PHENOMENON OF LOCAL SKIN REACTIVITY TO
BACTERIAL FILTRATES: FORMATION OF REACT-
ING FACTORS IN VIVO

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It has been reported in previous communications (1) that intra-
venous injections of mixtures of serum precipitinogens with precipitat-
ing antisera produced severe hemorrhagic necrosis in rabbit skin sites
prepared with bacterial filtrates. The reacting potency of the mix-
tures did not depend on the degree of turbidity formed. Also, clear
supernatant fluids obtained by centrifugalization of partially precipi-
tated sera elicited severe reactions. It was concluded from these and
other observations that the reacting potency of the mixtures was not
due to the mechanical effect of colloidal particles in the blood stream
but to some toxic factors liberated or formed in the sera through the
colloidal disturbance of the antigen-antibody interaction. The pur-
pose of the work embodied in the present paper was to determine
whether the reaction between the antigen and antibody induced in
vivo would bring about formation of reacting factors in the blood
stream.

Formation of Reacting Factors in Actively Sensitized Rabbits

In this part of the work rabbits were sensitized by single or repeated intravenous
injections of animal proteins. After various intervals of time they were prepared
by single intradermal injections of 0.25 cc. of undiluted bacterial “agar washings”
filtrate. The skin-preparatory potency of the filtrates employed was ascertained
in the usual manner prior to the experiments (2). 24 hours after the skin prepa-
ration the rabbits received single intravenous injections of the animal protein to
which they were previously sensitized (i.e., test injections). For repeated skin
preparations in retests with the same or various proteins, a different bacterial
filtrate was employed each time in order to avoid acquirement of specific active
immunity (3). They were B. typhosus, meningococcus Group III (44B strain),
meningococcus Group I (44 D strain) and B. coli “agar washings” filtrates, re-
### TABLE I
Formation of Reacting Factors in Sensitized Rabbits

<table>
<thead>
<tr>
<th>Sensitizing Antigen</th>
<th>Doses of sensitizing injections per kilo of body weight</th>
<th>Interval of time between sensitizing injections</th>
<th>Antigen and doses used for test injections per kilo of body weight</th>
<th>Result of test injections*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg albumin</td>
<td>5% solution†</td>
<td>5% solution</td>
<td>5% solution</td>
<td>—</td>
</tr>
<tr>
<td>Horse serum</td>
<td>1 cc.</td>
<td>undiluted</td>
<td>1.5 cc.</td>
<td>undiluted</td>
</tr>
<tr>
<td></td>
<td>Diluted 1:10</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Diluted 1:100</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Diluted 1:1,000</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Diluted 1:10,000</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Horse serum</td>
<td>Undiluted†</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>——</td>
<td>——</td>
<td>——</td>
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<td>&quot; &quot;</td>
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</tr>
<tr>
<td>&quot; &quot;</td>
<td>Diluted 1:100</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>Diluted 1:100</td>
<td>Diluted 1:100</td>
<td>1 7</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>Undiluted†</td>
<td>Undiluted†</td>
<td>—</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>—</td>
<td>—</td>
<td>1 1</td>
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<tr>
<td>&quot; &quot;</td>
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<td>—</td>
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<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*The numerator indicates the number of positive rabbits. The denominator indicates the number of negative rabbits. The sum of both indicates the total number of rabbits used.
†1 cc.
### TABLE I—Concluded

<table>
<thead>
<tr>
<th>Sensitizing Antigen</th>
<th>Doses of sensitizing injections per kilo of body weight</th>
<th>Interval of time between sensitizing injections</th>
<th>Antigens and doses used for test injections per kilo of body weight</th>
<th>Result of test injections*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
<td>4th</td>
</tr>
<tr>
<td>Horse serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“ “</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“ “</td>
<td></td>
<td></td>
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<tr>
<td>“ “</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Result of test injections: 0/2 = slight reaction; 2/1, 3/0, 0/3 = no reaction; 2/0 = reaction 2 days following 0 days for injection.
spective. These experiments are summarized in Table I. In this table and others of this paper, the expression positive rabbit means that there was obtained in the rabbit’s prepared skin site a severe hemorrhagic and necrotic lesion 4 to 5 hours after the test intravenous injection. The lesions which were intense in the majority of rabbits were characteristic of the phenomenon of local skin reactivity to bacterial filtrates (4). By negative rabbit is meant absence of skin reaction following the test intravenous injection.

