CHEMICAL STUDIES ON CELLULAR COMPONENTS OF
BORDETELLA PERTUSSIS

I. PURIFICATION AND PROPERTIES OF AGGLUTINOGEN

KAORU ONOUE, MASAYASU KITAGAWA, AND YUICHI YAMAMURA

Department of Biochemistry, School of Medicine, Kyushu University, Fukuoka, Japan

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ABSTRACT

Onoue, Kaoru, (Kyushu University, Fukuoka, Japan), Masayasu Kitagawa, and Yuichi Yamamura. Chemical studies on cellular components of Bordetella pertussis. I. Purification and properties of agglutinogen. J. Bacteriol. 82:648-656, 1961.—A method is described for the preparation of the agglutinogen of Hemophilus pertussis in phase I by methanol precipitation and ion-exchange chromatography.

High homogeneity of this agglutinogen was evidenced by chromatography, but a trace of impurity was detected by ultracentrifugal analysis and by an agar gel diffusion test.

The agglutinogen produced an allergic skin reaction in rabbits which had been immunized with whole phase I organisms.

Chemical studies indicate that the agglutinogen should be a simple protein in nature. The approximate molecular weight was determined to be 10,000 by Archibald’s method.

A soluble antigen with an agglutinin-absorbing activity was extracted from Bordetella pertussis in phase I by Flosdorf, Kimball, and Chambers (1939) and Flosdorf and Kimball (1940a), and partially purified by Flosdorf and Kimball (1940b) and Smolens and Mudd (1943). These studies showed that the agglutinin-absorbing principle, agglutinogen, was thermostable and not toxic for rabbits, indicating that it was separated from thermolabile toxin. They also demonstrated that the agglutinogen produced an allergic-type skin reaction in sensitized animals and human beings. Because of the practical need of a simple and specific method to test susceptibility to whooping cough and to ascertain the effect of vaccination against whooping cough, clinical use of the agglutinogen as a skin test reagent has been reported by many authors (Felton and Flosdorf, 1943; Flosdorf et al., 1943; Sauer and Markley, 1946; Felton and Flosdorf, 1946; Felton, Smolens, and Mudd, 1946; Halpern and Halpern, 1948; Miller, Ryan, and Havard, 1948; Kohn et al., 1950; Zenyoji, 1952; Someya, 1955).

Most of these results have shown that (i) the agglutinogen seemed to produce a specific skin reaction in any individuals with immunity to whooping cough from either previous attack of the disease or vaccination, and no reaction in those with no history of the disease or vaccination, and (ii) after intradermal testing, a definite increase in agglutinin titer was observed, indicating that the agglutinogen is a complete antigen.

Flosdorf and Kimball (1940b) suggested that agglutinogen might be a protein, because of positive protein tests and of strong antigenicity. They also found that it was relatively thermostable over a wide range of pH. At pH 1.5 or 11 there was complete loss of activity by heating 30 min at 100°C, but at 56°C less than 50% of the activity was lost. Such stability of the agglutinogen is unusual, if it is assumed to be proteinaceous. Recently, Zenyoji (1952) reported that agglutinin prepared by the method of Smolens and Mudd (1943) might be a ribonucleoprotein, because it gave positive protein and orcinol-HCl tests and showed an ultraviolet absorption maximum at 264 μm.

Homogeneity of the agglutinogen, in spite of its high activity, has not been examined sufficiently; therefore, the disagreement concerning the chemical nature of agglutinogen might be due to impurity of the substance. In the present study, using cellulose ion-exchange we were able to isolate an agglutinogen with high purity, which was proved chromatographically to be a single component, although slight contamination was detected by ultracentrifugal and immunological analysis. Chemical and physicochemical studies showed that it is a protein of low molecular weight, without nucleic acid.
AGGLUTINOGEN OF B. PERTUSSIS

