

SPONTANEOUS AND ACQUIRED ACTIVE IMMUNITY TO
THE PHENOMENON OF LOCAL SKIN REACTIVITY TO
BACTERIAL FILTRATES

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Studies reported thus far point to the fact that the toxic substances necessary for the phenomenon of local skin reactivity to bacterial filtrates are closely related in many respects to true exotoxins. The main evidence lies in the antigenicity and specific serum neutralizability in multiple proportions *in vitro* and *in vivo* of these factors (1). In this paper there are presented data concerning active immunity to the toxins under consideration and also additional experiments on their relation to the endotoxins.

EXPERIMENTAL

The various bacterial toxic substances employed were "agar washings" filtrates (2). The rabbits were tested and immunized with measured quantities of these filtrates, the titration of which has been previously described in detail (3).

Spontaneous Immunity to the Phenomenon of Local Skin Reactivity to Bacterial Filtrates

It was reported early in these investigations that a certain percentage of rabbits proved refractory to the phenomenon even when tested with large doses of reacting factors. The spontaneous resistance varied, however, with the microorganism employed. Thus, *B. typhosus* filtrates elicited reactions in not more than 85 per cent, while meningococcus filtrates gave positive results in nearly 100 per cent of the rabbits. Further experiments were made in order to determine whether there existed a spontaneous specific immunity to the phenomenon under discussion, as follows:—

Rabbits 8-30 and 8-01.—May 26, 1931, these rabbits were injected intradermally with 0.25 cc. of *B. coli* S rough Filtrate 1042 and May 27 intravenously with 200 reacting units of the same filtrate. There were no reactions obtained. May 28 these rabbits were prepared by single intradermal injections of 0.25 cc. of meningococcus 44 B Filtrate A-38 and 24 hours later injected intravenously with 200 reacting units of this filtrate. There appeared severe reactions.

Rabbits 8-67, 8-55 and 8-73.—These rabbits were tested in a similar manner with three filtrates, namely, *B. coli* S rough Filtrate 1042, meningococcus 44 B Filtrate A-38 and *B. typhosus* rough Filtrate 1067 during 6 consecutive days. The intravenous doses were 200 reacting units of each filtrate, respectively. The intravenous injections elicited no reactions in Rabbit 8-67. Rabbits 8-73 and 8-55 showed no reactions to *B. coli* and meningococcus, but developed severe reactions following the intravenous injection of *B. typhosus* rough filtrate.

These results demonstrate spontaneous active immunity as concerns the phenomenon. In one instance, the immunity was of a non-specific nature. In other instances, it appeared to be specific, inasmuch as the rabbits retested at short intervals of time with various filtrates, proved susceptible to one or two filtrates and resistant to the remaining ones. The following group of experiments brings further evidence in favor of the thesis.

Rabbits 6-00 to 6-10.—These rabbits were prepared by three simultaneous intradermal injections of *B. typhosus* T_L Filtrate A-36, *B. coli* S rough Filtrate 1042 and meningococcus 44 B Filtrate A-38. 24 hours later each rabbit received 400 reacting units of A-36¹ intravenously, per kilo of body weight.² Rabbits 6-00 to 6-03 showed reactions in three areas, Rabbit 6-04 had no reaction in "typhoid" area but was positive in "*coli*" and "meningococcus" areas. Rabbit 6-05 was positive in the typhoid and *coli* areas but showed no reaction in the meningococcus area. Rabbits 6-06 to 6-09 were negative in typhoid and *coli* areas but positive in the meningococcus areas; and Rabbit 6-10 showed no reaction in three areas.

As can be seen, rabbits prepared by simultaneous injections of *B. typhosus*, *B. coli* and meningococcus filtrates and injected intrave-

¹ It has been shown previously (4) that the skin-preparatory and reacting factors of various biologically and serologically unrelated microorganisms are able to substitute for each other, provided they have the power of eliciting the phenomenon for themselves.

² Inasmuch as there exists a reciprocal quantitative relationship between the skin and intravenous doses (2), large intravenous doses were necessary in order to obtain uniformly severe reactions when several areas of the skin were prepared. The respective doses were previously determined on a group of 6 rabbits for each filtrate employed.

nously with *B. typhosus* filtrates showed reactions in all areas in some instances, and only in one or two areas in others. The results again can be interpreted as spontaneous specific immunity as concerns the phenomenon.

