ELECTRON MICROSCOPIC STUDY ON THE DEVELOPMENT OF FLAGELLA OF A FRESHLY ISOLATED STRAIN OF *SALMONELLA ABORTUS EQUI*

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INTRODUCTION

Recently NISHI and KUMAGAI (1951) stated that most of the freshly isolated strains of *S. abortus equi* have the phase 1 whose antigenic components have not yet been determined, and by serial passages on artificial media, phase variation could be caused from phase 1 to naturally occurring phase 2 (H antigens: e n x). In the next year, NISHI and OHTSUKA (1952) made supplementary statement that their undetermined flocculent antigens of the fresh strains were closely related to the antigen "z_5" which has been known as the beta phase of the Schleissheim type. WATANABE et al. (1952) observed that in one of their stock cultures of this organism phase 3 "z_5" occurred under the natural condition as EDWARDS and BRUNER had also artificially succeeded in inducing phase variation with this organism.

KATO (1954), a colleague of this department, has demonstrated that the fresh strains of *S. abortus equi* are not flocculated in e n x-serum when they are grown in alkaline broth ranging from pH 7.5 to 8.0 and they will become flocculated as well as stock cultures after several serial passages on agar.

The present investigation was undertaken to illustrate the relationship between the flocculability in e n x-serum and flagellar development of *S. abortus equi* of freshly isolated and stock strains.

MATERIALS AND METHODS

Culture of S. abortus equi: Gastric contents of aborted fetus were used as the materials. Newly isolated colonies on agar plate were serially passed on agar slant and transferred into broth of various pH at every generation and H antigen (e n x) was titrated with e n x-serum.

Electron microscopy: After 15 hrs. incubation of each broth culture, one drop of

each culture respectively was absorbed with glass capillary pipettes and suspended in 0.3 per cent neutral formalin saline. Then one drop of this suspension was mounted on a formval membrane for making electron micrographs. Then the membranes were dried and washed three times with distilled water and chromium-shadowed. The micrographs were taken at an electronic magnification of ×2,000～×4,000.

Results

Behaviors of the freshly isolated cultures in agglutination are given in table 1. The fresh strains grown in alkaline broth of pH 7.5～8.0 did not flocculate with H (e n x) serum for the first several passages on agar slant, whereas fresh strain grown in broth of that acidic range and stock cultures grown in both alkaline and acid broth are flocculated with e n x-serum. Following several passages on agar, the fresh strains would become to be flocculated with alkaline broth cultures. The number of agar passages to become sensitive to e n x-serum are different depending on each of the strains. Strain N 4 needed two passages, strain N 1 nine passages, strains N 2 and N 3 seven and eight passages respectively.

When the fresh strains were passed serially on agar, they would become to be flocculated with e n x-serum even if they were grown in alkaline broth. At this time,

TABLE 1. *Changes Occurring in Flocculating Capacity of the Fresh Strains by Serial Passages on Agar*

NO. OF STRAINS	PH OF BROTH	NO. OF SERIAL PASSAGES ON AGAR							
		0	1	2	3	4	8	9	10
N 1	6.1	++	++	•	•	++	•	++	++
	7.0	++	++	•	•	++	•	++	++
	7.5	—	—	•	•	—	•	—	++
	8.0	—	—	•	•	—	•	—	+
N 2	6.1	++	++	•	•	++	++	++	++
	7.0	++	++	•	•	++	++	++	++
	7.5	—	—	•	•	—	++	++	++
	8.0	—	—	•	•	—	++	++	++
N 3	6.1	++	•	•	•	++	++	++	++
	7.0	++	•	•	•	++	+	++	++
	7.5	—	•	•	•	—	—	++	++
	8.0	—	•	•	•	—	—	++	++
N 4	6.1	•	++	+	+	++	•	•	•
	7.0	•	++	+	++	++	•	•	•
	7.5	•	—	—	++	++	•	•	•
	8.0	•	—	—	++	++	•	•	•

colonies were separated on agar plate from an agar slant and the flocculation of each colony was tested.

As the results, most of the colonies were flocculated but a small number still remained insensitive to e n x-serum. These insensitive colonies were subjected further serial passages on agar slant and they became to be flocculated after several passages. Thus, an individual bacterial cell of a colony does not have always similar sensitivity to e n x-serum, in the other words, the development of flagella is varied in each cell. Sooner or later, these insensitive colonies would be rendered sensitive to e n x-serum by the passages on agar. This would mean the adaptation of the organism on artificial media. As the control, many stock cultures of the organisms in this department were examined in the same way and none of them showed non-flocculation in either acid or in alkaline broth.

