PHENOMENON OF LOCAL SKIN REACTIVITY TO BACTERIAL FILTRATES IN ITS RELATION TO BACTERIAL HYPERSENSITIVENESS

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PLATE 56

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Observations reported in previous communications (1–3) allowed differentiation of two distinct and separate phases of the phenomenon of local skin reactivity to bacterial filtrates: (a) the change in tissue elicited by the preparatory injection of potent bacterial filtrates whereby it becomes susceptible to certain toxic principles, and (b) the injurious effect of the principles upon the vulnerable tissue. The toxic principles are bacterial substances identical or closely related to true exotoxins; mixtures of animal proteins with homologous antisera; and mixtures of bacterial antigens, incapable of eliciting the phenomenon by themselves, with homologous antisera. The intradermal reinjection of antigen-antibody mixtures into an area prepared with a bacterial filtrate produces no effect. In order to obtain a severe reaction at the prepared skin site, it is necessary to inject intravenously either the antigen-antibody mixture or to inject intravenously the antigen and the antibody separately. Intravenous injection of the antigen alone is sufficient to elicit reactions at the prepared sites of rabbits possessing actively acquired antibodies. In the experiments described below it was deemed of interest to determine whether the reaction could also take place if either the antigen or the antibody was present in the blood stream at the time when antibody or antigen was injected directly into the area previously prepared with the bacterial filtrate.

EXPERIMENTAL

As seen from the experiments summarized in Table I, rabbits were sensitized by single intravenous injections of normal horse serum, in a dose of 1 cc. per kilo
### TABLE I

**Rabbits Sensitized with Horse Serum. Hemorrhagic Necrosis in Skin Sites Prepared with Bacterial Filtrate and Re injected with Horse Serum**

<table>
<thead>
<tr>
<th>No. of rabbits</th>
<th>Intravenous sensitizing injections</th>
<th>Skin-preparatory injections</th>
<th>Time between sensitizing and preparatory injections</th>
<th>Skin test injections</th>
<th>Time between preparatory and test injections</th>
<th>Results*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1 cc. horse serum</td>
<td>0.25 cc. <em>B. typhosus</em> Filtrate 1776 1:100 0.5 cc. histamine diluted</td>
<td>7 days 0.5 cc. horse serum 0.5 cc. horse serum</td>
<td>24 2/0 0/2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>“ “</td>
<td>“ “</td>
<td>“ “</td>
<td>“ “ 0/3 0/3</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>“ “</td>
<td>“ “</td>
<td>“ “</td>
<td>“ “ 0/3 3/0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>“ “</td>
<td>0.5 cc. horse serum</td>
<td>7 days 0.5 cc. <em>B. typhosus</em> Filtrate 1776 0.25 cc. horse serum</td>
<td>24 0/3 -</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>“ “</td>
<td>“ “</td>
<td>“ “</td>
<td>“ “ 0/3 0/3</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

± = doubtful reaction.

* The numerator indicates the number of skin sites showing severe hemorrhagic necrosis; the denominator the number of negative areas.
of body weight. 1 week later they received injections of *B. typhosus* "agar washings" filtrate into one to three areas of the skin of the abdomen (i.e., upper and lower right and upper left quadrants, respectively). 24 hours afterwards the same skin areas were re-injected with normal horse serum. From 4 to 5 hours later the rabbits showed severe hemorrhagic necrosis at the site of the intradermal injections. The lesions were identical with those observed in the phenomenon of local skin reactivity to bacterial filtrates. Histological changes were also similar to those previously described (Fig. 1).

