusequi* SL 23 (Iino, 1962a). This strain is stable in antigenic phase-2 (antigen-*e*, *n*, *x*). Genetic analyses have shown that the curly character of this strain occurs by a mutation in a site of the structural gene of phase-2 flagellin, *H*₂, of strain SL 23.

For the selection of flagellar mutants, tests for spreading ability and transduction experiments, semi-solid medium containing 0.2% (w/v) agar and 8% (w/v) gelatin in broth was used throughout. On the semi-solid medium, a normal clone spreads and forms a swarm, while a curly clone grows confined at the region of inoculation. For antiserum selection, 0.2% (w/v) final concentration of anti-H serum of agglutination titre 5000 was added to semi-solid medium before pouring into Petri dishes.

Cellular motility was observed by phase-contrast microscopy of hanging drops of broth culture. To observe the flagella of living bacteria, dark-field microscopy of bacterial suspensions in methyl cellulose (Wako Pure Chemical Co., Ltd., Tokyo) solution was adopted following the procedure of Pijper (1957). Bacteria were grown in broth at 37° for 2 hr and the organisms at the middle log phase were used for the observation. Bacterial samples were prepared by mixing a drop of culture with the same volume of saline containing 0.5% (w/v) methylcellulose at the final viscosity of 2.8×10^{-2} poise (20°). The mixture was dropped on a 1.4 mm thick glass slide and 0.25 mm coverslip put on. Manicure enamel was used for sealing the coverslip. The dark-field microscope used was a 7-volt Ernst Leitz Wetzlar Nr. 457210, in combination with a Chiyoda paraboloid condenser of aplanat 1.4 N.A., and an immersion objective $\times 90$ (with iris) with an eyepiece $\times 10$.

Flagellar shape was observed by electron microscopy. Bacteria were grown in Penassay broth (Difco Lab., Inc., Detroit, Michigan) for 8 hr at 37° with shaking, harvested by centrifugation at 3000 rev./min. for 10 min. and finally resuspended in distilled water at pH 6.2. This suspension was ordinarily used without any fixative for the electron microscope observations of flagella. For the preparation of samples for electron microscopy, droplets of bacterial suspension were placed on collodion-coated grids, thereafter extra drops were dried in vacuum and shadowed with chromium at an angle of 20°. For negative staining the method of Brenner & Horne (1959) was used. The grids were examined in a JEM T 6S electron

microscope (Japan Electron Optics Laboratory Co., Ltd.) with a single condenser system and accelerating voltage of 60 kV. Micrographs were taken at initial magnification of $\times 5000$ on Sakura process hard plates. One micron diam. Bacto latex particles (Difco Lab., Inc., Detroit, Michigan) were used for the determination of magnification.

To obtain average and standard deviation values for wave-length, amplitude and spiral unit length, several waves in a single flagellum were observed on different organisms and a total 60 to 90 waves were measured per clone.

RESULTS

Detection and isolation of mutants

Curly type organisms of *Salmonella abortusequi* strain SJ 30 cannot spread on plates of semi-solid medium and when they are incubated for 10–20 hr at 37° grow at the area where the organisms were inoculated (Iino, 1962*a*). Occasionally it was found that spreading mutants which were present in the inoculum started to form swarms in this period (Pl. 1, fig. 1). After continued incubation, the number and size of the swarms increased until at 2 days at least some mutant swarms were invariably found on the plates; further increase was not observed, probably because spreading of the organisms was inhibited by changes of the media. The sizes of the developed swarms ranged from that of the normal motile type to that of the small satellite type.