On the other hand, the electron micrographs were studied at every step of passages on agar. As is indicated in fig. 1, abortion bacilli in gastric contents of aborted fetus reveal flagella. Micrographs of the fresh strains do not develop flagella when they are grown in alkaline media, whereas the stock strains develop flagella equally in both acid and alkaline media.

EDWARDS and BRUNER (1939) succeeded in inducing phase variation in *S. abortus equi* and concluded that supposedly monophasic *S. abortus equi* contains three phases. Phase 2 is the form in which the species naturally occurs. Phase 1 contains H antigenic component "a" and phase 3 contains "z_5". These induced phases are reversible.

The authors investigated the phases of 83 stock strains in this department with H sera (a, e n x, z_5). The results obtained are indicated in table 2.

TABLE 2. *The Phase of the 83 Stock Strains in this Department*

PERIOD OF PRESERVATION	NO. OF STOCK CULTURE	H SERA		
		a	e n x	z_5
32 Years	1	—	+	—
30 "	3	—	+	—
24 "	4	—	+	—
23 "	1	—	+	—
14 "	1	—	+	—
10 "	2	—	+	—
9 "	2	—	+	—
6 "	14	—	+	—
5 "	16	—	+	—
4 "	10	—	+	—
3 "	9	—	+	—
2 "	16	—	+	—
9 Months	2	—	+	—
6 "	2	—	+	—

Table 2 indicates that all stock cultures are in phase 2 (e n x). From above results, it would seem that S. *abortus equi* maintains phase 2 for long time without provoking phase variation under the natural conditions.

Discussion

The results presented indicate that the freshly isolated strains of S. *abortus equi* do not develop flagella in alkaline broth. The development of flagella of the fresh strains is highly suppressed in alkaline media. Consequently, the flocculation of broth culture with e n x-serum is not observed at all. Through the serial passages on agar slant, the organisms adapted on artificial media and the result was the fine development of flagella and flocculation in e n x-serum to the same degree as in the stock cultures.

As is cited in the introduction, Nishi and Ohtsuka misunderstood that the organism has another phase at the freshly isolated stages because of the insensitiveness of the bacilli to e n x-serum.

The observations reported here also might serve to emphasize the fact that S. *abortus equi* has very stable characters which do not provoke spontaneous phase variation even after long period preservation. Thus, the authors would qualify the statement that the organism exhibits naturally phase 2 and that phase variation scarcely occurs.

Summary

Electron micrographs of freshly isolated cultures of S. *abortus equi* were studied. The development of flagella of the fresh cultures was highly suppressed in alkaline media. Through the passages on agar, the bacilli would become to develop flagella as was the case in old stock cultures. The speed of adaptation on artificial media was somewhat different depending on the strains. All 83 stock strains of the organism preserved in this department revealed phase 2 without the occurring of phase variation.

References

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EXPLANATION OF PLATES

Electron micrographs of *Salmonella abortus equi*

Electron microscope of Japan Electron Optics Laboratory Co., Model JEM-4 CHD.

Cultures of 15 hrs. in broth.

PLATE I.

Fig. 1. Gastric content of an aborted fetus. Showing the peritrichous flagella in vivo (Electron magnification × 1,320)

Fig. 2. Fresh strain N 4 after single passage on agar, grown in broth of pH 6.1 (× 2,400).

Fig. 3. Same culture, grown in broth of pH 7.0 (× 1,320)

Fig. 4. " pH 7.5 (× 1,800)

Fig. 5. " pH 8.0 (× 1,800)

PLATE II.

Fig. 6. Fresh strain N 4 after 4th passage on agar, grown in broth of pH 6.1 (× 1,800)

Fig. 7. Same culture, grown in broth of pH 7.0 (× 1,800)

Fig. 8. " pH 7.5 (× 2,200)

Fig. 9. " pH 8.0 (× 1,800)

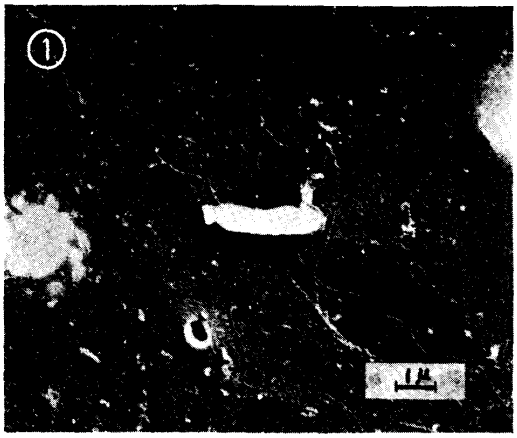
PLATE III.