As is seen from Table I, rabbits actively sensitized to animal proteins (i.e., blood sera and egg albumin) responded with formation of reacting factors in vivo upon intravenous reinjection of the same protein after an adequate incubation period.\(^1\)

The factors were apparently formed immediately after the test intravenous injection, inasmuch as the reactions in the prepared skin sites had developed to their fullest extent 4 to 5 hours later.

The state of active sensitization could be demonstrated in the manner described 7 to 8 days after a single intravenous injection and it persisted for longer periods of time (i.e., 12 days). Shorter incubation periods were inadequate (i.e., 2 days). The state could be also attained with diluted sera. A single injection of 1 cc. horse serum diluted 1:10 induced sensitization after 8 days. Higher dilutions of horse serum also elicited it but only upon repeated sensitizing injections and after longer incubation periods (i.e., dilution 1:100, 14 days\(^2\)). The human serum appeared to be of high antigenicity for rabbits because even 1 cc. of dilution 1:10,000 injected twice produced sensitization 8 days after the second sensitizing injection. The antigen for test injections could also be used in comparatively small amounts (i.e., 1 cc. horse serum diluted 1:100).

The experiments of Table I also demonstrated the specificity of the sensitization described. Rabbits sensitized to small amounts of horse serum, while showing reactions with the same serum, gave no reactions upon retests with human, guinea pig, rabbit, sheep and goat sera. Similarly, rabbits sensitized to human serum showed reactions

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\(^{1}\) The term sensitization employed in this paper is limited to the type described.

\(^{2}\) The term incubation period in the text and tables refers to the interval of time between the last sensitizing and the first test injection. When repeated sensitizing injections were made the true incubation period which was necessary, remained unknown.
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with human serum but failed to react to horse serum; and those sensitized to egg albumin did not react with horse serum. However, the specificity displayed by serum proteins of various animal species did not hold when repeated sensitization with large doses was extended over prolonged periods of time. Thus, rabbits sensitized by four intravenous injections of undiluted horse serum in doses of 1 to 4 cc., per kg of body weight, reacted with sheep serum after an incubation period of 27 days, although failing to respond to guinea pig serum. Sera of these rabbits obtained before the test injections were titrated for precipitins against sheep and guinea pig sera. They gave precipitation with sheep serum diluted 1:20 and no precipitation with guinea pig serum. It was shown, therefore, that the cross-reactions with heterologous sera were coincidental with cross-precipitations. In a recent paper, Hektoen and Cole offered an explanation for cross-precipitation obtained after employment of large doses of antigen (5).

Formation of Reacting Factors in Passively Sensitized Rabbits

The purpose of the work now to be taken up was to determine whether reacting factors would form in vivo in passively sensitized rabbits. Rabbits were prepared by a single intradermal injection of B. typhosus (TL) 'agar washings' filtrate and 28½ and 24 hours later injected intravenously, first, with the antigen, and secondly, with the antibody, respectively. Parallel experiments were done in which mixtures of the antigen with the antibody made in vivo were injected intravenously into prepared rabbits. The results are summarized in Table II.