MATERIALS AND METHODS

*B. pertussis.* Killed organisms of Sakurayashiki strain in phase I were supplied by Takeda Pharmaceutical Industries, Ltd., Osaka, Japan. Stock cultures grown on Bordet-Gengou agar medium were subcultured in Roux bottles on a semisynthetic culture medium containing Casamino acids and charcoal. Composition of the culture medium was as follows: Casamino acids, 10.0 g; NaCl, 2.5 g; KH2PO4, 0.5 g; MgCl2·6H2O, 0.6 g; FeSO4·7H2O (1% solution), 0.6 ml; CaCl2·2H2O (0.5% solution), 1.0 ml; CuSO4·5H2O (0.05% solution), 1.0 ml; L-cysteine (1% solution), 1.0 ml; “Agino-moto” (sodium salt of glutamic acid, Ajinomoto Company, Inc., Tokyo, Japan), 3.0 g; soluble starch, 1.5 g; yeast, 5.0 g; charcoal, 10.0 g; agar, 30.0 g; and distilled water, 1,000 ml. The medium was adjusted to pH 6.8. After incubation at 38 C for 48 hr, the organisms were harvested using 0.15 M sodium chloride solution, centrifuged, washed with saline, and re-centrifuged. The paste was suspended in saline. Merthiolate was added to a final concentration of 0.01%, and the mixture was stored at 4 C for several months.

*Preparation of agglutinogen by picric acid precipitation.* This was performed according to the method of Smolens and Mudd (1943), with slight modification. Killed organisms suspended in 0.15 M sodium chloride solution containing 0.1% Merthiolate were collected by adjusting the pH to 3.7 with 1 N HCl and centrifuging at 4,000 rev/min for 15 min; the resulting packed cells were weighed. After grinding the organisms with glass powder in a ball mill, three extractions were made at 50 to 56 C with dilute hydrochloric acid at pH 1.8. The period of time for each extraction was 17 to 20 hr. After extraction, clear supernatants were obtained by centrifugation at 18,000 rev/min for 20 min. The three extracts were combined to give a total volume of three to four times that of the original packed cells.

To the combined supernatant was slowly added 1 N NaOH. Precipitation began to occur at pH 4 and increased gradually with increasing pH to a maximum at pH 7. At this point, the precipitate was separated by centrifugation, washed three times with a small amount of distilled water (pH 7.0), and the washings combined with the supernatant. This was designated as crude extract.

An equal volume of saturated ammonium sulfate solution was added to the crude extract and allowed to stand overnight at 2 to 4 C. The precipitate was collected by centrifugation, washed once with half-saturated ammonium sulfate solution, and dissolved in a volume of water equal to one-tenth the volume of crude extract. The solution was put into a cellophane bag and dialyzed for 48 hr against three changes of 2 liters of distilled water. Any insoluble residue in the dialyzed solution was removed by centrifugation at 10,000 rev/min for 15 min.

In the next step, according to the method of Smolens and Mudd (1943), a one-half volume of saturated picric acid solution was added to the supernatant. After 3 hr, the resulting precipitate was dissolved in distilled water and dialyzed against distilled water until the yellow color had almost disappeared. The inner solution was centrifuged at 10,000 rev/min for 15 min, and the clear supernatant was freeze-dried.

*Preparation of agglutinogen by methanol precipitation.* In our modified method, methanol was used in place of picric acid to precipitate the agglutinogen.

The dialyzed clear solution of ammonium sulfate precipitate was cooled to 0 C, and methanol added slowly with stirring to a final concentration of 13%. At this point the solution was cooled to −5 C. After standing 30 min at −5 C, it was centrifuged at 10,000 rev/min for 7 min. Methanol was added slowly to the supernatant to a final concentration of 25%; the solution was allowed to stand for 30 min at −5 C and centrifuged at 10,000 rev/min for 7 min. The precipitate was dissolved in distilled water and dialyzed against cold distilled water overnight. A clear, pale-yellow solution was obtained by removing any insoluble material from the dialyzed inner solution. This was designated as methanol-precipitated agglutinogen. For storage, it was lyophilized.

*DEAE-cellulose column chromatography.* The anion-exchanger, diethylaminoethyl (DEAE)-cellulose, was prepared according to the method of Peterson and Sober (1956), using Toyo Roshi cellulose powder (100 to 200 mesh, Toyo Roshi Kaisha, Ltd., Tokyo, Japan).