The edges of seven swarms of independent origin and of types apparently different from each other and from the normal type, were isolated and streaked on nutrient agar plates, and one of the colonies which developed from each isolate was transferred to nutrient broth. Each broth culture was spotted on a plate of semi-solid medium and incubated for 8 hr at 37°. The developed swarms from the seven clones varied in size and texture in parallel with the mode of spreading of the swarms formed by each of the clones on the initial selective media (Table 1, column 3; Pl. 1, fig. 2). The spreading of clone 1 was about 70% of that of the original normal type, while clone 7 formed a compact colony surrounded by many tiny dotted colonies like satellite colonies reported by Quadling (1958). The remaining five colonies were intermediate types between clones 1 and 7. Clones 2 and 3 spread slower than the normal type and formed swarms having about half the diameter as compared to the normal. Clones 4, 5 and 6 spread more slowly than clones 2 and 3; the swarms formed by these clones were compact, and their diameters were one-sixth to one-seventh of the normal type.

Motility in broth

The mutant clones described in the foregoing section differed from each other with regard to the predominant type of cellular movement in broth culture (Table 1, column 4). Most of the organisms in clone 1 moved translationally and a few wriggled as shown in Fig. 1. In clone 2, the predominant organisms also moved translationally and some rotated. The translational movement in clone 2 was slower than that of the normal clone. The predominant type of movement in clone 3 was a wriggle, but this clone differed from the following clones in showing a circular movement, that is, the bacteria travelled in small circles with diameter of 2–3 μ (Fig. 1). Clones 4, 5 and 6

Table 1. *Characteristics of the flagella-shape mutants obtained from a curly strain, SJ 30, of Salmonella abortusequi.*

Type	Representative clone	Spreading ability*	Major type of movement	Flagellar shape observed by			Other remarks
				E.M.†	D.M.		
Normal	SL 23	1.00	Translation	Normal	Normal		—
Heteromorphous	Mutant 1	0.67	Translation and wriggle	Normal and curly	Normal		Tendency to stretch
Small-amplitude	Mutant 2	0.53	Translation and rotation	Small-amplitude	Small-amplitude and curly		—
Para-curly	Mutant 3	0.44	Wriggle and circular	Curly	Curly		Strong tendency of bundling
Short	Mutant 4, 5, 6	0.15	Wriggle and rotation	Uncertain	Invisible		Short and small number of flagella
Hooked-curly	Mutant 7	0.08	Rotation and wriggle	Hooked-curly	Hooked-curly		Aggregation in small amount as compared to SJ 30
Curly	SJ 30	0.00	Rotation	Curly	Curly		Aggregation in broth

* Diameter of a swarm grown on semi-solid medium after 8 hr incubation at 37°; the value of SL 23 was taken as 1.00.
† E.M. = electron microscopy; D.M. = dark field microscopy.

Table 2. *Wavelength, amplitude and spiral unit length of flagella-shape mutants of Salmonella abortusequi observed with electron microscope*

Type	Strain	WL* (μ)	SD	Amp. (μ)	SD	WL/ amp.	s.u.l. (μ)	SD
Normal	SL 23	2.90	0.30	0.51	0.11	5.9	3.00	0.38
	Clone 1 {L†	2.85	0.34	0.45	0.07	6.5	3.01	0.36
Heteromorphous	Clone 1 {S	1.51	0.07	0.38	0.02	4.0	1.83	0.19
	Clone 2	2.31	0.24	0.30	0.07	7.8	2.44	0.31
Small-amplitude	Clone 3	1.52	0.24	0.50	0.09	3.1	1.92	0.22
Para-curly	Clone 7	1.56	0.27	0.45	0.09	3.8	1.97	0.21
Hooked-curly	SJ 30	1.12	0.08	0.29	0.05	3.9	1.36	0.19
Curly								

* WL = wavelength; Amp. = amplitude; s.u.l. = spiral unit length; SD = standard deviation;
† L = larger flagellar wave; S = smaller flagellar wave.

were indistinguishable from each other by their motility. The organisms of these clones mostly wriggled but rotation and translation were also observed in them. Clone 7 was characterized by rotation in most of the organisms, each spinning around on itself; the rest wriggled. The organisms of clone 7 aggregate in broth, but in smaller amount compared with those of strain SJ 30.

Fig. 1

Fig. 1. Four representative types of movement of *Salmonella* organisms in liquid medium. A = translation; B = wriggle; C = rotation; D = circulation.