As seen from Table II, two separate intravenous injections of precipitinogen-containing serum (i.e., normal human serum) and precipitating antiserum (i.e., anti-human horse serum) elicited severe hemorrhagic necrosis in skin sites prepared with bacterial filtrates. No reactions were obtained when each of the sera was injected alone in the same dose; when either the anti-human horse serum or the normal human serum was reinjected alone, the anti-human horse serum elicited severe hemorrhagic necrosis in skin sites prepared with bacterial filtrates. The results are therefore in agreement with the interaction of passively acquired antigen and antibody.
### TABLE II

**Formation of Reacting Factors in Passively Sensitized Rabbits**

<table>
<thead>
<tr>
<th>Intravenous Injections</th>
<th>Interval between skin and intravenous injections</th>
<th>Interval between 1st and 2nd intravenous injections</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 cc. horse Serum 416</td>
<td>—</td>
<td>24 hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 &quot; human &quot; I</td>
<td>—</td>
<td>24 hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 &quot; horse &quot; 416</td>
<td>1 cc. horse Serum 416</td>
<td>23½ hrs.</td>
<td>0/6</td>
</tr>
<tr>
<td>1 &quot; human &quot; I</td>
<td>1 &quot; human &quot; I</td>
<td>23½ hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 &quot; SL</td>
<td>1 &quot; horse &quot; 416</td>
<td>23½ hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 &quot; 918</td>
<td>1 &quot; 416</td>
<td>23½ hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 &quot; B</td>
<td>1 &quot; 416</td>
<td>23½ hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 &quot; Sc</td>
<td>1 &quot; 416</td>
<td>23½ hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 &quot; 416</td>
<td>1 &quot; human &quot; XIII</td>
<td>23½ hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 &quot; 416</td>
<td>1 &quot; human &quot; XIV</td>
<td>23½ hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 &quot; anti-human horse Serum 7/405</td>
<td>—</td>
<td>24 hrs.</td>
<td>3/0</td>
</tr>
<tr>
<td>Mixture of 1 part human serum—1 part anti-human horse Serum 7/405</td>
<td>1 cc. anti-human horse Serum 7/405</td>
<td>23½ hrs.</td>
<td>3/0</td>
</tr>
<tr>
<td>1 cc. human Serum I</td>
<td>1 cc. anti-human horse Serum 7/486</td>
<td>23½ hrs.</td>
<td>3/0</td>
</tr>
<tr>
<td>1 &quot; diluted 1:10</td>
<td>1 cc. anti-human horse Serum 7/501</td>
<td>23½ hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 cc. human Serum I</td>
<td>1 cc. anti-human horse Serum 7/515</td>
<td>23½ hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 &quot; I</td>
<td>1 cc. anti-human horse Serum 7/522</td>
<td>23½ hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 &quot; I</td>
<td>1 cc. anti-human horse Serum 7/527</td>
<td>23½ hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 &quot; I</td>
<td>1 cc. anti-human horse Serum 7/540</td>
<td>23½ hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 &quot; I</td>
<td>1 cc. anti-human horse Serum 7/486</td>
<td>15 min.</td>
<td>2½ hrs.</td>
</tr>
<tr>
<td>Human serum 2 cc. Group III Pneumococcus Type III culture filtrate 3 cc.</td>
<td>1 cc. 5% human red blood cells Group II</td>
<td>23½ hrs.</td>
<td>2/6</td>
</tr>
<tr>
<td>1 cc. Antipneumococcus Type III horse serum</td>
<td>—</td>
<td>24 hrs.</td>
<td>0/4</td>
</tr>
<tr>
<td>3 cc. Pneumococcus Type III culture filtrate</td>
<td>—</td>
<td>24 hrs.</td>
<td>0/3</td>
</tr>
<tr>
<td>1 cc. human Serum I</td>
<td>1 cc. anti-human horse Serum 7/486</td>
<td>24 hrs. prior to skin injection</td>
<td>24 hrs.</td>
</tr>
</tbody>
</table>

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Positive rabbits were obtained when the interval of time between the injections of serum precipitinogen and precipitating antiserum was \( \frac{1}{2} \) hour, and also, in one experiment, when it was 23\( \frac{3}{4} \) hours. A 48 hour interval gave negative results.

