

# THE ENHANCEMENT OF INFECTION WITH STAPHYLOCOCCUS AUREUS IN MICE SENSITIZED BY BOVINE SERUM-PERTUSSIS MIXTURES\*

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CLINICALLY it is well recognized that allergic patients are subject to frequent infections of the sinorespiratory apparatus.<sup>1-3</sup> Sinusitis and bronchitis, in particular, have been noted. Less frequently one encounters bronchiectasis and a variety of pneumonitis, sometimes with bronchopulmonary stenosis.<sup>4</sup> Particular vulnerability to the common cold is also observed, although it may be difficult at times to differentiate this type of infection from allergic rhinitis. Most often the enhanced infectibility of the allergic patient is explained on mechanical grounds—increased mucus production, bronchospasm and stasis, and possibly impairment or loss of ciliary function.

That sensitization, per se, may modify the defensive mechanisms of the host has not been previously reported. The following experiments were designed to investigate the possible role that a generalized type of sensitization of the anaphylactic type may have on the infectibility of the host (the mouse) to a standard type of infection with a known strain of staphylococcus.

## METHODS AND MATERIALS

*Animals.*—Two strains of albino mice were utilized in these experiments. One was available from Carworth Farms, New City, New York; the other was the Rockefeller-Swiss strain of the Rockefeller Institute. Female mice, aged 6 to 10 weeks, were selected, as this group was reported to be more susceptible to sensitization. All the animals employed in a given experiment were of the same age and were provided with the same diet and housing.

*Antigens.*—Bovine serum available in sterile ampule form (Difco) was employed as the antigen throughout the experiment. Rabbit serum and egg albumen were employed only as challenging antigens to determine specificity.

*Adjuvants.*—Pertussis vaccine was employed as an adjuvant to enhance sensitization to the bovine serum.<sup>5</sup> Two types were used. One, available for human prophylaxis, was supplied through the kindness of Dr. Stanton M. Hardy of the Lederle Laboratories, Pearl River, New York. This contained approximately 60 billion cells per milliliter. The other, used for experimental purposes only, contained approximately 1,000 billion cells per milliliter, and was made available through the courtesy of Dr. J. Munoz of Sharp & Dohme, Glenalden, Pennsylvania. With few exceptions, approximately 12 billion organisms were injected along with the serum.

*Injections.*—For sensitization, the antigen and adjuvant were given intraperitoneally at the same time. Infective and challenging doses were administered intravenously.

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*Bacterial Cultures.*—A coagulase-positive strain of *Staphylococcus aureus* (Smith) was employed. This strain is described elsewhere and its infectibility is well known and predictable in mice.<sup>6</sup> It was used as an 18-hour-old culture in beef heart infusion-peptone broth.

*Infectibility.*—This was determined by the viable organisms available from organs. The technique is described elsewhere.<sup>6</sup>

*Anaphylaxis.*—Anaphylaxis, when induced, was either of the mild form manifested by sluggishness, mild dyspnea, and cyanosis of the ears and tails, or was severe and fatal. The severe and fatal anaphylactic reactions usually occurred within thirty to sixty minutes and were manifested by aggravation of the above symptoms, in addition to which were noted wobbling gait, loss of sphincter control, convulsions, paralysis or cycling movements of the legs, and death. In a few instances anaphylactic death occurred several hours after the challenge. No autopsies were performed. The survivors, on recovery, appeared normal in all respects.

The first experiment was designed to (1) demonstrate the induction of anaphylaxis in the mouse by sensitization with beef serum along with pertussis vaccine; (2) demonstrate the specificity of the sensitization; and (3) determine a minimum challenging dose which would not kill the animal and which ultimately could be employed along with the infecting agent in the ensuing experiments. The results are recorded in Table I.