The rabbits which showed no reactions in the typhoid areas, but gave positive ones in the remaining areas, apparently had cellular immunity of sufficient potency to prevent the occurrence of the state of reactivity to *B. typhosus* but had humoral immunity of insufficient potency to neutralize *B. typhosus* factors in the blood stream. The non-neutralized factors were then capable of producing injury in areas prepared with heterologous filtrates against which these rabbits had no cellular immunity.

Rabbit 6-10 which showed no reactions in three areas tested either was non-specifically resistant as concerns the phenomenon or possessed a high titer of antityphoid neutralizing antibodies in the blood stream. The rabbit was not retested with other bacterial filtrates.

*Active Acquired Immunity to the Phenomenon of Local Skin
Reactivity to Bacterial Filtrates*

Inasmuch as there apparently exists in rabbits a natural specific immunity to the toxic substances necessary for the phenomenon, it was of interest to determine whether immunity could be also actively acquired. In the experiments described below the rabbits were immunized by one or two intradermal injections of bacterial filtrates as follows:

Group of 14 Rabbits.—These rabbits each received a single intradermal injection of 0.25 cc. of undiluted *B. typhosus* T_L Filtrate A-36. 7 days later they each received again one intradermal injection of the same amount of the filtrate and 24 hours later intravenously 50 reacting units of the filtrate. 6 rabbits showed reactions and 8 rabbits remained negative (*i.e.* 43 per cent gave positive results). There were no deaths. Several control groups showed positive reactions in 80 per cent of the surviving rabbits and 50 per cent mortality following the intravenous injection of 50 reacting units.

Group of 24 Rabbits.—The rabbits of this group received each three single intradermal injections of 0.25 cc. of undiluted A-36 filtrate at 7 day intervals. 24 hours after the third intradermal injection they each received intravenously 200 reacting units of A-36, per kilo of body weight. One rabbit died, 15 rabbits were negative and 8 rabbits showed severe reactions (*i.e.* 4.2 per cent mortality and 33.3 per cent positive reactions).

With these doses, the control groups invariably showed about 85 per cent positive reactions and about 50 per cent mortality (2).

As is seen from these experiments, intradermal vaccination with *B. typhosus* culture filtrates induces active immunity to the phenomenon of local skin reactivity to *B. typhosus* and also confers resistance to the lethal effect of the toxic substances.

In further experiments there was tested the specificity of active acquired immunity with graded amounts of bacterial filtrates. As will be seen from the protocols, the rabbits were tested first with 1 unit of reacting factors. According to their response to this injection, they could be grouped as I—sensitive animals, *i.e.* showing reaction with 1 reacting unit; and II—refractory animals, *i.e.* showing no reaction with 1 reacting unit. The latter group could be further subdivided into II *a*—those which had only partial immunity, inasmuch as retests with 15 reacting units 1 week later produced reactions in them; and II *b*—those which did not show any reactions when later retested with larger doses of reacting units.

The following protocols represent experiments on rabbits of Group I.

Rabbit 7-38.—Mar. 18, 25, Apr. 2, 23 and 30 the rabbit received single intradermal injections of 0.25 cc. of undiluted *B. typhosus* T_L Filtrate A-53. Mar. 19, 26, Apr. 3, 24 and May 1, it received intravenously 1, 15, 30, 60 and 100 reacting units, respectively, of the same filtrate, per kilo of body weight. The intravenous injections of Mar. 19 and 26 were followed by severe reactions. The injection of Apr. 3 elicited only a doubtful reaction and those of Apr. 24 and May 1 gave no reactions. May 6 and 14 the rabbit received single intradermal injections of meningococcus 44 B Filtrate 1596 and May 7 and 15 intravenously 30 and 75 reacting units, respectively, of the same filtrate, per kilo of body weight. Both intravenous injections elicited severe reactions.