Fig. 2

Fig. 2. Distribution diagrams of the ratio of wavelength to amplitude of flagellar waves observed by electron microscope on each flagella-shape mutant. The broken lines denote the average ratios and N denotes the number of flagellar waves measured. WL = wavelength; amp. = amplitude.

Stability of the mutant clones

For the examination of the stability of the mutant clones, each of them was subcultured through broth. In each subculture, 0.01 ml. of the culture at the late log-phase of growth (10^7 organisms/ml.) was transferred to 10 ml. of fresh broth. Thus bacteria passed about 6.2 divisions in a single-step culture. The subcultivation was repeated five times for each clone. At the time of each subculture, a drop of culture was sampled from each clone, and cellular and clonal motilities were observed in broth and on semi-solid medium. Even after five successive subcultures,

each clone showed its characteristic cellular and clonal motility, indicating that the mutant characters were so stable that they could be maintained through successive subculture.

Flagellar shape observed by electron microscopy

Normal flagella of the wild strain SL 23 observed by the electron microscope have wavelength of $2.20\ \mu$ and amplitude of $0.53\ \mu$ (Pl. 2, fig. 11). The curly mutant, SJ 30, has flagella about one-half of the normal in wavelength. Consequently its

Fig. 3. Distribution diagrams of spiral unit length of flagellar waves observed by electron microscope on each flagella-shape mutant. The broken lines denote the average spiral unit length and N denotes the number of flagellar waves measured.

flagella are more curled compared with that of the normal (Pl. 3, fig. 18). Mutant clones characterized by motility were found to have characteristic flagellar shapes (Pls. 2 and 3). They were classified into five morphological types (Table 1, column 5). Wavelength, amplitude and spiral unit length of the representative strain of each type are listed in Table 2, and histograms of the ratio of wavelength to amplitude and spiral unit length are shown in Figs. 2 and 3. The five morphological types are:

(1) Heteromorphous (Pl. 2, fig. 13), represented by clone 1. Flagella of this type were mostly normal. Curly waves were observed mixed with normal ones in one flagellum in 2% of the organisms in the clone. Bacteria with only curly flagella have never been seen. The spiral unit length, flagellar wavelength and amplitude of normal flagella were the same as those of wild-type flagella (Pl. 2, fig. 11). With the curly mutant, its wave resembled para-curly. Irregular-shaped flagella with wavelength longer than the normal were often observed.

(2) Small-amplitude (Pl. 2, fig. 12), represented by clone 2. The small-amplitude mutant is characterized by flagellar shape with the ratio of wavelength to amplitude higher than the normal type (Leifson, 1960); the ratio is the highest among the flagella-shape mutants. Amplitude and wavelength of this type of flagella were about 60 and 80% of the normal, respectively (Table 2).

(3) Para-curly (Pl. 3, figs. 15, 16), represented by clone 3. Organisms of this type produce curly flagella having larger wavelength, amplitude and spiral unit length compared with the original curly strain (Pl. 3, fig. 18). Moreover, flagella of this type have more tendency to retain bundles than the curly (Mitani & Iino, 1965), and the bundle has a specific wavelength 60% longer than that of the component flagella.

(4) Short (Pl. 2, fig. 14), to which clones 4, 5 and 6 belong. As described in the foregoing paragraph, the motility behaviour of the clones belonging to this type were the same as each other. Organisms of this type carried short flagella and the number of flagella per bacterium was small. Consequently, the flagellar shape cannot be identified. The presence of many fragmented flagella in the field of the electron microscope suggests that these flagella were more fragile than those of other types.

(5) Hooked-curly (Pl. 3, fig. 17), represented by clone 7. Flagella of this type are curly but have longer spiral unit length and a curvature more bent than the original curly; consequently their waves are hook-shaped. They tend to coil with each other and to form bundles. From the measurements of wavelength, amplitude and spiral unit length, the difference between flagellar shapes of para-curly and hooked-curly were not clear, but the discrimination of these two types is shown by observation of flagellar curvature and the mode of spreading on semi-solid medium (Table 1; Pl. 1, fig. 2). The ratio of wavelength to amplitude of hooked-curly flagella was diversely distributed compared to that of other mutants, while their spiral unit length was almost constant (Figs. 2, 3). This may indicate that the hook-shaped flagella have a tendency to stretch.