**TABLE II**

*Precipitation Reactions with Sera of Rabbits Showing Hemorrhagic Necrosis upon Injection of Horse Serum into Vulnerable Skin Sites*

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Sensitizing injection per kilo of body weight</th>
<th>Time between sensitizing and test injections (days)</th>
<th>Preparatory injections</th>
<th>Test injection (hrs)</th>
<th>Time between preparatory and test injections (hrs)</th>
<th>Intensity of hemorrhagic necrosis</th>
<th>Precipitation tests with various horse serum dilutions*</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-57</td>
<td>Horse serum 1 cc.</td>
<td>7</td>
<td>Meningococcus Group III filtrate</td>
<td>Horse serum</td>
<td>24</td>
<td>Strongly positive</td>
<td>± 4 + 4 + 4 + 4 +</td>
</tr>
<tr>
<td>3-36</td>
<td>u u</td>
<td>7</td>
<td>u u u</td>
<td>u u u</td>
<td>24</td>
<td>u u u</td>
<td>1 + 1 + +</td>
</tr>
<tr>
<td>2-75</td>
<td>u u</td>
<td>7</td>
<td>u u u</td>
<td>u u u</td>
<td>24</td>
<td>u u u</td>
<td>4 + 4 + 4 + 4 + 1 +</td>
</tr>
<tr>
<td>4-80</td>
<td>u u</td>
<td>7</td>
<td>u u u</td>
<td>u u u</td>
<td>24</td>
<td>No reaction</td>
<td>± 4 + 4 + 1 +</td>
</tr>
<tr>
<td>1-07</td>
<td>u u</td>
<td>7</td>
<td>u u u</td>
<td>u u u</td>
<td>24</td>
<td>Strongly positive</td>
<td>2 + 4 + 4 + 4 + 4 + 1 +</td>
</tr>
</tbody>
</table>

Amount of precipitate is recorded by plusses. ± indicates a slight turbidity. – shows absence of precipitation.

* The rabbit serum was used undiluted.

As Table I shows, injection of normal horse serum into skin areas prepared 24 hours previously by injection of horse serum, histamine diluted 1:1000 and sterile meat infusion broth did not elicit any hemorrhagic necrosis. Moreover, the horse serum failed to elicit the reaction in areas prepared with bacterial filtrates devoid of skin-preparatory potency (i.e. *Streptococcus viridans* culture filtrate) (Figs. 2 and 3).

It was necessary to allow a definite incubation period for the pre-
<table>
<thead>
<tr>
<th>No. of rabbits used</th>
<th>First intradermal injection</th>
<th>Second intradermal injection</th>
<th>Time between intradermal injections</th>
<th>Dose and material of intravenous injections</th>
<th>Time of intravenous injections</th>
<th>Results*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper right quadrant</td>
<td>Lower right quadrant</td>
<td>Upper right quadrant</td>
<td>Lower right quadrant</td>
<td>hrs.</td>
<td>2 cc. Pneumococcus Type III filtrate</td>
</tr>
<tr>
<td>6 0.25 cc. meningococcus Group III Filtrate 1797</td>
<td>—</td>
<td>—</td>
<td>0.5 cc. Antipneumococcus Type III horse serum</td>
<td>—</td>
<td>24</td>
<td>—</td>
</tr>
<tr>
<td>3 “ “</td>
<td>—</td>
<td>—</td>
<td>0.5 cc. horse serum</td>
<td>—</td>
<td>24</td>
<td>“ “</td>
</tr>
<tr>
<td>3 “ “</td>
<td>—</td>
<td>—</td>
<td>0.5 cc. Antipneumococcus Type I horse serum</td>
<td>—</td>
<td>24</td>
<td>“ “</td>
</tr>
<tr>
<td>3 “ “</td>
<td>—</td>
<td>—</td>
<td>0.5 cc. Antipneumococcus Type II horse serum</td>
<td>—</td>
<td>24</td>
<td>“ “</td>
</tr>
<tr>
<td>3 “ “</td>
<td>—</td>
<td>—</td>
<td>0.5 cc. Pneumococcus Type I filtrate</td>
<td>—</td>
<td>24</td>
<td>“ “</td>
</tr>
<tr>
<td>3 “ “</td>
<td>—</td>
<td>—</td>
<td>0.5 cc. Antipneumococcus Type III horse serum</td>
<td>—</td>
<td>6</td>
<td>2 cc. Pneumococcus Type III horse serum</td>
</tr>
<tr>
<td>3 0.5 cc. Antipneumococcus Type III horse serum</td>
<td>—</td>
<td>—</td>
<td>0.25 cc. Meningococcus Group III Filtrate 1797</td>
<td>—</td>
<td>24</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>0.25 cc. meningococcus Group III Filtrate 1797</td>
<td>0.5 cc. rabbit serum</td>
<td>24</td>
<td>10 cc. horse serum</td>
<td>Simultaneously with 2nd intradermal injection</td>
<td>0/3</td>
</tr>
<tr>
<td>3</td>
<td>&quot; &quot;</td>
<td>0.25 cc. B. typhosus Filtrate 1792</td>
<td>0.5 cc. horse serum</td>
<td>24</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>3</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>24</td>
<td>5.1 cc. of mixture of 1 part of horse serum and 49 parts of anti-horse rabbit Serum S42</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>3</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>24</td>
<td>10 cc. horse serum</td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