Rabbit 8-48.—Mar. 4, 12, 19, 26, Apr. 2 and 22 the rabbit received single intradermal injections of *B. typhosus* A-53 filtrate. Mar. 5, 13, 20, 27, Apr. 3 and 23 it received intravenously 1, 15, 30, 75, 100 and 150 reacting units, respectively, of the same filtrate, per kilo of body weight. The first intravenous injection elicited a severe reaction. The remaining intravenous injections produced no reactions. Apr. 30, May 7 and 14 the rabbit was prepared by single intradermal injections of 0.25 cc. of meningococcus 44 B Filtrate 1596 and May 1, 8 and 15 it received intravenously 50, 75 and 100 reacting units, respectively, of the same filtrate, per kilo of body weight. The intravenous injection of May 1 elicited a severe reaction, that of May 8, a doubtful reaction and the injection of May 15 gave no reaction. May 20 the rabbit received intradermally 0.25 cc. of *B. coli* S Filtrate A-43 and

May 21 intravenously 75 reacting units of the same filtrate, per kilo of body weight. There was, then, obtained a severe reaction.

Rabbit 7-17.—Mar. 31, Apr. 7, 20, 28, May 4, 12, 20 and 26 the rabbit was injected intradermally with single doses of 0.25 cc. of *B. typhosus* Filtrate A-53 and Apr. 1, 8, 21, 29, May 5, 13, 21 and 27 intravenously with 1, 15, 30, 60, 100, 200, 300 and 350 reacting units, respectively, of the same filtrate, per kilo of body weight. The rabbit gave severe reactions with 1 and 15 reacting units, no reactions with 30, 60, 100 and 200 units and strong reactions with 300 and 350 reacting units.

Rabbit 2-92.—Apr. 7, 15, 21 and 29 the rabbit received intradermally 0.25 cc. of meningococcus 48179 filtrate and Apr. 8, 16, 22 and 30 intravenously 1, 15, 30 and 60 reacting units, respectively, of the same filtrate, per kilo of body weight. The rabbit showed severe reactions to each intravenous injection.

Rabbit 9-44.—The rabbit was treated similarly to Rabbit 2-92. The first two injections elicited severe reactions. The third injection gave no reaction. The fourth injection produced a strong reaction.

As will be seen, Rabbit 7-38 was sensitive to 1 and 15 *B. typhosus* reacting units. 2 weeks after the first injection there was obtained a doubtful immunity to 30 units and 3 and 4 weeks later the rabbit showed complete immunity to as many as 60 and 100 units. The immunity thus established was specific, inasmuch as retests with meningococcus filtrate elicited severe reactions. Rabbit 8-48 at first sensitive to 1 reacting unit of *B. typhosus* promptly became immune to these factors. Here again, the immunity was specific, since the rabbit remained sensitive to meningococcus reacting factors. In addition, it was possible to immunize it also to these factors within 2 weeks. The rabbit, thus immunized to reacting factors of two microorganisms, remained, however, sensitive to a third microorganism; *i.e.*, *B. coli*. Rabbit 7-17 illustrates an active acquired immunity to graded amounts of *B. typhosus* reacting factors. The immunity was incomplete since retests with 300 and 350 units elicited strong reactions. On the other hand, Rabbit 2-92 demonstrates failure to induce active immunity, and Rabbit 9-44 shows a partial acquired immunity during the course of immunization to 30 reacting units, with subsequent susceptibility to larger doses on retests in a week.

The experiments demonstrate that rabbits highly sensitive to the phenomenon, *i.e.* those reacting to 1 intravenous unit, may acquire an active specific immunity to it by combined intradermal and intravenous injections of toxic bacterial filtrates. As might be expected the

rabbits showed individual variations in their response to the process of immunization. Some of them failed to acquire a state of immunity, whilst others showed various grades of it. It becomes evident, therefore, that in order to demonstrate the existence of this immunity a sufficiently large group of animals and graded amounts of toxic material should be employed.

Experiments with Group II *a* are described below.

Rabbit 5-27.—Feb. 25, Mar. 5, 12 and 19 the rabbit was injected intradermally with 0.25 cc. of undiluted meningococcus Filtrate 48179 and Feb. 26, Mar. 6, 13 and 20 intravenously with 1, 15, 30 and 60 reacting units, respectively, of the same filtrate, per kilo of body weight. There were no reactions following intravenous injections of Feb. 26, Mar. 13 and 20. The intravenous injection of Mar. 6 produced a severe reaction.