Dark-field microscopy

Under the dark-field microscope, most of the bacteria were motile when observed immediately after mounting on slides. But the number of motile bacteria decreased gradually with the passage of time. When the organisms were moving rapidly bundled flagella were blurred and had the appearance of a smooth straight tail as reported by Pijper (1957). While the organisms were moving slowly the spirals of bundled flagella were clearly seen. The organisms moved forward by making the bundled flagella as the axis (Fig. 1). The shape of bundled flagella showed a wavelength of $2.20\ \mu$ and an amplitude $0.53\ \mu$ in normal bacteria while in the curly mutant the respective figures were 0.91 and $0.29\ \mu$ (Pl. 1, figs. 3, 10). The average wave-

lengths and amplitudes of the bundles of each mutant clone are listed in Table 3. The characteristic type of flagellar shape of each mutant was the same, between electron microscopy and dark-field microscopy, for all clones. One of the remarkable differences between the figures seen by electron microscopy and dark-field microscopy was that dimorphism was observed in clone 2 in the dark field but not by the electron microscope (Pl. 1, figs. 5, 6). During 30–60 min. after preparation of the bacterial suspension in 0.5% methylcellulose solution, flagellar bundles observed in clone 2 were mostly those with the longer wavelength. Thereafter the number of the bundles with the shorter wavelength gradually increased to more than 50% of the observed bundles. By continuous observation of single organisms having bundles of long wavelength, change from long type to short type was observed to occur in a single bundle. The detail of this change will be reported elsewhere. The shape of a flagellum of clone 2 by the electron microscope was comparable to that of the bundle with long wavelength (Tables 2, 3).

Table 3. *Wavelength and amplitude of flagella-shape mutants of Salmonella abortusequi observed with dark-field microscope*

Type	Strain	WL* (μ)	SD	Amp. (μ)	SD	WL/ amp.
Normal	SL 23	2.20	0.09	0.53	0.06	4.2
Heteromorphous	Clone 1	2.24	0.12	0.46	0.03	4.9
Small-amplitude	Clone 2 {	L†	1.69	0.14	0.32	5.3
		S	0.95	0.10	0.45	2.1
Para-curly	Clone 3	1.08	0.06	0.31	0.04	3.5
Hooked-curly	Clone 7	0.98	0.06	0.42	0.03	2.4
Curly	SJ 30	0.91	0.06	0.29	0.08	3.1

* WL = wavelength; Amp. = amplitude; SD = standard deviation; † L = larger flagellar wave; S = smaller flagellar wave.

In contrast to clone 2, dimorphism observed with clone 1 by the electron microscope was not seen by dark-field microscopy: the shape of flagellar bundle in clone 1 by dark field was like with that of a normal flagellum of the clone as seen by the electron microscope. The bundles of clones 3 and 7 corresponded to spiral of each flagellar wave as observed on the bundle formation of curly flagella of *Salmonella abortusequi* strain SJ 30 (Mitani & Iino, 1965). The spiral of the flagellar bundles of clone 3 by dark-field microscopy did not resemble the curvature of the bundle as observed by the electron microscope but that of a unit flagellum.

The ratio of wavelength observed with the electron microscope to that by dark-field microscopy was 1.3 to 1.6 in all clones. On the contrary, the ratio of amplitude by electron microscope to that by dark-field microscopy was not significantly different except in clone 3 (Tables 2, 3). In clone 3 the ratios of wavelength and of amplitude by electron microscope to those by dark-field microscopy was greater than 1. Clone 7 organisms formed a spiral of bundled flagella with the same wavelength and longer amplitude compared with that of clone 3; while after flattening on a grid, vertical extension was not seen as it was with clone 3. This may explain the overbending of the wave in a flagellum of clone 7. Organisms of strain SJ 30 and of clone 7, which aggregated in broth, were very often found to bind each other with their flagellar bundles. Bundled flagella were not detected in clones 4, 5 or 6 by dark-field microscopy (Pl. 1, fig. 8).

