Susceptibility of *Haemophilus paragallinarum* to Bactericidal Activity of Normal and Immune Chicken Sera

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**ABSTRACT.** Bactericidal (BC) activity of fresh or heated, normal (NCS) and immune (ICS) chicken sera was investigated with encapsulated and nonencapsulated variants dissociated from serotype HA-1 strain 221 of *Haemophilus paragallinarum*. The BC titer was expressed as the highest serum-dilution reducing viable cell count by 50% after reaction time of 3 hr. Nonencapsulated organisms were shown to be sensitive to the BC activity of fresh NCS, in which no specific antibodies were demonstrable, but encapsulated organisms resisted the BC activity. Fresh ICS with the detectable hemagglutination-inhibition (HI) and agglutination antibodies were bactericidal for both encapsulated and nonencapsulated organisms, but the activity was completely lost by heating at 56°C for 30 min. The BC activity appears to be due to the antibodies against HA-L hemagglutinin and/or L agglutinogen located on outer membrane of the cell, but not to the antibodies against capsular or other somatic antigens except for the HA-L and L antigens.—**KEY WORDS:** bactericidal activity, chicken, *Haemophilus paragallinarum.*

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*Haemophilus paragallinarum* causes acute upper respiratory tract infection in chickens, resulting in significant economic loss. Prevention of the disease can adequately be accomplished by vaccination with inactivated whole cells. Little is known, however, on antigens and other substances which are responsible for pathogenicity and immunogenicity of the organisms. Various studies showed that encapsulated organisms formed iridescent colonies (hi variant [17]) and were virulent in chicks, whereas nonencapsulated ones formed noniridescent colonies (ni-A variant [17]) and were avirulent [5, 8, 17, 20]. Therefore, virulence of *H. paragallinarum* seems to be related to a capsular substance or a substance specifically produced from the encapsulated organisms [17].

The report concerning the antigen structure of serotype HA-1 [11] (serotype I [20]) strains of *H. paragallinarum* showed that the strain had hemagglutinins [17, 18], designated as HA-L, HA-HL and HA-HS, and agglutinogens, designated as L, HL and HS [21]. These somatic antigens seemed to be located on outer membrane of the bacterial cell [18].

The chicks having the hemagglutination-inhibition (HI) antibodies against HA-L hemagglutinin and agglutinins against L agglutinogen (L agglutinins) were significantly protected against the challenge exposure with homologous strains of *H. paragallinarum*, whereas the chicks lacking the antibodies were not [5–10, 22]. Recently, Sawata et al. [19] showed that the capsular antigen of *H. paragallinarum* does not produce protective activity. Thus, the HA-L and/or L antigens seem to be protective antigen, although antigenic relationship between HA-L and L antigens remains to be clarified.

In 1932, Ward and Wright [25] reported that a bactericidal (BC) effect could be produced on capsulated *Haemophilus influenzae* by fresh human serum or by anti-*H. influenzae* horse serum in the presence of guinea pig complement. Since then, there were many
Table 1. Antigen structure of variants hi and ni-A dissociated from serotype HA-1 strain 221 of H. paragallinarum

<table>
<thead>
<tr>
<th>Variant</th>
<th>Capsular antigen determined by IHA-inhibition</th>
<th>Agglutinin&lt;sup&gt;b) &lt;/sup&gt;determined by RPA</th>
<th>Hemagglutinin&lt;sup&gt;c) &lt;/sup&gt;determined by HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>hi</td>
<td>+</td>
<td>L</td>
<td>HL</td>
</tr>
<tr>
<td>ni-A</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

a) Somatic antigen.
b) Lack of agglutinability in the presence of capsules, but the activity appeared soon after treatment with hyaluronidase.

IHA = Indirect-hemagglutination; RPA = rapid plate agglutination; HI = hemagglutination-inhibition; L = heat-labile, trypsin-sensitive, hyaluronidase-resistant; HA-L = heat-labile, trypsin-sensitive, hyaluronidase-resistant, active against glutaraldehyde-fixed erythrocytes; HL and HA-HL = heat-labile, trypsin-resistant; HS and HA-HS = heat-stable, trypsin-resistant.

Reports concerning the BC effect of the serum against H. influenzae [1, 12, 23]. Resistance to systemic infection with H. influenzae type b has been associated with serum BC activity for the organisms [23]. It is unknown whether specific chicken antibodies have BC activity against H. paragallinarum. The present study deals with susceptibility of the encapsulated and nonencapsulated variants, which are hi and ni-A dissociated from a serotype HA-1 strain of H. paragallinarum, to BC activity of normal (NCS) and immune (ICS) chicken sera.