In the majority of experiments of Table II antigen was injected first and the antibody second. No reactions were elicited when the order of intravenous injections was reversed. Precipitation titrations with the serum precipitinogen and precipitating antiserum showed that there were required larger amounts of antibody than antigen for the precipitation. Thus, human serum diluted 1:1,000 precipitated with undiluted anti-human horse serum, whilst the antibody diluted 1:40 already failed to precipitate with undiluted and diluted 1:10 human serum. Inasmuch as unquestionably a part of the injected serum rapidly disappears from the blood stream, it is conceivable that in the latter experiment the reactions did not take place because an insufficient amount of antibody remained in the blood stream at the time of the subsequent injection of the antigen.

As is also seen from Table II, there were tested various bleedings of Horse 7. Since every bleeding did not give positive results it seemed of interest to determine their precipitation titers (Table III).

As is seen from a comparison of the respective results of Tables II and
III, no relationship could be established between the precipitin contents and the ability of the sera to induce formation of reacting factors upon combination with the precipitinogen in vivo. The results of Table II demonstrate in addition that the interaction of inactive Pneumococcus Type III culture filtrates with Anti-pneumococcus Type III horse serum induced formation of reacting factors in vivo. This observation is corroborated by previously reported results on the reacting potency of mixtures of the same filtrates and sera made in vitro (6).

There was obtained suggestive evidence that the factors could also be formed through the interaction of hemagglutinins with human red blood cells. The results are not sufficiently conclusive to warrant special consideration in this paper. Further work is in progress.

No clear-cut results could be obtained as yet concerning formation of reacting factors in vivo through the interaction either of homologous (i.e. rabbit) antiserum with heterologous serum or heterologous antiserum with homologous (i.e. rabbit) serum. Further experiments are also under way.

Effect of Reacting Factors Formed in Vivo upon Skin Sites Prepared by Non-Bacterial Substances

In order to emphasize the essential rôle of bacterial filtrates in the phenomenon under discussion the experiments embodied in Table IV were done.

As seen from Table IV, no reactions were obtained when rabbits were injected intradermally, either with the serum precipitinogen or the precipitating antiserum, and after various intervals of time reinjected intravenously with the precipitating antiserum in the case of the former, and with the serum precipitinogen in the case of the latter. Moreover, whilst mixtures of serum precipitinogen with precipitating antiserum injected intravenously elicited severe hemorrhagic necrosis in skin sites prepared with a potent bacterial filtrate, they had no effect upon sites prepared either with the precipitinogen or the precipitins. Also, an intravenous injection of horse serum into rabbits actively sensitized to the serum produced no effect either upon areas in which there was induced inflammation with turpentine, or in areas prepared with horse serum. As is seen from experiments
### TABLE IV
Effect of Reacting Factors Formed in Vivo upon Sites Prepared with Non-Bacterial Substances

<table>
<thead>
<tr>
<th>Preliminary treatment</th>
<th>Skin-preparatory injection*</th>
<th>Test intravenous injection per kilo of body weight</th>
<th>Interval between skin and intravenous injection</th>
<th>Results†</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Human serum</td>
<td>1 cc. anti-human horse Serum 4/405</td>
<td>1 hr.</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>4</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>6</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>24</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>2 cc. of mixture of 1 part of 4/405—1 part human serum diluted 1:4</td>
<td>24</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>Anti-human horse Serum 7/405</td>
<td>&quot;</td>
<td>24</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>B. typhosus culture filtrate</td>
<td>&quot;</td>
<td>24</td>
<td>3/0</td>
</tr>
<tr>
<td></td>
<td>Anti-human horse Serum 7/405</td>
<td>1 cc. human serum</td>
<td>24</td>
<td>0/3</td>
</tr>
<tr>
<td>1 cc. horse serum intravenously 1 wk. prior to skin injection</td>
<td>B. typhosus culture filtrate</td>
<td>1 &quot; horse &quot;</td>
<td>24</td>
<td>3/0</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>Turpentine diluted 1:5</td>
<td>24</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>Horse serum</td>
<td>24</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>25 B. typhosus reacting units</td>
<td>24</td>
<td>0/3</td>
</tr>
</tbody>
</table>

* 0.25 cc. of undiluted material was used for each intradermal injection.
† The numerator indicates the number of positive rabbits, the denominator the number of negative rabbits. The sum indicates the total number of rabbits tested.
‡ Abscesses, no hemorrhagic necrosis.