Flagellar antigens of the mutants

By the slide agglutination test, the H-antigens of all the seven mutants examined were identified as *e*, *n*, *x*. For further details of their antigenicity, anti-*e*, *n*, *x* sera were obtained against *Salmonella abortusequi* strain SJ 30 and each of the five mutant clones, representing each flagellar type. The agglutination titre of the anti-*e*, *n*, *x* sera to the *e*, *n*, *x* type organisms was examined before and after reciprocal absorption of all pairs of mixtures. The titres with the antisera before absorption were all 2^{14} ; after absorption, agglutination was not observed at the dilution of $1/2^3$ to $1/2^{16}$. That is, the *e*, *n*, *x* antigens of strain SJ 30 and its five mutant strains behaved identically in the absorption-agglutination test.

Genetic analysis

Transductions were done with P 22 phage from the five mutant clones, representing each flagellar type, to a phase-1 monophasic strain of *Salmonella typhimurium* sw 1166 *i*: (genotype *H1'H2¹⁻²ah2⁻*; Iino, 1962*b*). Antigen type recombinants were screened on semi-solid medium containing anti-*i* serum. The developing transductional clones, i.e. swarms, were isolated on nutrient agar plates and their flagellar antigen typed by slide agglutination. They were further transferred to broth and

Table 4. *Transduction from the flagella-shape mutants of Salmonella abortusequi* SJ 30 (*a*): *e*, *n*, *x* to *S. typhimurium* sw 1166 *i*: (1,2)

SJ 30 is curly in both phase-1 and phase-2. The culture used for the present experiment is stable in phase-2. sw 1166 is a phase-1 monophasic (*ah2⁻*) type. Recombinants were selected on plates of semi-solid medium containing anti-*i* serum.

Donor	No. of phase-2 type transductional clone				
	Total	M- <i>e</i> , <i>n</i> , <i>x</i>	N- <i>e</i> , <i>n</i> , <i>x</i>	M-1, 2	N-1, 2
Mutant: clone 1	12	11	0	0	1
Mutant: clone 2	14	13	1	0	0
Mutant: clone 3	6	6	0	0	1
Mutant: clone 4	5	5	0	0	0
Wild: SL 23	12	0	10	0	2
None	0	0	0	0	0

M = mutant type corresponding to phase-2 of donor; N = normal type.

semi-solid medium, and the motility of the component organisms examined; the results are listed in Table 4. The major types developed were *a* and *i*: *e*, *n*, *x*. Movement of the former in broth was rotational as expected from the genotype of the donor (Iino, 1962*a*). The latter showed motility in *e*, *n*, *x*-phase as that of the phase-2 organisms of the donor strain. In transduction from clone 7, *i*: *e*, *n*, *x* type recombinant was not detected, probably because the *e*, *n*, *x* types among the transductional clones were as poorly motile as the donor strain and could not form distinctive swarms on the screening media. In transduction from SJ 30 to clone 7 no swarms developed, on semi-solid medium. These results indicate that each characteristic flagellar shape of the mutant clones 1, 2, 3 and 4 was co-transduced with the phase-2 antigen, *e*, *n*, *x*. As a minor type, *i*: 1, 2 swarms were detected on the screening

plates. The motility of the component organisms were normal without exception. In the control plate, to which only recipient organisms were inoculated, *i*: 1, 2 swarms did not grow. Moreover, the mutation of strain sw 1166 to diphasic, *ah2*⁺, type was not observed. The *i*: 1, 2 swarms may, therefore, represent the recombinant between *ah2* and *H2*. Normal flagellar type in phase-2 of these recombinants indicated that the flagellar shape determinant of the corresponding donor mutants was more closely associated with phase-2 antigen type determinant with *ah2*.