MATERIALS AND METHODS

Bacterial strains: The hi and ni-A variants were used. The variants were dissociated [17] from a hemagglutination serotype HA-1 [11] (an agglutination serotype 1 [20]) strain 221 [4], and their antigen structure is summarized in Table 1. Morphologic, biologic, serologic, and immunologic properties of the variants were described previously [5, 17].

Antigens: The hi and ni-A variants were grown in S broth medium [7, 8] at 37°C for 12 hr, the cells were harvested by centrifugation at 10,000×g for 30 min at 4°C, and the following antigens were prepared according to the procedures described previously [17]: nontreated (NT); hyaluronidase (Sigma)-treated (HU); 2 M saline and trypsin (P-L Biochemical)-treated (SAT); and heated at 121°C for 2 hr at pH 7.0 (HE) antigens. All the antigens were adjusted the concentration to 1×10<sup>11</sup> cells/ml by spectrophotometry (650 nm, 1×10<sup>10</sup> cells/ml = optical density of 0.386) with a spectrophotometer model 6/20, (Coleman Instruments), and finally added with 0.01% thimerosal for rapid plate agglutination (RPA) and hemagglutination-inhibition (HI) tests. Each thimerosal-inactivated antigen, adjusted the concentration to 5×10<sup>9</sup> cells/ml by spectrophotometry, was used for preparation of immune chicken serum (ICS) after absorption with 900 μg/ml of Al(OH)<sub>3</sub> [8].

Extracted antigens: Formalin-saline-extracted (FSEX), and formaline-saline-extracted and phenol-treated (FSEX-P) antigens were prepared by the method described previously [19]. The FSEX-P antigen was used for the indirect hemagglutination (IHA) test to detect anticapsular antibody [19]. The FSEX and FSEX-P antigens adjusted to correspond to 1:16 of IHA-inhibition titer [19] against 221-hi-NT antiserum (IHA titer against hi-FSEX-P antigen was 1:1.280) were used for ICS preparation.

Normal chicken serum: Normal chicken serum was obtained from 13-week-old, specific pathogen-free (SPF), line S chicks (Nissuikei Co., Yamanashi, Japan) known to be
free from *Haemophilus* organisms. Pooled NCS was dispensed in 1 ml aliquots within 2 hr after the collection, and kept at −80°C.

**Immune chicken serum:** Immune chicken serum was prepared by injecting SPF chicks intramuscularly at 10 and 13 weeks of age, each 1 ml/chick, with NT, HU, SAT or HE antigen (5 × 10⁹ cells/ml) from hi variant, and sera were collected at 16 weeks of age. The FSEx and FSEx-P antigens were injected, each 1 ml/chick, intravenously in chicks at 13, 14, 15 and 16 weeks of age [19], and sera were collected at 18 weeks of age. Sera collected from individual chicks in each injection group were pooled, and then dispensed in 1 ml aliquots within 2 hr after the collection. They were kept at −80°C. Antibody titers of each ICS tested by the various serologic tests were listed in Tables 4 and 5.

**Sera:** Fresh or heated (56°C for 30 min) NCS and ICS were tested for their BC activities on hi and ni-A variants. All the tests were conducted within 1 month after collection of sera.

**Cell suspension:** After 5 hr incubation at 37°C, organisms grown on S agar medium [7, 8] were suspended in 0.02 M phosphate-buffered solution containing 0.85% NaCl (PBS), pH 7.0. The suspension was adjusted to a concentration of 5 × 10⁴ colony-forming units (CFU)/ml by spectrophotometry.

**Bactericidal assay:** Bactericidal assay was done as follows: Test serum was diluted serially in fivefold steps with fresh NCS. To 0.8 ml each of the diluted serum, 0.2 ml of the cell suspension (5 × 10⁴ CFU/ml) was added. The mixture (1 × 10⁴ CFU/ml) was incubated in a water bath at 37°C for 5 hr. A portion (0.025 ml) of the sample was taken for viable cell count at 0, 1, 2, 3 or 5 hr after incubation. The time was referred to as “reaction time” hereinafter. The sample was spread on S agar medium and the plate was incubated at 37°C for 16 hr. After incubation, viable cell count was done.