| 3 | 0.5 cc. B. typhosus Filtrate 1792 | 0.5 cc. anti-human horse serum | 24 | 20 cc. horse serum | Simultaneously with intradermal injection | 3/0 | - |
| 3 | " " | " " | 0.5 cc. human serum | 24 | 2 cc. human serum | " " | " " | 3/0 |
| 3 | " " | 0.25 cc. B. typhosus Filtrate 1792 | 0.5 cc. human serum | 24 | 6 cc. anti-human horse Serum 486 | " " | " " | 0/3 |
| 3 | " " | " " | " " | 24 | 6 cc. anti-human horse Serum 486 diluted 1:10 | " " | " " | 0/3 |
| 3 | " " | " " | " " | 24 | 20 cc. anti-human horse Serum 486 | " " | " " | 3/0 |

* The numerator indicates the number of skin sites showing severe hemorrhagic necrosis; the denominator the number of negative areas.
paratory effect of a potent bacterial filtrate. The results were negative if the injections immediately followed each other or were carried out within 6 hours. The interval of time successfully employed was 24 hours. Intervals of time between 6 and 24 hours have not been studied as yet.

The sensitization of rabbits was accomplished by single intravenous injections 7 days prior to the tests. Shorter incubation periods were insufficient (i.e. 3 days).

The reaction observed was specific. Reinjection of prepared areas with sheep, guinea pig and human serum in rabbits sensitized by single injections of horse serum elicited no reactions.

Some of the rabbits were bled on the day of preparatory injections and the sera tested for precipitins against horse serum. The results are recorded in Table II.

As is seen from Table II, there was no apparent parallelism between the precipitin titer values of the various sera and the incidence of the reactions. These were easily obtained in most of the rabbits tested in the manner described. Occasionally rabbits failed to give the phenomenon. The percentage of negative animals has not been ascertained as yet.

The experiments recorded in Table III were carried out in order to determine whether the reaction could be passively transferred to normal rabbits.

The antibodies employed were anti-horse rabbit sera, anti-human horse sera and Antipneumococcus Type III horse sera. The antigens were normal rabbit and human sera and Pneumococcus Type III culture filtrate. The anti-horse and anti-human sera were made according to methods described previously (1, 3). The antipneumococcus sera were obtained from the New York Board of Health through the courtesy of Miss G. Cooper. The pneumococcus antigen was a filtrate of a culture in meat infusion broth pH 7.8 containing 0.3 per cent glucose and 0.3 per cent maltose. On repeated retests, this filtrate proved incapable of eliciting any reaction when injected intravenously, in a dose of 4 cc. per kilo of body weight, into rabbits prepared with the B. typhosus and meningococcus filtrates. In the experiments described here, a dose of only 2 cc. was employed.

One or two skin sites were each injected with 0.25 cc. of B. typhosus or meningococcus "agar washings" filtrate. 24 hours later the prepared skin sites were injected with the antibody-containing sera and simultaneously injected intravenously with the homologous antigens. 4 to 5 hours after the intravenous injection there appeared severe hemorrhagic necrosis in the injected skin sites.
In other experiments the antigens were injected into the skin and the antibodies intravenously. These experiments were positive provided a sufficiently large amount of antibody was injected intravenously. Thus, a dose of 6 cc. per kilo of body weight, of anti-human horse serum gave negative results, whilst 10 cc. gave strong reactions. Also a dose of 10 cc. per kilo of body weight of anti-horse rabbit serum was necessary for the elicitation of the reaction.