Transductions were next done from the four mutant clones 1, 2, 3 and 4, to a phase-1 curly mutant strain of *Salmonella typhimurium* sw 577 *i*: 1, 2 (Iino, 1962*a*). The purpose of this experiment was to examine the possibility of modification of phase-1 curly character by the mutant genes of these clones. Transductional clones were isolated, inoculated into broth and their motilities examined; further sub-culture to broth was made. To check whether phase-1 antigen-*i* remained as curly or was modified, the cultures were diluted appropriately and plated together with anti-*e*, *n*, *x* serum on semi-solid medium. The results are summarized in Table 5; the alternative phase of *e*, *n*, *x* was curly with antigen type *i*. That is, the mutation affected only flagellar type in phase-2.

Table 5. *Flagellar type of the recombinants obtained by transductions from the flagella-shape mutants of Salmonella abortusequi* sv 30 (*a*): *e*, *n*, *x* to *S. typhimurium* sw 577 *i*: 1, 2

sw 577 is curly in phase-1 and normal in phase-2. The recombinants in which phase-2 flagellin gene, *H2*^{1,2}, was replaced by *H2*^{*e,n,x*}, were selected on plates of semi-solid medium containing anti-*i* serum.

Donor	No. of phase-2 type transductional clone examined			Flagellar type in phase-1
	Total	Normal- <i>e</i> , <i>n</i> , <i>x</i>	Mutant- <i>e</i> , <i>n</i> , <i>x</i>	
Mutant: clone 1	21	0	21	Curly- <i>i</i>
Mutant: clone 2	98	0	98	
Mutant: clone 3	63	0	63	
Mutant: clone 4	5	0	5	
Wild: SL 23	39	39	0	

DISCUSSION

Bacterial flagella have been known to have characteristic shapes for each bacterial strain. The flagellar morphology of various bacteria has been studied extensively with the light microscope with stained materials; the accumulated information was reviewed by Leifson (1960). The flagellar shapes which have been reported are normal, curly, small-amplitude, coiled semi-coiled, and straight. The occurrence of subclones with flagellar shape different from that of the parental clone, probably because of mutation, has sometimes been noticed (Leifson & Palen, 1955). The present investigation has shown that a curly flagellar strain of *Salmonella abortusequi* can mutate at least to five different flagellar types, namely, heteromorphous, small-amplitude, para-curly, short and hooked-curly. The small-amplitude mutant appears to correspond in shape to that described by Leifson (1960) by this name. Para-curly and hooked-curly are to be regarded as subtypes of curly: they have not been differentiated by the foregoing light-microscope study. Among the curly types reported in a previous paper (Iino, 1962*a*), curly-*i* flagella of *Salmonella typhimurium*

belong to the original curly and curly-*a* flagella of *S. abortusequi* to hooked-curly. The curly type observed by Pijper, Nefer & Abraham (1956) and Leifson, Carhart & Fulton (1955) under dark-field or light microscope may correspond to para-curly or hooked-curly in the present report: in all of them the ratios of normal wavelength/curly wavelength were in the range 1.8–2.2 to 1 while in the curly type flagella of SJ 30 it was 2.4–2.5 to 1.

The comparative observation of flagellar shape with the electron microscope and by dark-field microscopy has indicated that the characteristics of the shape of flagella on living organisms are preserved well in samples dried for electron microscopy. Although after being dried the spiral of a flagellum is generally flattened to form a wave with wavelength about 30–50% longer than that of the spiral, the amplitude of the wave is not significantly different in the two preparations. An exceptionally greater value of the amplitude after flattening as compared with that of the flagellar spiral was observed in the para-curly mutant of *Salmonella abortusequi*. This may reflect a physico-chemical character of the flagella of this type. Irregularly shaped flagella of heteromorphous clone observed by electron microscopy may be also due to the tendency to stretch during the preparation of the bacterial samples.