TABLE I. SENSITIZATION OF CARWORTH FARMS MICE TO BEEF SERUM

| DAY OF CHALLENGE FOLLOWING SENSITIZATION | CHALLENGING ANTIGEN (ML.) | SURVIVORS                                            |                    |
|------------------------------------------|---------------------------|------------------------------------------------------|--------------------|
|                                          |                           | SENSITIZED                                           | CONTROLS           |
|                                          |                           | 0.05 ML. PERTUSSIS (LEDERLE)<br>0.5 ML. BOVINE SERUM | 0.05 ML. PERTUSSIS |
| 14                                       | 0.2 Bovine serum          | 0/5                                                  | 5/5                |
|                                          | 0.1 Bovine serum          | 0/5                                                  | 5/5                |
|                                          | 0.05 Bovine serum         | 2/5                                                  | 5/5                |
|                                          | 0.02 Bovine serum         | 1/5                                                  | —                  |
|                                          | 0.005 Bovine serum        | 1/5                                                  | —                  |
|                                          | 0.001 Bovine serum        | 5/5                                                  | —                  |
| 19                                       | 0.001 Bovine serum        | 2/5                                                  | 5/5                |
|                                          | 0.001 Bovine serum        | 4/5                                                  | —                  |
|                                          | 0.0005 Bovine serum       | 5/5                                                  | —                  |
|                                          | 0.2 Rabbit serum          | 5/5                                                  | 5/5                |
|                                          | 0.01 Rabbit serum         | 5/5                                                  | 5/5                |
|                                          | 0.2 1% Egg albumen        | 5/5                                                  | —                  |

Eighty-five mice of the Carworth Farms strain were employed. Fifty-five of them were sensitized with the combined pertussis-serum mixture, while the remaining thirty serving as controls were given the pertussis vaccine only. It is apparent here (Table I) that sensitization is of a high order and that, with sufficient antigen, anaphylactic death can readily be induced. The serum, however, when employed in the order of 0.001 or 0.0005 ml. failed to produce death (with one exception) and this was the dose subsequently selected for administration along with the infecting agent. That the sensitization was specific was indicated when challenging doses of either rabbit serum or egg albumen failed to induce anaphylaxis.

In the second sensitization experiment, fifty-five female mice of the Rockefeller-Swiss strain were employed to note any variation of response due to difference in strain. In addition, for control purposes, serum alone without pertussis was employed and found to be sensitizing but to a lesser degree than the combined serum-pertussis vaccine. The results are recorded in Table II.

TABLE II. SENSITIZATION OF ROCKEFELLER-SWISS MICE TO BEEF SERUM

| DAY OF CHALLENGE FOLLOWING SENSITIZATION | CHALLENGING ANTIGEN (ML. BOVINE SERUM) | SURVIVORS                                            |                      |                                         |                   |
|------------------------------------------|----------------------------------------|------------------------------------------------------|----------------------|-----------------------------------------|-------------------|
|                                          |                                        | SENSITIZED                                           |                      | CONTROLS                                |                   |
|                                          |                                        | 0.05 ML. PERTUSSIS (LEDERLE)<br>0.5 ML. BOVINE SERUM | 0.5 ML. BOVINE SERUM | PERTUSSIS VACCINE (LEDERLE)<br>0.05 ML. | SALINE<br>0.5 ML. |
| 21                                       | 0.1                                    | 0/5                                                  | 2/5                  | 5/5                                     | 5/5               |
|                                          | 0.01                                   | 0/5                                                  | 3/5                  | 5/5                                     | 5/5               |
|                                          | 0.003                                  | 3/5                                                  | 4/5                  | —                                       | —                 |
|                                          | 0.001                                  | 2/5                                                  | —                    | —                                       | —                 |

For the third experiment, which was designed to investigate the role of prior sensitization upon infectibility, eighty female mice of the Rockefeller-Swiss strain, all 6 weeks old, were employed. These were divided into two equal groups: the first group was sensitized with the bovine serum-pertussis combination, while the second group received only pertussis vaccine. The infecting agent was the "Smith" strain of staphylococcus, previously employed in this laboratory, whose infectibility was well known in mice.<sup>6</sup> The staphylococcus was introduced intravenously simultaneously with the challenging dose of bovine serum (0.001 ml.) previously estimated to produce no anaphylactic death. The challenge and infection were introduced on the tenth, eighteenth, and twenty-third days following initial sensitization. Twenty-four hours later the animals were sacrificed; the organs were emulsified, and from the emulsion loopfuls were streaked on plates and incubated for eighteen to twenty-four hours. This procedure has been employed frequently in this laboratory and the approximate number of colonies available from the organs is known and predictable.<sup>6</sup>

The results of the experiment, as recorded in Table III, definitely indicate the enhancement of infectibility to the staphylococcus by prior sensitization. High colony counts were obtained from the kidney, which is in accord with previous observations,<sup>6, 7</sup> the highest, however, were from the kidneys of the sensitized mice. Furthermore, there is a correlation between degree of sensitization and degree of infectibility. The highest colony counts were noted after the third week following sensitization, when sensitization was at its highest.