DEAE-cellulose (5 g) was suspended in 50 ml of 0.3 M potassium phosphate buffer (pH 7.7), stirred gently for 10 min to avoid air bubbles, and allowed to settle. After decanting the supernatant, the suspension was poured into a glass tube of 1.3 cm diameter with a sintered glass
filter. After settling, the column was washed with 70 ml of the same buffer, then with 200 ml of 0.005 M sodium phosphate buffer, pH 7.7. The height of the column was usually 22 to 25 cm.

About 200 mg of methanol-precipitated agglutinogen dissolved in 5 to 10 ml of 0.005 M sodium phosphate buffer (pH 7.7) were charged on the column. After washing with the same buffer to remove nonadsorbing materials, the elution was made with sodium phosphate buffer, pH 7.1, by increasing the ionic strength of the buffer. Each 4 or 8 ml of effluent was collected by an automatic fraction collector. The protein concentration was determined in each tube by estimating an absorption at 277 nm, or by the copper-Folin reaction according to the modified method of Kabat and Mayer (1948).

The eluted proteins were precipitated by adding 32 g of ammonium sulfate per 100 ml of effluent; and the precipitates were dissolved in the minimal amount of distilled water, dialyzed against distilled water, and then lyophilized.

**Immunization of rabbits.** Rabbits were immunized with killed organisms of *B. pertussis* in phase I, Sakurayashiki strain. Intravenous injections were made twice a week for 4 weeks. Each dose was 0.8 ml, 0.8 ml, 0.8 ml, 1.5 ml, 1.5 ml, 1.6 ml, 1.0 ml, and 1.0 ml of cell suspension (7.1 × 10^9/ml), respectively. Two to four weeks after the last inoculation, the rabbits were bled and the agglutinin titers of the sera were tested. These rabbits were used for intradermal tests with agglutinogen.

**Preparation of antipertussis globulin.** Six weeks after the intradermal testing, rabbits received one booster injection (1.5 ml of cell suspension, intravenously); 2 weeks later, they were bled. An equal volume of saturated ammonium sulfate solution was added to the serum. After standing overnight at 4 C, the precipitate was separated by centrifugation and washed with half-saturated ammonium sulfate solution. It was dissolved in 0.85% saline, dialyzed against distilled water, and lyophilized.

**Agglutinin-absorption test.** This test was performed, according to the procedure of Flosdorf and Kimball (1940c), as follows: A test sample was diluted with 0.85% saline buffered with 0.004 M sodium phosphate buffer (pH 7). To each 1.2 ml of the dilution was added 0.075 ml of diluted (1:25) rabbit immune serum (original agglutinin titer, 1:37,200). The mixture was incubated at 37 C for 1 hr and overnight at 4 C. After removal of the precipitate, if necessary, serial twofold dilutions of absorbed sera were made with buffered saline, using 0.5-ml volumes. To each tube was then added a 0.5-ml suspension of killed *B. pertussis* organisms in phase I, Sakurayashiki strain (1 × 10^10/ml); the tubes were incubated at 37 C for 2 hr. After incubation overnight at 4 C, the agglutinin titers of absorbed sera were read.

To estimate the increase in activity during the course of purification, the ratio of the agglutinin-absorbing activity of the sample in each purification step to that of crude extract was calculated as follows:

\[
\text{Ratio} = \frac{C_r}{S}
\]

where \( C_r \) is the minimal amount (dry weight) of crude extract and \( S \) is the minimal amount of the sample in each purification step which absorbs agglutinin completely in the above described technique.

**Ultracentrifugal analysis.** This analysis was kindly performed by K. Kakiuchi (Institute for Protein Research, Osaka University), using a Hitachi UCA-I ultracentrifuge (Hitachi, Ltd., Tokyo, Japan). Determination of molecular weight was made according to Archibald's method (Archibald, 1947; Klainer and Kegeles, 1956).