By dark-field microscopy, dimorphism in the spiral of flagellar bundles was observed by Pijper & Abraham (1954) in *Sarcina urea* and *S. agilis*. The dimorphous spirals they reported were normal and curly. Similar type of dimorphism was observed in the present work in clone 2 of *Salmonella abortusequi* in which both small-amplitude and curly flagellar bundles appeared. Further, this dimorphism occurred by transition from small-amplitude to curly in already-formed bundles. The dimorphism in clone 2 was not seen with the electron microscope: the flagella were all of small-amplitude, even in the sample prepared from organisms showing curly bundled flagella by dark-field microscopy.

The characteristic modes of movement of the organisms of each mutant of *Salmonella abortusequi* indicate that the flagellar shape influences the mode of locomotion. Generally speaking, curly type organisms manifested abnormal movement, such as rotation and circulation in broth, and their speed of spreading in semi-solid medium was slower than that of the normal type. The motility behaviour of the small-amplitude type was intermediate of normal and curly. Whether or not the minor differences in flagellar shape are directly reflected in differences of spreading ability among three curly types is uncertain. It may be worth noting that curly type organisms tended to aggregate by the linking of flagella in liquid medium, and the degree of aggregation differed among the different curly types: the higher the degree of aggregation, the lower the ability to spread.

Based on current information, we may summarize the sequential steps of flagellar formation as follows: (1) synthesis of flagellin polypeptide from the component amino acids; (2) folding of the flagellin polypeptide into flagellin monomer; (3) polymerization of flagellin monomer to flagellar fibre. By analogy with other protein-forming systems, the first step is presumed to occur on the active ribosomes, where messenger-RNA transcribed from the structural gene of flagellin determines the whole amino acid sequence of flagellin polypeptide, and immediately following the first step, the second step may occur on or near the site of the first step reaction (Rich, Warner & Goodman, 1963). The third step must proceed at the basal

granule of each flagellum. Theoretically, reactions involved in all of these three steps can contribute to the determination of the specific shape of the flagella finally produced. Genetic analysis of curly mutants in *Salmonella abortusequi* indicates that the genetic change of flagellar shape from normal to curly occurred by one step mutation in the structural gene of flagellin; that is, the genetic information of the flagellar shapes are primarily tendered in the form of amino acid sequence of the component flagellin (Iino, 1962*a*; Enomoto & Iino, 1963). On the other hand, the change of flagellar shape has been known to be caused by certain environmental factors, for example, the change of pH value (Leifson, 1960). Further, it has been observed that flagella reconstituted from normal flagellin monomers are changed to curly flagellar fibres *in vitro* when they are stored in certain environmental conditions (Asakura *et al.* 1964). These observations suggest that flagellin of the same amino acid composition can form more than one type of flagella-shape under different conditions. Preliminary genetic analysis on the five flagellar shape mutants used in the present work has indicated that at least in four, the mutant characters are phase-2 flagella specific and transduced together with the phase-2 H-antigen type. In transduction with the remaining one, hooked-curly, the recombinants of the mutant character were not obtained, probably because they spread slowly on semi-solid medium used for the screening. The most plausible explanation of these results is that the change of flagellar shape occurred by mutation in the structural gene of phase-2 flagellin, consequently by the replacement of an amino acid in flagellin polypeptide leading to the alteration of the conformation of flagellin monomer and of the mode of polymerization. The clear-cut examples of such second site reversion were given in *td* locus of *Escherichia coli* by Helinski & Yanofsky (1962).

In the heteromorphous mutant of *Salmonella abortusequi*, dimorphism of flagellar shape was observed in a clone: curly flagella appeared occasionally among the normal. The strain used is stable as regards the flagellar phase (Iino, 1962*b*). Therefore the involvement of flagellar phase variation in the dimorphism is excluded. When the curly waves appear they are always mixed with the normal in an organism or even in a single flagellum. Consequently, the possibility is also excluded that curly flagella are formed by organisms mutated from the clone. The following two possibilities remain to explain this flagella-shape dimorphism. The first one is that flagellin produced by the heteromorphous organisms is a single kind but can make two kinds of polymer, normal and curly, under the environmental condition provided by the basal granules of flagella in the mutant strain, though the choice of the alternative is greatly biased to the former. The second is that curly flagellin is occasionally produced by the degeneracy of the genetic code responsible for the heteromorphism.