**Determination of BC titer:** Viable cell count by sensitization with heated NCS (C) or test serum (T) at time 0 and at the reaction time “n” was shown as C₀, Cₙ, T₀ and Tₙ, respectively. Bactericidal activity (%) of each sample was calculated as follows: 100 − (C₀/T₀ × Tₙ/Cₙ) × 100. The serum showing ≥50% of the BC activity was considered positive for BC effect. The BC titer was expressed by the highest serum-dilution that had the BC activity of ≥50% after reaction time of 3 hr. Bactericidal assay was repeated at least three times with each sample.

**Serologic tests:** The RPA method was used for agglutination test, and the result was read according to the criteria described previously [21]. The hi-HU (L agglutinin), hi-SAT (HL) and hi-HE (HS) antigens were used for the RPA test [21]. The HI test was performed by the method described previously [11] by using glutaraldehyde (Eastman Kodak)-fixed erythrocytes (RBC) from 60-day-old, line S chicks. The hi-HU (HA-L hemagglutinin) and ni-A-SAT (HA-HL) antigens were used for the HI test [18]. The IHA test was performed by the method described previously [19].

**Absorption test:** The antigens adjusted to a concentration of 1 × 10¹² cells/ml by spectrophotometry were used as absorbent. The bacterial sediment by centrifugation at 10,000 × g for 30 min at 4°C was resuspended in fresh test serum, and absorption was done at 0°C for 1 hr. After the absorption, agglutinin, IHA and BC titers remaining in the supernatant were titrated as mentioned above.

**RESULTS**

**Kinetics of viable cells in NCS:** Viable cell count of nonencapsulated organisms (ni-A variant) decreased slowly in the presence of fresh NCS in which no specific antibodies were detected by the HI, RPA and IHA tests. In contrast, that of encapsulated organisms (hi variant) gradually increased (Fig. 1 and Table 2). Thus, it was revealed that ni-A
variant was sensitive to the BC activity of fresh NCS, whereas hi variant was resistant. Heat-treatment destroyed the BC activity of NCS on ni-A variant.

Kinetics of viable cells in ICS: Viable cell counts of both hi and ni-A variants decreased slowly when incubated with fresh ICS (Fig. 1). In contrast, they increased gradually when ICS had been heated at 56°C for 30 min. The results indicated that both hi and ni-A variants were sensitive to fresh ICS, and heat-treatment of ICS abolished the BC activity (Table 2).

Reaction time influenced BC activity (Table 2), and 3 hr to 5 hr of incubation time were required to obtain positive BC effect.
Table 2. Bactericidal activity of chicken sera on variants hi and ni-A dissociated from serotype HA-1 strain 221 of *H. paragallinarum*

<table>
<thead>
<tr>
<th>Chicken sera used</th>
<th>Reaction time at 37°C and BC activity (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>hi variant</th>
<th>ni-A variant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hr</td>
<td>3 hr</td>
<td>5 hr</td>
</tr>
<tr>
<td>Fresh NCS</td>
<td>0</td>
<td>-53</td>
<td>-33</td>
</tr>
<tr>
<td>Fresh ICS&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26</td>
<td>75</td>
<td>91</td>
</tr>
<tr>
<td>Heated ICS&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-23</td>
<td>-62</td>
<td>-247</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentage of inactivated organisms was calculated as follows: 100−(C0/T0×Tn/Cn)×100. See text.

<sup>b</sup> The serum having BC activity of ≥50% was considered positive for BC effect (underlined).

<sup>c</sup> Immunized with nontreated (NT) antigen prepared from 221-hi variant (221-hi-NT antigen). The serum has both the HI antibody titer of 1:80 against HA-L hemagglutinin and the L. agglutinin titer of 1:40.

BC=Bactericidal; NCS=normal chicken serum; ICS=immune chicken serum.

Table 3. Serum BC titers of fresh and heat-treated chicken sera on hi variant of *H. paragallinarum* serotype HA-1

<table>
<thead>
<tr>
<th>Chicken sera used</th>
<th>Additional chicken sera&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reaction time of 3 hr at 37°C and BC titers&lt;sup&gt;b&lt;/sup&gt; in serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh NCS</td>
<td>No</td>
<td>&lt;1:5</td>
</tr>
<tr>
<td>Fresh ICS&lt;sup&gt;c&lt;/sup&gt;</td>
<td>No</td>
<td>1:125</td>
</tr>
<tr>
<td>Heated ICS&lt;sup&gt;c&lt;/sup&gt;</td>
<td>No</td>
<td>&lt;1:5</td>
</tr>
<tr>
<td>Heated ICS&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Fresh NCS</td>
<td>1:125</td>
</tr>
</tbody>
</table>

<sup>a</sup> Test serum: additional serum (1:1).