Rabbit 6-78.—Feb. 25, Mar. 5, 12 and 19 the rabbit received single intradermal injections of 0.25 cc. of undiluted meningococcus Filtrate 48179 and Feb. 26, Mar. 6, 13 and 20 intravenously 1, 15, 30 and 60 reacting units, respectively, of the same filtrate, per kilo of body weight. The intravenous injections of Feb. 26, Mar. 13 and 20 gave no reactions. The intravenous injection of Mar. 6 elicited a severe reaction. Mar. 26 the rabbit received intradermally 0.25 cc. of undiluted *B. coli* S Filtrate A-43 and Mar. 27 intravenously 25 reacting units of the same filtrate, per kilo of body weight. No reaction resulted. Apr. 2, it was injected intradermally with 0.25 cc. of *B. typhosus* T_L Filtrate A-36 and Apr. 3 intravenously with 50 reacting units of A-36. There was no reaction obtained. Apr. 9 and 16 it received single intradermal reactions of 0.25 cc. of undiluted meningococcus 44 D filtrate, and Apr. 10 and 17 intravenously 50 and 100 reacting units, respectively, of the same filtrate, per kilo of body weight. No reactions appeared.

Rabbit 6-82.—Apr. 13, 21, 28 and May 4, the rabbit received single intradermal injections of meningococcus Filtrate 46508 and Apr. 14, 22, 29 and May 5, intravenously 1, 15, 30 and 60 reacting units, respectively, of the same filtrate, per kilo of body weight. May 11 the rabbit was prepared with 0.25 cc. of undiluted *B. typhosus* rough T_L filtrate and injected intravenously with 50 reacting units of the same filtrate, per kilo of body weight, the following day. May 20 it was prepared with *B. coli* S Filtrate A-43 and May 21 intravenously with 100 reacting units of the same filtrate, per kilo of body weight. The intravenous injection of Apr. 22, elicited a severe reaction. There were no reactions following the remaining intravenous injections.

Rabbit 4-21.—May 2, 8, 15 and 24 the rabbit received single intradermal injections of 0.25 cc. of undiluted meningococcus 44 D filtrate and May 3, 9, 16 and 25 intravenously 1, 15, 30, 75 and 100 reacting units, respectively, of the same filtrate, per kilo of body weight. The second intravenous injection (*i.e.* 15 reacting units) gave a strong reaction. The results of all the other injections were negative.

Rabbits 6 and 8-0.—Both rabbits were treated in the same manner, as follows:—

The single skin injections of 0.25 cc. of meningococcus Filtrate 49206 were made Apr. 2, 10, 17, 24 and May 1. The intravenous injections of 1, 15, 30, 60 and 100 reacting units, respectively, of the same filtrate, per kilo of body weight, were given 24 hours after each skin injection. The 2 subsequent weeks they were prepared with 0.25 cc. of undiluted *B. typhosus* Filtrate A-53 and 24 hours after each skin injection tested intravenously with 50 and 120 reacting units, respectively, of A-53, per kilo of body weight. The 2 following weeks the rabbits were similarly tested with 100 and 200 reacting units, respectively, of *B. coli* S Filtrate A-43. Rabbit 6 gave a positive reaction to 15 reacting units of the meningococcus filtrate and Rabbit 8-0 to 15 and 30 reacting units of this filtrate. The remaining injections produced no reactions.

Rabbits 6-44, 6-39 and 6-50.—Feb. 6, 13, 20, 27 and Mar. 6, the rabbits were each injected intradermally with 0.25 cc. of undiluted *B. typhosus* 159 filtrate. Feb. 7, 14, 21, 28 and Mar. 7, they were injected intravenously with 1, 15, 30, 60 and 100 reacting units, respectively, of the same filtrate, per kilo of body weight. Mar. 15 they were injected each intradermally with 0.25 cc. of undiluted meningococcus 44 D filtrate and 24 hours later intravenously with 25 reacting units of the same filtrate, per kilo of body weight. In these rabbits only injections of Feb. 14 elicited reactions, the remaining injections gave no reactions.

As will be seen the rabbits had a spontaneous partial immunity inasmuch as they showed no reactions with 1 reacting unit, but proved sensitive to 15 units of the same filtrate, when retested 1 week later. The sensitivity was followed by a prompt immunity which was of a non-specific nature. The animals gave no reactions to reinjections with larger amounts of the same filtrates as well as with large doses of other filtrates (*i.e.* *B. coli*, *B. typhosus*, *B. typhosus* rough and meningococcus of other serological groups).