Of Tables I and IV, an intravenous injection of horse serum into sensitized rabbits invariably produced severe lesions at the sites prepared with a bacterial filtrate.

Thus, the experiments clearly showed that the antigen-antibody interaction elicited in the manner described, while producing no effect...
upon normal, sensitized and inflamed tissues, was responsible for severe injury to tissues prepared with bacterial filtrates.

Effect of Intradermal Reinjection of Antigen-Antibody Mixture upon Sites Prepared with Bacterial Filtrates

There remained the question whether the antigen-antibody interaction taking place outside the blood stream would also bring about a similar injurious effect upon skin sites prepared with bacterial filtrates. The following experiments were done.

Six rabbits each received a single intradermal injection of 0.25 cc. of undiluted B. typhosus (TTL) "agar washings" filtrate. 24 hours later the same areas were each reinjected with 0.5 cc. of a mixture consisting of one part of anti-human horse Serum 7/527 and one part of human serum diluted 1:4. The same mixture was also injected intravenously into three rabbits prepared by a single intradermal injection of the above B. typhosus filtrate 24 hours before. The rabbits reinjected intradermally showed no hemorrhagic necrosis during the following 48 hours of observation. The rabbits reinjected intravenously had severe hemorrhagic and necrotic lesions at the prepared skin sites 4 to 5 hours later.

These experiments demonstrate that the sine qua non of the phenomenon is that the antigen-antibody mixture be brought into contact with the prepared skin areas via the blood stream.

DISCUSSION AND CONCLUSIONS

The data presented in this paper offer a new method for the demonstration of antigen-antibody interaction. It is easily carried out, as follows:

A rabbit sensitized to some animal protein receives an intradermal injection of 0.25 cc. of undiluted bacterial filtrate of ascertained skin-preparatory potency. 24 hours after the skin preparation the rabbit is injected intravenously with the same animal protein. From 4 to 5 hours later there appears severe hemorrhagic necrosis at the prepared skin site.

Inasmuch as the necessary state of sensitization can be obtained by a single intravenous injection of a protein and a 1 week incubation period is sufficient, the method offers the advantages of speed and simplicity. The readings are reliable and clear-cut since the incidence of positive rabbits is high (i.e. with some proteins about 85 per cent
of animals tested after a single sensitizing injection) and the severe hemorrhagic necrosis makes the reaction unmistakable. The test is highly sensitive and strictly specific, unless there are made repeated sensitizing injections of large doses of the antigen. Anaphylactic shock in rabbits is difficult to elicit, and the test injections of the proteins do not have lethal effect upon these animals.

It is also possible to elicit severe reaction in the prepared skin site of non-sensitized rabbits upon separate intravenous injections of antigen and antibody (i.e., passive transfer).

The test described is clearly differentiated from the Arthus phenomenon by the following important features.

(1) A single sensitizing injection is sufficient. (2) The reaction can be obtained in rabbits which are not ready as yet for the Arthus phenomenon. (3) Skin preparation with a bacterial filtrate of ascertained skin-preparatory potency is essential. (4) The reaction is limited to the prepared skin site. (5) The antigen-antibody interaction has to take place in the blood stream.

It has been previously shown (1) that similar reactions can be obtained in prepared rabbits by an intravenous injection of antigen-antibody mixture made in vitro. Considerable evidence has been accumulated to prove that the injury was not due to the mechanical effect of the precipitate in the blood stream but to a toxic principle formed through the antigen-antibody interaction. In this paper there is presented additional proof of the fact, inasmuch as the precipitation titer bears no relation to the resulting toxic effect of separately injected serum precipitinogens and precipitating antisera.