<sup>b</sup> BC titer was expressed as the highest serum dilution that presented the BC activities of ≥50% after reaction time of 3 hr.

<sup>c</sup> Same ICS used in Table 2.

Table 4. Bactericidal effect of various fresh ICS prepared from 221-hi variant of *H. paragallinarum* on the hi variant

<table>
<thead>
<tr>
<th>Antiserum against</th>
<th>IHA titers with hi-FSEX-P antigen&lt;sup&gt;b&lt;/sup&gt;</th>
<th>HI titers with © hi-HU antigen&lt;sup&gt;b&lt;/sup&gt; (HA-L)</th>
<th>Hi-A-SAT antigen&lt;sup&gt;b&lt;/sup&gt; (HA-HL)</th>
<th>RPA titers with hi-HU antigen&lt;sup&gt;b&lt;/sup&gt; (L)</th>
<th>hi-SAT antigen&lt;sup&gt;b&lt;/sup&gt; (HL)</th>
<th>hi-HE antigen&lt;sup&gt;b&lt;/sup&gt; (HS)</th>
<th>Reaction time of 3 hr at 37°C and BC titers in serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>hi-NH</td>
<td>&lt;1:5</td>
<td>1:640</td>
<td>1:640</td>
<td>1:40</td>
<td>1:20</td>
<td>&lt;1:5</td>
<td>1:125</td>
</tr>
<tr>
<td>hi-HU</td>
<td>&lt;1:5</td>
<td>1:80</td>
<td>1:320</td>
<td>1:40</td>
<td>1:20</td>
<td>1:5</td>
<td>1:125</td>
</tr>
<tr>
<td>hi-SAT</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
<td>1:1,280</td>
<td>&lt;1:5</td>
<td>1:10</td>
<td>1:5</td>
<td>&lt;1:5</td>
</tr>
<tr>
<td>hi-HE</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
<td>1:5</td>
<td>1:125</td>
</tr>
<tr>
<td>hi-FSEX&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1:1,280</td>
<td>1:40</td>
<td>1:5</td>
<td>1:10</td>
<td>1:5</td>
<td>&lt;1:5</td>
<td>1:625</td>
</tr>
<tr>
<td>hi-FSEX-P&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1:1,280</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
<td>&lt;1:5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Capsular antigen.

<sup>b</sup> Somatic antigen.

<sup>c</sup> Formalin-saline-extracted antigen.

<sup>d</sup> Formalin-saline-extracted and phenol-treated antigen.
Table 5. The effect of absorption of fresh ICS with various antigens originated from the hi variant of *H. paragallinarum* on BC activities

<table>
<thead>
<tr>
<th>Antiserum against</th>
<th>Absorbed with a)</th>
<th>IHA titers with hi-FSE-P antigen b)</th>
<th>HI titers with</th>
<th>RPA titers with</th>
<th>Reaction time of 3 hr at 37°C and BC titers in serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>hi-HU antigen (HA-L)</td>
<td>hi-HU antigen (HA-HL)</td>
<td>hi-HU antigen (L)</td>
<td>hi-SAT antigen (HL)</td>
</tr>
<tr>
<td>hi-NT</td>
<td>None</td>
<td>&lt;1:5</td>
<td>1:640</td>
<td>1:40</td>
<td>1:20</td>
</tr>
<tr>
<td></td>
<td>hi-NT</td>
<td>&lt;1:5</td>
<td>1:5</td>
<td>1:5</td>
<td>1:20</td>
</tr>
<tr>
<td></td>
<td>hi-HU</td>
<td>&lt;1:5</td>
<td>1:5</td>
<td>1:5</td>
<td>1:20</td>
</tr>
<tr>
<td></td>
<td>hi-SAT</td>
<td>&lt;1:5</td>
<td>1:640</td>
<td>1:40</td>
<td>1:20</td>
</tr>
<tr>
<td></td>
<td>hi-HE</td>
<td>&lt;1:5</td>
<td>1:640</td>
<td>1:40</td>
<td>1:20</td>
</tr>
<tr>
<td>hi-FSEx</td>
<td>None</td>
<td>1:1, 280</td>
<td>1:40</td>
<td>ND</td>
<td>1:10</td>
</tr>
<tr>
<td></td>
<td>hi-HU</td>
<td>1:1, 280</td>
<td>&lt;1:5</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

a) Absorbed with $1 \times 10^{12}$ cells/ml.
b) Capsular antigen.
c) Somatic antigen.
d) Not done.