**Agar gel diffusion test.** This test was performed according to the method of Oucherlony (1948).

**Quantitative determination of phosphorus.** Phosphorus content was determined according to the method of King (1932).

**RESULTS**

**Purification of agglutinogen by precipitation with methanol.** Agglutinogen was purified by either picric acid precipitation or methanol precipitation as described. The agglutinin-absorbing activity in each step of the procedure is shown in Table 1. An approximate 5- to 10-fold increase in specific activity was observed both for the picric acid-precipitated and the methanol-precipitated agglutinogen.

Both purified samples gave positive tests for protein or amino acids, in tests such as: sulfosalicylic acid, biuret, ninhydrin, Sakaguchi, Miller, and Adamkiewicz. As shown in Table 1, the
orcinol-HCl reaction was positive for picric acid-precipitated agglutinogen, indicating that it might contain ribonucleic acid, but it was negative for the methanol-precipitated agglutinogen. Recovery of the sample in each step from 100 g (wet weight) of whole organisms in a typical case is listed in the last column of the table. The yield of the methanol-precipitated agglutinogen was usually 80 to 90 mg from 100 g (wet weight) of whole organisms, corresponding to 5% of the crude extract by dry weight.

The picric acid-precipitated agglutinogen showed an ultraviolet absorption spectrum maximum at 275 μm and a ratio of extinction at 275 to 260 μm of 1.11, while the methanol-precipitated agglutinogen showed an absorption maximum at 276 μm and a ratio of 1.35. Nitrogen content of the agglutinogen precipitated by picric acid was 14.52%.

In the course of the preparation of agglutinogen by picric acid precipitation, it was difficult to remove picric acid completely from the picric acid precipitate by dialysis against water for as long as 5 to 7 days. Therefore, methanol precipitation was conveniently used in the following experiments.

**Purification of agglutinogen by chromatography.** In preliminary experiments, it was found that stepwise elution was more effective to separate agglutinogen than gradient elution. As seen in Fig. 1, two peaks appeared in fraction F2, and the agglutinin-absorbing activity was concen-

### Table 1. Activity, chemical reaction, and recovery of preparation in each step

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Agglutinin-absorbing activity (ratio)*</th>
<th>Oracinol-HCl test</th>
<th>Diphenylamine test</th>
<th>Molisch test</th>
<th>Recovery&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1700</td>
</tr>
<tr>
<td>AS-50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>370</td>
</tr>
<tr>
<td>SM&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5-10</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>160</td>
</tr>
<tr>
<td>MeOH 13-25&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>85</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ratio of the activity of each preparation to the activity of crude extract.

<sup>b</sup> Recovery of solid material from 100 g wet weight of whole organisms.

<sup>d</sup> Precipitate from crude extract at half-saturation with ammonium sulfate.

<sup>e</sup> Picric acid-precipitated agglutinogen.

<sup>f</sup> Methanol-precipitated agglutinogen.

**FIG. 1. Chromatography of methanol-precipitated agglutinogen on a DEAE-cellulose column.** Methanol-precipitated agglutinogen (869 mg) was chromatographed on a column (1.3 by 23 cm). The flow rate was 10 ml per hr; 8 ml were collected in each of no. 1 to 24 tubes, and 4 ml in no. 25 to 85. Total recovery of protein was 88% as measured by the copper-Folin reaction. Solid line, optical density at 277 μm; dotted line, optical density at 650 μm in the copper-Folin reaction.
treated in the first moving peak (designated F2-1), whereas the activity of the second peak was unappreciable. Remaining proteins were eluted with 0.3 m potassium phosphate buffer, pH 7.7.

The results of the agglutinin-absorption test of each fraction are shown in Table 2. The specific activity of F2-1 was increased 4- to 8-fold, compared with methanol-precipitated agglutinogen.

The highly active F2-1 also produced a definite skin reaction in animals sensitized with whole organisms. The induration was produced specifically in sensitized animals and not in nonsensitized animals. The reaction reached its maximum at 20 to 24 hr after injection. As seen in Table 3, the activity of F2-1 was more potent than methanol-precipitated or picric acid-precipitated agglutinogen.