The part of the phenomenon under consideration in this paper deals with toxic factors formed in vivo through antigen-antibody interaction. These factors are capable of inducing via the blood stream severe injury in tissues prepared by potent bacterial filtrates. As was pointed out in previous papers (7) and also shown again in this paper, the preparatory injection of a bacterial filtrate brings about a transient state of vulnerability in the animal cell. Vulnerability may or may not be associated with active inflammation. A great number of observations have afforded unquestionable proof that the primary

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3 The generalization to the animal cell is justified because the state of vulnerability was demonstrated in animals of various species and in various organs and tissues (11).
injection may induce vulnerability in spite of complete absence of primary erythema and may fail to elicit it in the face of active inflammation produced either by certain inactive bacterial filtrates or by non-bacterial inflammatory substances (i.e. broth, turpentine, gum tragacanth, egg albumin, horse serum, blood plasma, India ink, agar, gelatine, sodium arsenate, heparin, histamine, spirits of nitroglycerine, eserine hydrochloride, trypan blue, adrenaline, pituitrin, silicic acid, charcoal, infusorial earth, paraffin oil and caseine). Recently Opie (8), Menkin (9) and Cannon and Pacheco (10) emphasized fixation of colloidal particles in the blood stream by inflamed tissues. If one should grant that some sort of undetectable inflammation is invariably elicited by skin injection of a bacterial filtrate, then it is easily understood why the reacting factors formed or introduced into the blood stream localize in the prepared area. However, this alone does not explain why a severe reaction takes place only in areas prepared with bacterial filtrates of ascertained skin-preparatory potency and fails to occur in other inflamed areas. For these reasons, it must be concluded beyond any doubt that the bacterial filtrates described (i.e. those of high skin-preparatory potency) possess in addition the ability of inducing a certain state of vulnerability. Thus, the vulnerable cell becomes a reagent for the detection of apparently widely separated groups of toxic principles, all with one characteristic feature in common; i.e., the production of injury via the blood stream. These principles are as follows:

1. **Neutralizable and Antigenic Exotoxic Substances.**—In previous communications it has been demonstrated that bacterial substances identical or closely related to true exotoxins are capable of eliciting severe injury in the vulnerable cell. The observations lead to the demonstration of a new category of antigenic and neutralizable exotoxins with a distinct difference between them and true exotoxins in the mechanism of the effect. The classical toxins are capable of primary local injury, whilst those under discussion inflict injury via the blood stream on vulnerable tissues. It has been previously assumed (13) that these toxins act as agents which produce a disturbance in the colloidal state of the blood. As a result, certain toxic factors are formed in vivo which attack the vulnerable cell.

2. **Formation of Reacting Factors Resulting from Intravenous Injection of Agar.**—It has been shown by Sickles (12) and corroborated by
the present author (1) that agar, whilst possessing no skin-preparatory
potency, when injected intravenously is capable of eliciting the
reaction in areas prepared with potent bacterial filtrates. Inasmuch
as the agar produces the reaction only via the blood stream, it may be
concluded that it also acts as an agent producing a disturbance in the
colloidal state of the blood, necessary for the formation of reacting
defactors injurious to the vulnerable cell. It has been demonstrated by
Bordet (13) that addition of agar to blood serum induces formation of
anaphylatoxins. It has been further observed by Novy and De Kruif
(14) that agar also forms anaphylatoxins in vivo. Thus, correlative
studies on anaphylatoxins and the phenomenon under discussion
suggest themselves.

3. Formation of Reacting Factors Resulting from in Vivo Interaction
of Non-Bacterial Antigens with Antibodies.—As described in this paper,
the antigen-antibody combination in vivo brings about formation of
toxic factors which are detected by the vulnerable cell. It also re-
mains to determine whether the toxic principles formed by the antigen-
antibody combination of anaphylaxis and Arthus phenomenon are
similar to the reacting factors of the phenomenon under discussion.
It is conceivable that identical toxic factors may produce different
effects in various tissues, the vulnerable cell of this phenomenon re-
sponding with hemorrhagic necrosis.