**Bactericidal titers of chicken sera on hi variant:** BC titer of fresh ICS was 1:125, whereas the titers of fresh NCS and heated ICS were <1:5 (Table 3). Bactericidal activity of heated ICS could be restored by the addition of fresh NCS.

**Bactericidal effect of various fresh ICS on hi variant:** Antisera prepared against hi-NT, hi-HU and hi-FSEx antigens had BC antibody titers of 1:25 to 1:625 (Table 4). The HI antibodies against hi-HU antigen (HA-L hemagglutinin) and agglutinins against hi-HU antigen (L agglutinogen) were also detected in these antisera. In contrast, antisera against hi-SAT, hi-HE or hi-FSE-P antigen, lacking both the HI antibodies against HA-L hemagglutinin and L agglutinins, did not show BC activities (<1:5). Thus, a correlation was observed between the presence of HI antibody and L agglutinins and that of BC activity in the test sera.

**Bactericidal effect of fresh ICS absorbed with various antigens on hi variant:** Fresh ICS against hi-NT (hi-NT antiserum) or hi-FSEx (hi-FSEx antiserum) antigens had BC titers of 1:125 to 1:625 (Table 5). The BC activity of these antisera was completely removed (<1:5) by the absorption with hi-NT or hi-HU antigens having both HA-L and L antigens. In the same way, both HI antibodies against HA-L hemagglutinin and L agglutinins were abolished (<1:5). In contrast, the BC antibodies could not be removed from the hi-NT antiserum by absorption with the hi-SAT or hi-HE antigen which did not possess HA-L or L antigens.

**DISCUSSION**

Bactericidal action of mammalian serum is known to involve activation of the complement system. There are two pathways for the activation. One is the alternative pathway which can act independently at the presence of an antibody [3, 24]. Another is the classical pathway, on which complement system is activated by interaction between an antibody and an antigen [3, 16]. The complement system of the chicken has been reported to be responsible for serum hemolytic activity in a manner analogous to that of the mammals [15]. Although avian complement activation pathways have not been established, present results strongly suggest that BC action of chicken sera definitely involves complement components, since the BC activity was completely lost by heating at 56°C for 30 min and
could be restored by the addition of fresh NCS (Table 3).

The results of the present investigation showed that fresh NCS and ICS inactivated \textit{H. paragallinarum} variant \textit{in vitro}, and that significant correlation was observed between the presence of capsule on the test organisms and the susceptibility of the organisms to the BC activity of NCS. Furthermore, the results indicated that a complement-dependent BC effect might be involved in the BC reaction(s) of specific antibodies. The same mechanism appears to operate on the encapsulated or nonencapsulated organisms. Nonencapsulated ni-A variant of \textit{H. paragallinarum} bacteria was sensitive to BC factors in fresh NCS in which no antibodies against the tested bacteria were demonstrable by the HI, RPA and IHA tests. The BC effect of normal sera on nonencapsulated \textit{H. influenzae} bacteria is well known [2] and has been attributed to activation of the alternative pathway [3, 24]. Our results show that the factor causing the BC activity is heat-labile (at 56°C for 30 min).

Previously, the virulence of \textit{H. paragallinarum} was shown to correlate well with the amount of capsular substance by using seven variants dissociated from serotype HA-1 strains [17, 19]. In the present investigation, it was shown that susceptibility of \textit{H. paragallinarum} to the BC activity of fresh NCS significantly differed on the encapsulated and nonencapsulated organisms. It might reflect a difference in virulence in chicks among the variants [17]. Moreover, capsule of \textit{H. paragallinarum} might act as a natural defence substance against BC power of complement activated through the alternative pathway. In fact, the difference in the bacterial recovery period was observed \textit{in vivo} on the encapsulated and nonencapsulated organisms of \textit{H. paragallinarum} [17].

It has been known that complement concentration, inoculum size, and reaction time had an influence on the BC titers of pre-immune and hyperimmune rabbit sera to \textit{H. influenzae}. Transfors and Dahlberg [2] reported that the kinetics of the BC effect on \textit{H. influenzae} was different in preimmune and hyperimmune sera, and the latter needed a longer reaction time of 2 hr. Also in our present study, reaction time had an influence on the BC activities of NCS and ICS. In general, positive BC activity was observed after 3 hr incubation at 37°C. Complement concentration may be a variable factor in our present BC assay, though NCS and ICS employed herein contained sufficient amount of complement for the BC assay.