**Table 2. Agglutinin-absorption tests of preparation in each step and of fractions separated by chromatography on DEAE-cellulose**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Conc of absorbent (µl/ml)</th>
<th>Agglutinin titer of absorbed serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>500</td>
<td>1 0 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1 ± 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>1 ± 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>2 1 0 0 0</td>
</tr>
<tr>
<td>MeOH a</td>
<td>13-25 500</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1 ± 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>1 1 0 0 0</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>1 1 ± 0 0 0</td>
</tr>
<tr>
<td>F1 b</td>
<td>500</td>
<td>2 2 1 1 ± 0</td>
</tr>
<tr>
<td>F2-1 a</td>
<td>125</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>31.2</td>
<td>± 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>15.6</td>
<td>1 0 0 0 0</td>
</tr>
<tr>
<td>F3 b</td>
<td>250</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>1 1 0 0 0</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>2 1 1 0 0</td>
</tr>
<tr>
<td>Control: absorbed with saline</td>
<td>3 3 2 2 1 0</td>
<td></td>
</tr>
</tbody>
</table>

* Methanol-precipitated agglutinogen.
  * These fractions were separated by chromatography on DEAE-cellulose, as illustrated in Fig. 2.

FIG. 2. Rechromatography of F2-1 on a DEAE-cellulose column. F2-1 (25 mg) was chromatographed on a column (0.9 by 22 cm); 5.5 ml were collected per tube. The flow rate was 5 ml per hr.

FIG. 3. Ultraviolet absorption spectrum of RF2-1. RF2-1 was dissolved in 0.8% saline at a concentration of 1 mg/ml, and the absorption was measured in a Beckman DK-2 spectrophotometer, using a 1.0-cm cell.

F2-1 was rechromatographed on a DEAE-cellulose column. As shown in Fig. 2, a single sharp peak was eluted with 0.04 m sodium phosphate buffer, pH 7.1. The recovery of protein in this peak was 98% of the total protein. The agglutinin-absorbing activity of each tube was measured to confirm the homogeneity of this fraction, and the results are shown in Table 4. The first and second tubes (no. 15, 16), containing about 70% of the total protein in this peak, show the same specific activity, indicating the high homogeneity of this fast moving portion; the remaining tubes (no. 17, 18), containing about 30% of the
TABLE 3. Intradermal testing with agglutinogen

<table>
<thead>
<tr>
<th>Test antigen</th>
<th>Dose</th>
<th>Sensitized</th>
<th>Nonsensitized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. 1</td>
<td>No. 2</td>
</tr>
<tr>
<td>MeOH* 13-25</td>
<td>50</td>
<td>13X14</td>
<td>14X15</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8X8</td>
<td>9X8</td>
</tr>
<tr>
<td>F2-1*</td>
<td>50</td>
<td>11X13</td>
<td>14X14</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>10X10</td>
<td>11X11</td>
</tr>
<tr>
<td>SM*</td>
<td>50</td>
<td>8X8</td>
<td>11X11</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8X8</td>
<td>7X8</td>
</tr>
</tbody>
</table>

* Doses of test antigen in 0.1 ml saline were injected intradermally into rabbits which had been immunized with *B. pertussis* cells in phase I.
* After 24 hr, the size of induration and erythema was measured. Normal rabbits were used as controls. Numerator: size of induration; denominator: size of erythema.
* Methanol-precipitated agglutinogen.
* Agglutinogen separated by chromatography on DEAE-cellulose.
* Picric acid-precipitated agglutinogen.

The ultracentrifugal pattern of RF2-1 showed a slow moving main component and a trace of a faster moving component (Fig. 4). The approximate molecular weight of the agglutinogen was measured by Archibald’s method (Archibald, 1947; Klainer and Kegeles, 1956), and was calculated to be 10,000 ± 500.