Fig. 10. Stock strain E 9, grown in broth of pH 6.1 (× 1,320)

Fig. 11. " pH 7.0 (× 1,200)

Fig. 12. " pH 7.5 (× 1,200)

Fig. 13. " pH 8.0 (× 1,320)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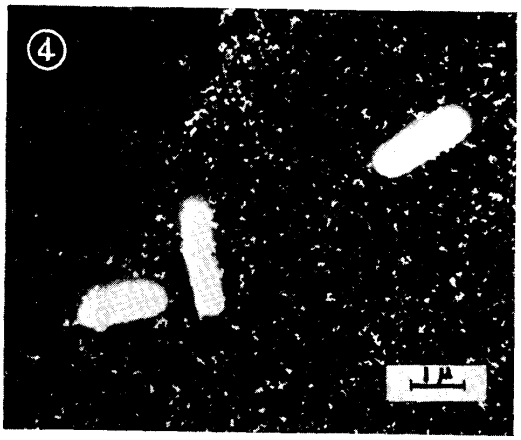
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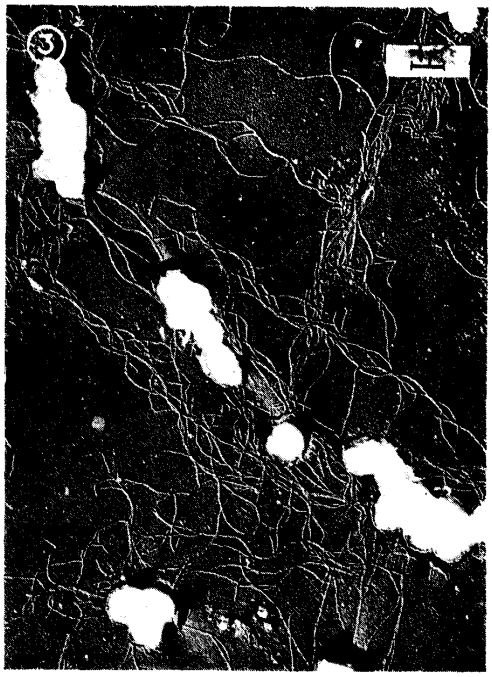
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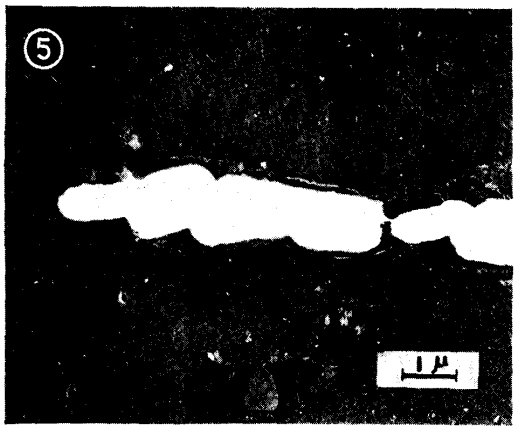