In view of recent observations in this laboratory that pertussis vaccine has a protective effect against infection with a variety of agents,<sup>8</sup> it is possible that, with the use of a different adjuvant, the degree of infection might have been even higher. As little as 0.0006 ml. of the pertussis vaccine (Sharp & Dohme) was found to be protective.

The protective as well as the adjuvant action of pertussis vaccine is readily demonstrable in the fourth experiment, in which varying amounts of the vaccine were employed. As in the previous experiments, the sensitizing agent was beef serum. The pertussis vaccine used as the adjuvant was changed to a more concentrated one (Sharp & Dohme). All the challenges

TABLE III. THE EFFECT OF SENSITIZATION ON STAPHYLOCOCCAL INFECTION IN MICE

| CHALLENGING<br>AND INFECTIVE<br>DOSE                                    | DAY OF CHAL-<br>LENGE FOLLOW-<br>ING SENSI-<br>TIZATION | NUMBER OF COLONIES CULTURED FROM ORGANS* |                                                         |      |      |      |                    |     |     |     |
|-------------------------------------------------------------------------|---------------------------------------------------------|------------------------------------------|---------------------------------------------------------|------|------|------|--------------------|-----|-----|-----|
|                                                                         |                                                         | ORGAN                                    | SENSITIZED                                              |      |      |      | CONTROLS           |     |     |     |
|                                                                         |                                                         |                                          | 0.5 ML. BOVINE SERUM<br>0.05 ML. PERTUSSIS<br>(LEDERLE) |      |      |      | 0.05 ML. PERTUSSIS |     |     |     |
| 0.1 ml. staphy-<br>lococcus<br>("Smith")<br>0.001 ml. bo-<br>vine serum | 10                                                      | Kidney                                   | 4                                                       | 14   | 85   | 0.2  | 1                  | 12  |     |     |
|                                                                         |                                                         | Liver                                    | 53                                                      | 140  | 148  | 27   | 39                 | 103 |     |     |
|                                                                         |                                                         | Spleen                                   | 53                                                      | 87   | ?    | 12   | 14                 | 57  |     |     |
|                                                                         |                                                         | Lung                                     | 13                                                      | 22   | 31   | 0.2  | 2                  | 57  |     |     |
|                                                                         | 18                                                      | Kidney                                   | 8                                                       | 55   | 160  | 2900 | 0.1                | 1   | 5   | 35  |
|                                                                         |                                                         | Liver                                    | 24                                                      | 210  | 310  | 1100 | 5                  | 48  | 56  | 230 |
|                                                                         |                                                         | Spleen                                   | 2                                                       | 2    | 30   | 78   | 8                  | 12  | 76  | 101 |
|                                                                         |                                                         | Lung                                     | 3                                                       | 6    | 17   | 210  | 0                  | 0   | 0.4 | 2   |
|                                                                         | 23                                                      | Kidney                                   | 560                                                     | 3200 | 3500 |      | 25                 | 80  | 750 |     |
|                                                                         |                                                         | Liver                                    | 78                                                      | 170  | 300  |      | 12                 | 17  | 47  |     |
|                                                                         |                                                         | Spleen                                   | 30                                                      | 33   | 84   |      | 4                  | 5   | 80  |     |
|                                                                         |                                                         | Lung                                     | 0                                                       | 1    | 4    |      | 0.1                | 1   | 1   |     |

\*The figures given are to be multiplied by  $10^4$  for the total colonies available from the kidney, liver, and spleen, and by  $10^3$  for the lung.

were made on the twenty-sixth day following sensitization. Some deaths (anaphylactic) occurred within four hours after challenge, particularly in those animals treated with higher concentrations of pertussis vaccine. The low number of colonies available in this group from the kidneys and lungs of the infected mice that survived reflects the protective action of the pertussis vaccine (Table IV). Where pertussis was employed in lower concentrations,

