STUDIES ON THE ANTIGENIC PATTERNS OF BACTERIAL FLAGELLA

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INTRODUCTION

It is well known that there are two kinds of *Salmonella* organisms in respect of flagellar antigens; the one is monophasic and the other is diphasic. The distinction is based upon whether organisms form colonies showing uniform behavior in respect of flagellar agglutinability or dissociating into two kinds of colonies different from each other. Further consideration reveals the existence of two apparently different antigenic patterns in flagella of *Salmonella* organisms, namely flagella with only one antigenic factor, for example *S. paratyphi A* has only one flagellar antigen *a* in its first phase, and the flagella, as the second type, with more than one antigenic factor, as for example *g, m* in *S. enteritidis* or *e, n, x*... in *S. abortus equi*. In the latter case, the factors never dissociate into different colonial types and are always found in coexistence, therefore it is to be considered that each bacterial body is attached with the flagella containing every antigenic factor. Though it is evidenced that the H-antigen represents flagellar substances, there is no direct answer to the question as to whether the flagella of each bacterial body are uniform in respect of antigenic patterns, i. e. a single flagellum contains each of the antigenic factors altogether or there are several kinds of flagella differing from each other in respect of antigenic factors contained in them. The present investigation has been conducted to make this question clear utilizing purified flagellar suspension.

EXPERIMENTAL METHODS

The strains used: *S. enteritidis* 1891 and *S. abortus equi* beta 202.

The method of purification for flagella: The method previously described in the paper¹⁾ was followed.

[illegible]

A similar experiment was performed with anti-(*f*, *g*) serum and the same flagellar material. Table 1-(b) presents the result of this experiment, which also indicates that the flagella with antigenic pattern *g*, *m* were entirely precipitated by the antibody (*g*) in anti-(*f*, *g*) serum in the antibody-excess zone.

Table 1
(b)

Serum (<i>f</i> , <i>g</i>) dilution	Antigen (<i>g</i> , <i>m</i>) dilution								
	2 ¹	2 ²	2 ³	2 ⁴	2 ⁵	2 ⁶	2 ⁷	2 ⁸	2 ⁹
5 × 2 ⁰	+	+	+	+	+	+	+	+	—
5 × 2 ¹	+	+	+	+	+	+	+	+	—
5 × 2 ²	+	+	+	+	+	+	+	+	—
5 × 2 ³	+	+	+	+	+	+	+	+	—
5 × 2 ⁴	+	+	+	+	+	+	+	+	—
5 × 2 ⁵	—	—	—	—	—	—	—	—	—

From the above mentioned experimental results, it is concluded that the flagellar material with antigenic pattern *g*, *m* of *S. enteritidis* 1891 contains both *g* and *m* factors in a single flagellum together, and that a flagellum possessing only one single antigenic factor, either *g* or *m*, never exists in the case of this organism.

2. Experiments with purified flagella of *Salmonella abortus equi* beta 202.

The result of precipitation reaction using varying amounts of purified flagellar suspension of *Salmonella abortus equi* beta 202 with varying dilution of anti-(*e*, *h*) serum is presented in Table 2. In this case, the original flagellar suspension contained 0.45 mg per ml of nitrogen. From the results, it has become clear that the flagella with antigenic pattern *e*, *n*, *x* are completely precipitated by the antibody (*e*) in anti-(*e*, *h*) serum in the antibody-excess zone. The above fact indicates that each flagellum of *Salmonella abortus equi* beta 202 contains all of the antigenic factors *e*, *n* and *x*. In other words, the antigenic pattern *e*, *n*, *x* is applicable to a single flagellum, and that there is no flagellum of this organism found possessing only one or two of these antigenic factors *e*, *n* or *x*.