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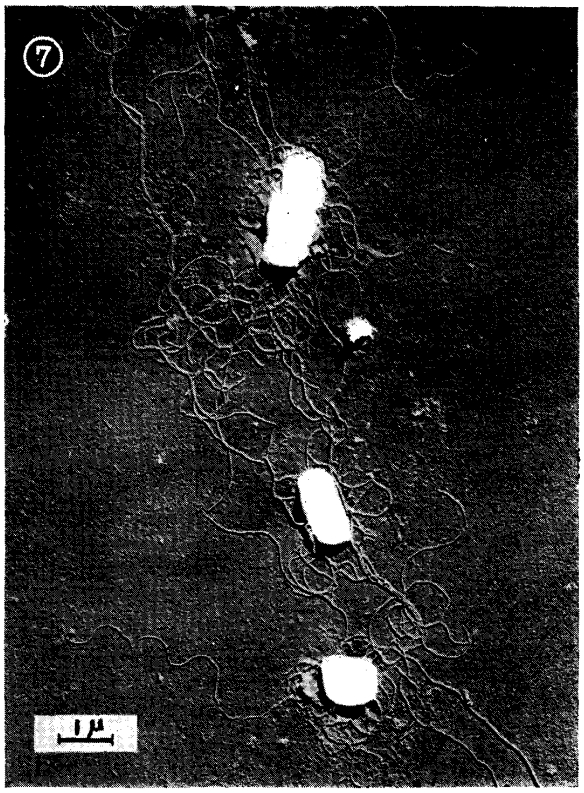
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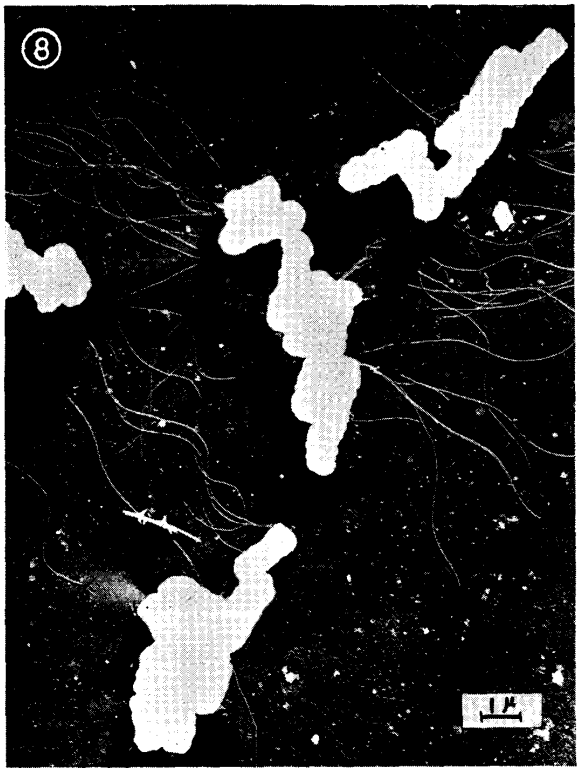
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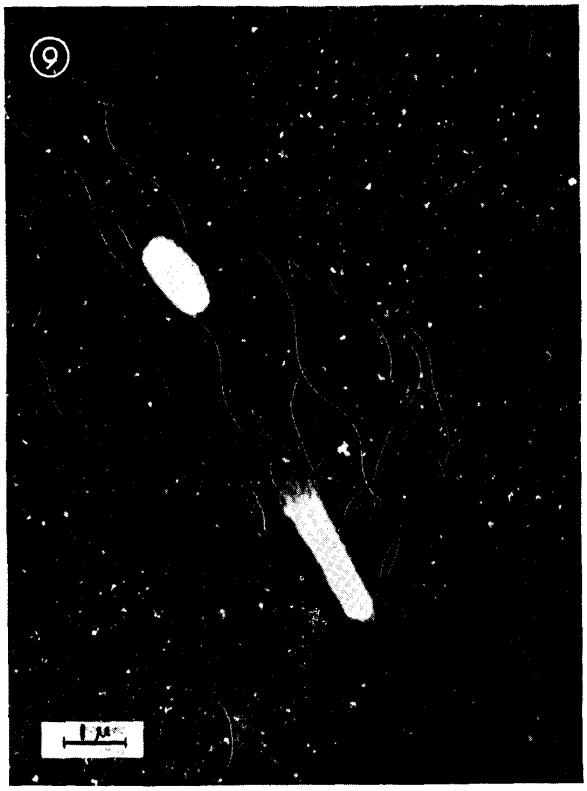
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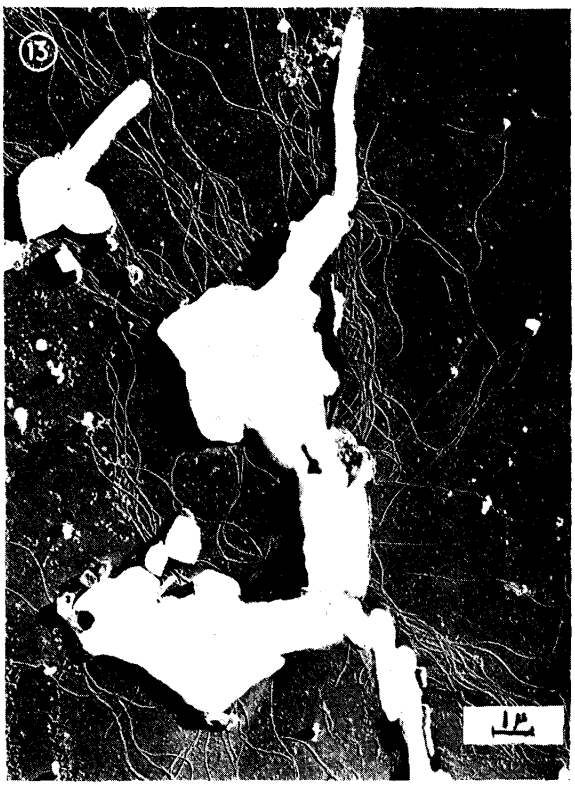
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