TABLE IV. THE EFFECT OF PRIOR SENSITIZATION WITH BOVINE SERUM-PERTUSSIS VACCINE ON INFECTION OF MICE WITH STAPHYLOCOCCI

| SENSITIZATION            |                               | STAPHYLOCOCCUS INFECTION<br>(0.1 ML. "SMITH" STRAIN IN 0.001 ML. BOVINE SERUM) |                                   |     |     |      |     |         |     |      |           |
|--------------------------|-------------------------------|--------------------------------------------------------------------------------|-----------------------------------|-----|-----|------|-----|---------|-----|------|-----------|
| BOVINE<br>SERUM<br>(ML.) | PERTUSSIS<br>VACCINE<br>(ML.) | DEATH<br>WITHIN<br>4 HOURS                                                     | STAPHYLOCOCCUS COLONIES IN ORGANS |     |     |      |     |         |     |      |           |
|                          |                               |                                                                                | LUNGS                             |     |     |      |     | KIDNEYS |     |      |           |
| 0.5                      | 0.02                          | 3/5                                                                            | 4                                 | 10  |     |      |     | 2       | 12  |      |           |
|                          | 0.006                         | 0/5                                                                            | 10                                | 10  | 10  | 300  | 300 | 1       | 70  | 400  | 1100 3000 |
|                          | 0.0006                        | 1/5                                                                            | 100                               | 240 | 250 | 3000 |     | 5       | 100 | 1700 | 5000      |
|                          | 0                             | 0                                                                              | 30                                | 30  | 30  | 290  | 500 | 11      | 17  | 100  | 300 1200  |

Note: All challenges were made on the twenty-sixth day following sensitization.

fewer anaphylactic deaths occurred, and higher colony counts were obtained from both lungs and kidneys. In the absence of pertussis vaccine, no anaphylactic deaths occurred and the staphylococcus infection was less severe.

## DISCUSSION

The induction of hypersensitivity of the anaphylactic type in the mouse has been difficult in the past.<sup>9</sup> Compared with the guinea pig, sensitization has been sporadic and often variable with the strain employed. Utilizing large volumes of horse serum (4 c.c.), it has been possible to increase the number of mice succumbing to anaphylaxis. This method, introduced by Mayer and Brousseau<sup>10</sup> and subsequently employed by Nelson, Fox, and Freeman,<sup>11</sup> was criticized by Solotorovsky and Winsten<sup>21</sup> because of the profound change in blood volume induced in this fashion. These workers employed alum-precipitated antigen with excellent results. Since the introduction of pertussis vaccine as an adjuvant by Parfentjev and Goodline,<sup>13</sup> however, such sensitization is now readily achievable. Reports on this technique are now available from several laboratories.<sup>5, 14</sup> Utilizing this sensitizing tool along with bovine serum, it was possible to induce anaphylaxis in two strains of mice and to observe the effect of prior sensitization upon infectibility with the staphylococcus.

The role that pertussis inoculation plays in the induction of hypersensitivity when administered along with a specific antigen is not clearly understood. Originally it was thought that the capability of the vaccine to enhance the mouse's susceptibility to histamine was the important factor, assuming that histamine release was involved in anaphylaxis of the mouse.<sup>13</sup> This, however, does not seem to be the case, since the peak of histamine sensitivity is reached on the fourth day following inoculation with the vaccine, whereas hypersensitivity reaches its peak by the third week.<sup>15</sup>

The role that pertussis vaccine plays when injected into the mouse is a complex one.<sup>8</sup> It is an adjuvant, enhancing hypersensitivity to other antigens<sup>5</sup>; it produces simultaneously sensitivity to the nucleoproteins of the vaccine as well as immunity to infections with pertussis<sup>15</sup>; and apparently it modifies the host's resistance to infection with other agents.<sup>16</sup> In the case of gram-negative infections, Parfentjev demonstrated that prior vaccination with pertussis made mice vulnerable to infection with *Pasteurella* and *Bacillus proteus*<sup>16</sup> and to *Shigella* endotoxin.<sup>17</sup> Observations by Dubos and Schaedler,<sup>8</sup> on the other hand, show pertussis vaccination to exert a protective effect against a variety of organisms, as was demonstrable in these studies with staphylococci. Pertussis vaccination may enhance the susceptibility of mice to infection with influenza virus.<sup>17</sup>

Dubos and Schaedler<sup>8</sup> have shown that the action of pertussis is biphasic, that immediately following injection (up to five hours) infection is enhanced, but thereafter there is an increased resistance to infection lasting as long as six weeks.

Despite the protective role of the pertussis (originally employed only for its usefulness as an adjuvant), the experiments described herein indicate that prior sensitization of mice makes them vulnerable to a standard type of infection with staphylococci. These organisms were selected because standard

techniques were available and the degree of infectibility was predictable. It is possible that other infective agents may exert similar action.

The mechanism by which a general type of sensitization enhances infection is not known. In view of the intimate relationship between allergy and immunity (both are forms of antigen-antibody reactions), it is not surprising that one may affect the other.

Whether these experimental observations truly reflect clinical experience awaits further study.

#### CONCLUSIONS

Sensitization of two strains of mice by the simultaneous injection of bovine serum and pertussis vaccine made these mice more vulnerable to infection with staphylococci.

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