Table 2

Experiment with the flagella of *Salmonella abortusequi* (*e, n, x...*)

Serum (<i>e, h</i>) dilution	Antigen (<i>e, n, x...</i>) dilution								
	2 ¹	2 ²	2 ³	2 ⁴	2 ⁵	2 ⁶	2 ⁷	2 ⁸	2 ⁹
5×2 ⁰	+	+	+	+	+	+	+	+	—
5×2 ¹	+	+	+	+	+	+	+	+	—
5×2 ²	—	—	—	—	—	—	—	—	—

Table 3

Experiment with the mixed flagella ((*g, m*) and (*e, n, x...*))

First Serum	Antigen ((<i>g, m</i>) 1:16 (<i>e, n, x...</i>) 1:8)	Supernatant	Second Serum	Supernatant
(<i>m, t</i>) 1:20	+	crowdy	(<i>e, h</i>) 1:5	clear +
(<i>e, h</i>) 1:5	+	crowdy	(<i>m, t</i>) 1:20	clear +

3. Experiments with a mixed suspension of the flagella from both *S. enteritidis* 1891 and *S. abortus equi* beta 202.

As seen from the above mentioned experiments, the dilution factor of antiserum, at which precipitation reaction turns up positive with purified flagellar suspension, is pretty higher than in H-agglutination reaction with bacterial suspension. Therefore a question arises as to whether the complete precipitation of flagella in antibody-excess zone in the above mentioned experiments was a purely specific antigen-antibody reaction or even those flagella containing no antigen corresponding to the antibodies in antiserum used were precipitated together with the flagella reacting with their corresponding antibodies in the serum by clumping effect. The following experiments were performed to give an answer to this question.

A purified flagellar suspension of *Salmonella enteritidis* 1891 (1:4 diluted from the original suspension with nitrogen content of 0.39 mg per ml) and that of *Salmonella abortus equi* beta 202 (1:2 diluted from the original suspension with nitrogen content of 0.45 mg per ml) were mixed in equal amount and 0.5 ml of the mixture was added with 0.5 ml of 1:10 diluted anti-(*m, t*) serum. In this case, although precipitation

occurred, the supernatant did not become completely clear, indicating that precipitation reaction was not complete. The reactant suspension was subjected to centrifugation at 3,000 R.P.M. and 0.5 ml of the supernatant was mixed with 0.5 ml of anti-(*e, h*) serum at 1:2.5 dilution. Precipitation reaction occurred and the supernatant became entirely clear this time. It has become clear herefrom that turbidity in the supernatant after the first reaction was due to the flagella capable of reacting with anti-(*e, h*) serum.

In case, the mixed flagellar suspension was added first with anti-(*e, h*) serum, although precipitation reaction occurred, the supernatant was also not clear. This reactant was centrifuged and the supernatant was mixed with anti-(*g, m*) serum. This time, precipitation reaction was complete and the supernatant became completely clear.

From these experiments, it was concluded that, when mixed suspension of flagella different from each other in respect of antigenic pattern was mixed with antiserum corresponding to only either one of these antigens possessed by the flagella used, the flagella possessing antigens other than those homologous to the antiserum were never precipitated together. Therefore it is clear that the precipitation reaction observed in the experiments 1) and 2) are considered to be entirely specific.

DISCUSSION

The question as to whether antigenic factors are located in each single flagella of a single bacterial body, and consequently each flagella of a single bacterial body has one and the same antigenic pattern or several kinds of flagella showing varying antigenic patterns were attached to a single bacterial body was subjected to serological analysis utilising purified flagellar suspension.

Our experimental results confirmed that the first assumption of those above mentioned is true. In other words, among the schematic diagrams of Fig. 1, the diagram (a) is showing the true picture.

(a)

(b)

(c)

SUMMARY

An analytical study of antigenic patterns of bacterial flagella was conducted using purified flagellar suspension of *Salmonella* organisms.

REFERENCE

Uchida, H. and Fukumi, H.: On the purification of bacterial flagella, *Jap. J. med. Biol. Sci.*, in press.