The encapsulated \textit{H. paragallinarum} bacteria (hi variant) were resistant to the BC factors in fresh NCS, thus the bacteria might lack the ability to activate the complement through the alternative pathway. In contrast, the bacteria were sensitive to the BC factors in fresh ICS. The present results apparently showed that the antisera having the HI antibodies against the HA-L hemagglutinin and L agglutinins produced the BC titers, whereas those lacking the antibodies did not (Table 4). The BC activity was completely removed in an absorbed antiserum without any loss of the anticapsular antibody determined by the IHA test [19] (Table 5). The anticapsular antibody titers did not decrease by absorption with the 221-hi-HU (whole cell) antigen. The absorption, however, significantly decreased the HI, RPA and BC antibody titers. The somatic [18] L and/or HA-L antigens, therefore, may be target site antigen(s) of the antibodies for the BC reaction. These two somatic antigens may be responsible for production of the BC antibodies to \textit{H. paragallinarum} in chicks. The HI antibodies against HA-L hemagglutinin as well as L agglutinins may share BC activity, which may be a key to specific humoral resistance to \textit{H. paragallinarum} serotype HA-1.

In general, the target site antigen of the BC antibodies is believed as a protective antigen of the test organisms [23, 25]. In our pre-
vious reports [5–10, 22], we showed that the protective activity of *H. paragallinarum* resided in the heat-labile somatic HA-L and/or L antigens. As shown herein, these two somatic antigens of *H. paragallinarum* appeared to be a target site antigen. Antibodies related to the protection may be detectable by the serum BC reaction *in vitro*.

In *H. paragallinarum*, both the anticapsular antibodies and the antibodies against other heat-stable somatic antigens except for the L and HA-L antigens may not have a BC effect. In fact, capsular [19] and other somatic antigens except for the L and HA-L antigens [5, 6, 9, 19] completely lacked protective activity. In contrast, *H. influenzae* anticapsular antibodies have a BC effect [1, 23]. In addition, antibodies against heat-stable somatic antigens also seemed to be important for the BC effect of certain sera against encapsulated *H. influenzae* [1, 12]. The target site antigen may differ between *H. paragallinarum* and *H. influenzae*.

*H. paragallinarum* strains were classified into serotypes by the serotype-specific agglutinin by Sawata et al. [21]. The presence of two immunotypes 1 and 2 corresponding to Sawata’s serotypes 1 and 2 [21] was reported by Kume et al. [8]. Rimler et al. [14] reported the presence of three immunotypes A, B and C corresponding to Page’s serotypes A, B and C [13]. However, Kume et al. [9] could not demonstrate the presence of three immunotypes among the Page’s serotype A, B and C strains. Recently, Kume et al. [11] classified *H. paragallinarum* strains into seven serotypes by the serotype-specific haemagglutinin HA-L [17, 18]. The classification based on the serotype-specific HA-L haemagglutinin system was found superior to that based on the agglutination test in its specificity. However, correlation between HA-L haemagglutinin specificity and immunologic specificity of *H. paragallinarum* has not been studied well. The BC assay method developed herein may be employed as an useful tool to analyse the immunotypes *in vitro* instead of the *in vivo* protection test.

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要約

正常および免疫誘血の殺菌作用に対する Haemophilus paragallinarum の感受性: 澤田 早・久米碧子 (1) 中井豊次 (2) 北里研究所附属家畜衛生研究所 (3) 北里研究所 —— Haemophilus paragallinarum 血清型 HA-1・221株から作出した変異株に対する正常 (NCS) および免疫 (ICS) 誘血血清の殺菌作用を調べた。供試菌に対する抗体を欠く新鮮 NCS の殺菌作用に対して荚膜非保有菌は、感受性を、荚膜保有菌は抵抗性を示した。荚膜保有菌および非保有菌は、種々の抗体を有する新鮮 ICS の殺菌作用に感受性を示した。殺菌作用は、56℃ 30 分の加熱によって失活したが、新鮮 NCS の添加で回復し、補体の関与が示唆された。殺菌および殺菌抗体吸収試験の成績から、ICS の殺菌作用は、菌体外膜に存在する易発性、トリプシン感受性、ヒアルロンダーゼ耐性の L 細胞集素および HA-L 赤血球凝集素に対する抗体によることが確認された。