A number of experiments, some of which are recorded in Table III, clearly demonstrated the specificity of the passive transfer described. In the work with Pneumococcus Type III filtrate, there was observed serological type specificity as well.

No incubation period was required for the passive sensitization, inasmuch as the experiments were successful when the injections of the antibody and the antigen were made simultaneously. As in the case of actively sensitized rabbits (page 861) it was necessary to allow a definite incubation period for the preparatory effect of the bacterial filtrates.

**RESUMÉ AND DISCUSSION**

The observations reported in this paper demonstrated that antigen-antibody interaction in a tissue rendered vulnerable, induced severe hemorrhagic necrosis in this tissue, provided either the antigen or the antibody was present in the blood stream at the time of the interaction. The antigens employed were animal proteins (i.e. blood sera of various animals) in experiments with actively and passively sensitized rabbits; and Pneumococcus Type III culture filtrates in the experiments with passively sensitized rabbits. The pneumococcus filtrates used were incapable of inducing the phenomenon by themselves.

The antibodies were actively and passively acquired. Active sensitization was induced by a single intravenous injection of the animal protein 7 days prior to the local injections. Shorter periods were inadequate. In passive sensitization experiments a small amount of antibody was found to be sufficient if it was injected locally and the antigen was given intravenously. When the antigen was injected locally and the antibody intravenously, large doses of the latter were required. In precipitation reactions, as pointed out previously (3), there were required larger amounts of antigen than antibody. Thus, human serum when diluted 1:1000 precipitated with undiluted anti-
human horse serum employed in these experiments, whilst the latter
diluted 1:40 failed to precipitate with undiluted 1:10 human serum.
As shown by Opie (4), large amounts of antibody are required for pas-
sive transfer of the Arthus phenomenon. Inasmuch as, in addition,
the antibody disappears rapidly from the circulation, it is easily under-
stood why it was necessary to introduce a large amount of antibody in
the experiments described above.

In passively sensitized rabbits no incubation period was required
after the injection of the antibody. The experiments were consist-
ently successful if the antigen and the antibody were injected simul-
taneously in the manner described. If some time was allowed to
elapse between the injections, the outcome of the experiments was
negative.

The consensus of opinion is that passive transfer of bacterial hyper-
sensitiveness has not been definitely demonstrated as yet (Doerr,
Zinser (7)). Some claims are suggestive (Bail, Helmholtz, Zinser and
Mueller, Gay and Claypole, Meyer and Christiansen, Julianelle (8)) but,
as stated by Zinser (7), they do not possess the convincing regularity
of passive transfer of anaphylaxis. The experiments embodied in
this paper demonstrate that passive transfer of hypersensitiveness to
a bacterial antigen (i.e. pneumococcus) can be easily accomplished
provided the interaction of passively acquired antibodies with the
antigen takes place intravascularly at the site of a tissue of induced
vulnerability.

The specificity of the antigen-antibody interaction has been demon-
strated. There was observed serological group specificity in experi-
ments with pneumococcus filtrate.

The state of vulnerability was induced by certain bacterial filtrates,
the skin-preparatory potency of which was proved by titrations (5).
It could not be induced with horse serum, meat infusion broth and
streptococcus culture filtrates in rabbits sensitive to horse serum. In
order to obtain the vulnerable state, in addition to employing potent
bacterial filtrates, it was necessary to allow a definite period to elapse,
the optimum period being 24 hours, 6 hours being insufficient. Thus,
there was clearly demonstrated that the sine qua non of the phenome-
on is that a state of vulnerability be induced at the site of the anti-
gen-antibody interaction. This change elicited by bacterial filtrates
has been discussed at length in previous communications (1, 3). The skin-preparatory factors, (i.e. those inducing the vulnerability) are bacterial products found only in certain bacterial culture filtrates. They are soluble, obtained under conditions of insignificant cell autolysis and produce either only a slight primary inflammation or no inflammation. The inflammatory property of bacterial filtrates is totally unrelated to these factors.