When the flagella were visible by dark-field microscopy, they were always presumed to be bundled (Mitani & Iino, 1965), as a single *Salmonella* flagellum would scatter insufficient light to make it visible (Stocker, 1956). The difference of curvature of flagellar bundles between dark-field microscopy and electron microscopy was observed in the para-curly mutant. In this type, the difference in the curvature was also present between flagellar bundle and its component flagella as seen with the electron microscope. The curvature of the flagella is more like that of the flagellar bundle observed with dark-field microscopy. This type of flagella-shape variation in a clone indicates that certain types of the already formed flagella transform their

curvature when they are dried and flattened on a collodion membrane for the electron microscope observation. The treatment of drying and flattening may cause tension between the unit flagellum and this may make the flagella stretch.

Flagella of the short mutants of *Salmonella abortusequi* are pronounced by the decrease of both number per organism and length. As regards the interrelation between the type of flagellin and the ability of polymerization, marked differences of the speed of polymerization among the different types of flagellin has been observed *in vitro* (Asakura, Eguchi & Iino, unpublished data). Therefore, there is a possibility that the flagellin of these mutants has exceedingly poor ability of polymerization among the mutant flagellins.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. A plate of semi-solid medium on which the organisms of curly flagellar strain SJ 30 of *Salmonella abortusequi* were brushed and incubated for 2 days at 37°. The organisms of SJ 30 grew only at the site of inoculation, from where flagella-shape mutants appeared as various types of swarms.

Fig. 2. Spreading ability of representative clones of flagella-shape mutants shown by the growth on a plate of semi-solid medium. (a) Clone 1, (b) clone 3, (c) clone 2, (d) clone 4, (e) clone 7, (f) SL 23: normal type, (g) SJ 30: parental curly type.

Fig. 3. Normal flagellar bundle of SL 23 in 0.5% methylcellulose solution observed under dark-field microscope. $\times 2160$.

Fig. 4. Flagellar bundle of clone 1 observed as in Fig. 3.

Fig. 5. Small-amplitude flagellar bundle of clone 2 observed as in Fig. 3.

Fig. 6. Curly flagellar bundle transformed from the small-amplitude in clone 2 observed as in Fig. 3.

Fig. 7. Para-curly flagellar bundle of clone 3, observed as in Fig. 3.

Fig. 8. Flagellar bundle cannot be observed on clone 4 under dark-field microscope. $\times 2160$.

Fig. 9. Hooked-curly flagellar bundle of clone 7, observed as in Fig. 3.

Fig. 10. Flagellar bundle of parental curly strain SJ 30 observed as in Fig. 3. Linking of flagellar bundles is seen.

PLATE 2

Fig. 11. Flagella of wild strain SL 23 observed under electron microscope, after being stained by PTA for 10 min. and shadowed by chromium. $\times 14,400$.

Fig. 12. Small-amplitude flagella of clone 2 observed under electron microscope, after being stained by PTA for 10 min. $\times 14,400$.

Fig. 13. Flagella of clone 1 observed as in Fig. 12.

Fig. 14. Short flagella of clone 4 observed as in Fig. 12.

PLATE 3

Fig. 15. Flagellar bundle of clone 3, which has specific wavelength longer than the unit flagellum, observed as in Fig. 12.

Fig. 16. Flagellar bundle and its component flagella of clone 3, observed as in Fig. 12. Notice the difference between the wavelength of bundled flagella and a component flagellum.

Fig. 17. Hooked-curly flagella of clone 7, observed as in Fig. 12.

Fig. 18. Flagella of parental curly strain SJ 30 observed under electron microscope after chromium shadow was given on the unfixed and dried organisms. $\times 14,400$.