4. Formation of Reacting Factors Resulting from in Vivo Interaction
of Bacterial Antigens with Antibodies.—It has been previously reported
that mixtures of inactive pneumococcus filtrates with homologous
antisera are capable of eliciting severe reactions in skin sites with
induced vulnerability. In this paper it has been shown that separate
(½ hour apart) intravenous injections of the inactive filtrate and the
serum lead to formation of reacting factors in vivo with the resulting
severe injury to the vulnerable skin area. These observations are
interesting from the point of view of immunopathology. The state
of vulnerability can be induced not only by bacterial filtrates but by
active infections as well (15). For this reason it is admissible to
assume that the reacting factors formed through the interaction of the
antigen secreted by the diseased focus and the actively acquired anti-
bodies may produce, via the blood stream, severe injury in vulnerable
tissues and organs. This mechanism of production of injury offers
a new working hypothesis for problems concerning relapse of infections, complications, metastatic lesions and generalized spread of disease. There is also suggested the possibility that focal reactions of tuberculin hypersensitiveness may be based on this mechanism. Tuberculin combined with tuberculous antibodies in vivo may bring about the formation of reacting factors. These factors would induce severe injury in tuberculous foci, inasmuch as the latter were shown to possess the state of vulnerability necessary for the phenomenon (16).

Bordet observed that reacting factors of heterologous bacterial filtrates were capable of reproducing the phenomenon under discussion at the site of a tuberculous lesion. He called this non-specific allergy. In view of the observations reported here one arrives at an explanation of specific and non-specific allergic manifestations in tuberculous lesions in which both may be an expression of the same mechanism. Thus, in terms of the phenomenon, active infections elicit a state of vulnerability in the diseased tissue. Severe injury then can be induced in this tissue by means of potent heterologous bacterial filtrates, since it has been shown (7) that preparatory and reacting factors of unrelated bacterial species can substitute for each other, provided they are potent (i.e., non-specific allergy). Also injury can be produced in infected tissues by reacting factors formed through the interaction of the injected specific antigen with the actively acquired antibodies of the infected animals (i.e., specific allergy or bacterial hypersensitiveness).

Inasmuch as the interaction of animal protein with the homologous antibody forms reacting factors injurious to tissues made vulnerable by bacterial filtrates or infections, there remains the question of the influence of serum sickness upon the course of infectious diseases.

To recapitulate, the essential mechanism of the phenomenon of local skin reactivity to bacterial filtrates consists in the alteration of tissues by bacterial filtrates or infections whereby they become vulnerable to a variety of toxic principles which act via the blood stream. These toxic principles are as follows:

Neutralizable and antigenic exotoxic substances (true toxins?); toxic factors formed through a colloidal disturbance in the blood stream (anaphylatoxins?); toxic factors resulting from the in vivo interaction of non-bacterial antigens with homologous antibodies (true
anaphylaxis?) and toxic factors resulting from the \textit{in vivo} interaction of bacterial antigens with specific antibodies (bacterial hypersensitivity?).

Thus the phenomenon of local skin reactivity to bacterial filtrates must be considered in various fields of immunology. It offers interesting possibilities for correlative studies.

**SUMMARY**

In this paper there is described a new method for the demonstration of antigen-antibody combination, as follows:

A rabbit sensitized a week previously to some animal protein receives a skin-preparatory injection of a potent bacterial filtrate. 24 hours after the skin preparation the rabbit is injected intravenously with the same animal protein. From 4 to 5 hours later there appears severe hemorrhagic necrosis at the prepared skin site.

The incidence of positive results is high. A single sensitizing injection and 1 week incubation period are sufficient. The test is highly specific unless repeated sensitizing injections of large doses of antigen are made. The necessary sensitization can be elicited with minute quantities of animal proteins.

It is also possible to elicit severe reactions in the prepared skin sites of non-sensitized rabbits upon separate intravenous injections of non-bacterial and bacterial antigens and homologous antibodies (\textit{i.e.}, passive transfer).

The relation of the phenomenon of local skin reactivity to bacterial filtrates to various fields of immunology is discussed in the light of the above observations.

**BIBLIOGRAPHY**