The reaction is severe and obtained shortly after the introduction of antigen and antibody. The hemorrhagic necrosis is somewhat similar in histological features to the Arthus phenomenon (4, 6). However, in control experiments horse serum was injected into two skin sites of rabbits which were sensitized by a single intravenous injection of horse serum 1 week previously. One skin site was prepared by an injection of *B. typhosus* filtrate 24 hours previously. The other skin site was unprepared. The prepared skin site showed severe hemorrhagic necrosis whilst the unprepared area was only slightly inflamed (Figs. 1 and 2). Thus, the injection of the antigen into a skin site prepared with a bacterial filtrate in a rabbit which had received a single sensitizing injection induced promptly hemorrhagic necrosis. In the Arthus phenomenon necrosis appears upon the injection of antigen into a rabbit which has been sensitized by six or seven injections of the protein in the course of several weeks (6).

*Additional Comment*

As seen from data presented in this and previous papers, tissues made vulnerable by bacteria or their soluble products undergo severe injury when acted upon by toxic principles resulting from intravascular antigen-antibody interaction. The interaction can be obtained in one of the following ways:

By separate intravenous injections of the antigen and the antibody; by intravenous injection of the antigen into an animal possessing actively acquired homologous antibodies; by injection of the antigen into the vulnerable area with a simultaneous intravenous injection of the antibody; and by injection of the antigen into the vulnerable area in rabbits possessing actively acquired antibodies. In the latter case, there apparently occurs intravascular production of the toxic principles at the site of the locally injected antigen through the interaction with the circulating antibodies.
In the attempt to utilize the above facts for the explanation of focal and skin reactions of bacterial hypersensitiveness, it may be assumed that the infected foci being in a state of vulnerability throw off soluble bacterial products which induce the state also in the skin of the infected animal. Upon parenteral introduction of the specific antigen there would occur an intravascular interaction between the injected antigen and the antibodies acquired in the course of the infection, with the resulting formation of toxic principles. The toxic principles would elicit injury in infected tissues (i.e. focal reactions). In intradermal tests, the toxic principles produced through the interaction of the locally injected antigen and circulating antibodies would elicit a local reaction (i.e. bacterial skin hypersensitiveness). It might be expected that the severity of focal and local reactions would depend on the degree of vulnerability of the infected tissues and of the skin of the infected animal and also on the amount of toxic principles formed, which in turn would depend on the titer of actively acquired antibodies and amount of antigen injected.

It seems that the introduction of the notion of cell vulnerability as essential prerequisite for response to specific antigen-antibody interaction together with the demonstration of intravascular toxic principles resulting from the interaction, offer new possibilities for studies on bacterial hypersensitiveness.

SUMMARY

The observations reported in this paper demonstrate that the intravascular interaction of bacterial and animal protein antigens with homologous antibodies at the site of a tissue made vulnerable by bacterial filtrates induces prompt severe hemorrhagic necrosis in this tissue. In the light of these observations there is offered an explanation of the mechanism underlying focal and skin bacterial hypersensitiveness.

BIBLIOGRAPHY


EXPLANATION OF PLATE 56

FIG. 1. Section of a skin site injected with *B. typhosus* "agar washings" filtrate and 24 hours later with horse serum in a rabbit sensitized by a single intravenous injection of horse serum 1 week previously. Skin removed 5 hours after the second intradermal injection. Note necrobiosis, rupture of blood vessel wall and severe hemorrhage. Hematoxylin and eosin. × 160.

FIG. 2. Section of a skin site injected with streptococcus culture filtrate and 24 hours later with horse serum in a rabbit sensitized by a single intravenous injection of horse serum 1 week previously. Skin removed 5 hours after the second intradermal injection. Note slight inflammation. Hematoxylin and eosin. × 320.

FIG. 3. Section of a skin area injected with horse serum in a rabbit sensitized by a single intravenous injection of horse serum 1 week previously. Skin removed 5 hours after the intradermal injection of horse serum. Note slight inflammation. Hematoxylin and eosin. × 320.