Agar gel diffusion test. Agar gel diffusion test of purified agglutinogen was performed according to the method of Ouchterlony (1948). A definite sharp line (Fig. 5) was observed between RF2-1 and the globulin fraction of anti-*B. pertussis* serum or anti-*B. pertussis* phase I whole serum which had been absorbed with phase III organisms. Another very faint line was also found between RF2-1 and anti-*B. pertussis* globulin after 7 days. This result suggested that a trace of contamination might exist in this preparation.
Table 4. Agglutinin-absorption test of eluate in each tube in the rechromatography of F2-1

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>Eluate</th>
<th>Agglutinin titer of absorbed serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Optical density (277 μm)</td>
<td>850</td>
</tr>
<tr>
<td>15</td>
<td>0.083</td>
<td>0.042</td>
</tr>
<tr>
<td>16</td>
<td>0.083</td>
<td>0.042</td>
</tr>
<tr>
<td>17</td>
<td>0.083</td>
<td>0.042</td>
</tr>
<tr>
<td>18</td>
<td>0.083</td>
<td>0.042</td>
</tr>
<tr>
<td>Control: absorbed with saline</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

* Each 4.52 ml of eluate was collected separately. Eluates in tubes no. 15, 16, 17, and 18, which contained most of the eluted protein, were diluted to equal protein concentration and their agglutinin-absorbing activity determined. Protein concentration was determined by measuring optical density at 277 μm.

Discussion

Agglutinogen prepared by the method of Smolens and Mudd (1943) has been used widely as a skin test antigen, but the purity and chemical nature of this antigen has not been established satisfactorily. Hink and Johnson (1947) claimed that the Smolens-Mudd agglutinogen did not completely dissolve in saline; they developed a new preparation by selective denaturation. However, this has been proved to be an antigen mixture, and is therefore not suitable for chemical analysis. Kuwashima and Masui (1950) obtained an agglutinogen by a combination of the above two methods, but no chemical analysis was made.

In the present work the agglutinogen was isolated by methanol precipitation and chromatography on a DEAE-cellulose column. The high purity of this agglutinogen was suggested by rechromatography, but the agar gel diffusion test and ultracentrifugal analysis showed that it contained a trace of contamination. The molecular weight of the agglutinogen was calculated to be 10,000, but this value may be slightly higher for pure agglutinogen because of the trace of contamination with a high molecular weight substance, as suggested by ultracentrifugal analysis.
Zenyoji (1952) reported that Smolens-Mudd's agglutinogen showed a single peak in electrophoresis, had a nitrogen content of 10.6% and a phosphorus content of 1.22%, showed an ultraviolet absorption spectrum maximum at 264 μm, and gave a positive orcinol-HCl test. He concluded from these results that the chemical entity of Smolens-Mudd's agglutinogen might be a ribonucleoprotein. But in our work, the nitrogen content of the purified agglutinogen was 14.76%, the phosphorus content was negligible, the absorption maximum was at 275 μm, the ratio of absorption at 275 to 260 μm was 1.35, and the orcinol-HCl test for purified agglutinogen was negative at a concentration of 0.4%. These results indicate that the agglutinogen of B. pertussis in phase I should be a simple protein without ribonucleic acid.

The increased activity of the agglutinogen purified by DEAE-cellulose column chromatography was about 30-fold compared with crude extract.

The purified agglutinogen produced a specific skin reaction of definite induration and erythema in sensitized rabbits, but no induration in nonsensitized rabbits; the reaction reached its maximum at 20 to 24 hr after injection. It was also shown that the skin reactive activity of purified agglutinogen was more potent than picric acid-precipitated agglutinogen.

Thus, it can be concluded that agglutinogen is a proteinaceous antigen of relatively low molecular weight, with both agglutinin-absorbing and skin-reactive activities.

ACKNOWLEDGMENTS

The authors wish to thank S. Someya and his collaborators for assaying the agglutinin-absorbing activity of the agglutinogen, and K. Kakuchi for determining the molecular weight of the agglutinogen. The authors are indebted to Takeda Pharmaceutical Industries, Ltd., for supplying a culture of B. pertussis.

LITERATURE CITED


MILLER, J. J., JR., M. L. RYAN, AND E. HAVARD.