The following protocols deal with Group II *b*.

Rabbits 9-74, 8-3, 2-78, 3-58, 3-54 and 4-94.—The rabbits received each single intradermal injections of *B. typhosus* T_L Filtrate A-36 on Mar. 5, 13, 20 and 27. On Mar. 6, 14, 21 and 28 they received intravenously 1, 15, 30 and 100 reacting units, respectively, of the same filtrate, per kilo of body weight. No reactions followed these intravenous injections. Apr. 3, they received each single intradermal injections of 0.25 cc. of undiluted meningococcus 44 D filtrate and Apr. 4 intravenously 25 reacting units of the same filtrate, per kilo of body weight. No reactions appeared. Apr. 10 they were each injected intradermally with 0.25 cc. of *B. coli* S Filtrate A-43 and Apr. 11 intravenously with 75 reacting units of the same filtrate, per kilo of body weight. There were no reactions obtained.

The results of these experiments are difficult to interpret. It is obvious, however, that these rabbits possessed a non-specific immunity as concerns the phenomenon under consideration. Inasmuch as none of the injections elicited reactions, in contrast to Group II *a*, it is impossible to determine whether the state of non-specific immunity was acquired or spontaneous.

Relation of Endotoxins to the Factors Necessary for the Phenomenon of Local Skin Reactivity to Bacterial Filtrates

Burnet (5) compared the phenomenon-producing potencies of "agar washings" filtrates and disintegrates made according to Besredka's method and found that the latter were stronger. However, his experiments cannot be considered conclusive for the following two reasons.

1. The disintegrates were prepared from unwashed suspensions of bacteria. It has been shown by the present author that the washings contain highly potent toxic principles (2).

2. There were not made quantitative titrations to the end-point in order to compare the relative potencies of the various preparations.

With these considerations in mind the following experiments were done.

1. Meningococcus Preparations.—Meningococcus Group I 44 D strain (Wadsworth) was inoculated into 1 per cent rabbit blood broth pH 7.4. After 22 hours of incubation the supernatant broth culture, free from red blood cells, served as the inoculum. On the surface of 0.7 per cent glucose veal infusion agar in Kolle flasks 4 cc. of the inoculum was poured. After 24 hours of incubation the growth of 10 Kolle flasks was each washed off with 4 cc. of 0.9 per cent NaCl solution containing 0.4 per cent phenol. The suspension was centrifuged three times, and resuspended each time in the same volume of 0.9 per cent NaCl solution containing 0.4 per cent phenol. Washings 1, 2 and 3 were the respective supernatant fluids obtained after each centrifugalization. The washed sediment was then suspended again in 40 cc. of 0.9 per cent NaCl solution and disintegrated by freezing in dry ice and thawing three consecutive times. The suspension of killed meningococcus was the meningococcus disintegrate employed in the experiments about to be described.

2. B. typhosus Preparations.—Three washings were obtained from *B. typhosus* cultures grown on 10 Kolle flasks, in a similar manner.³ The washed sediment, however, was mixed with 0.4 gm. of NaCl, which had previously been sterilized

³ The inoculum was a 24 hour old plain veal infusion broth pH 7.4 culture of *B. typhosus* T_L. The Kolle flasks contained plain veal infusion agar pH 7.4.

TABLE I
Comparative Potencies of Disintegrates and "Agar Washings" Filtrates

Preparation	Amount injected intravenously per kg./wt.	Total No. rabbits tested	Positive rabbits	Negative rabbits	Death
Meningococcus disintegrate	1 cc. undiluted material	3	1	2	0
" "	1 " diluted 1:2	3	0	3	0
" "	1 " " 1:10	3	0	3	0
" "	1 " " 1:25	3	0	3	0
" "	1 " " 1:100	3	0	3	0
" "	1 " " 1:250	3	0	3	0
" "	1 " " 1:600	3	0	3	0
" Washings 3	1 " " 1:10	3	2	1	0
" " 3	1 " " 1:25	3	0	2	1
" " 3	1 " " 1:100	3	0	3	0
" " 3	1 " " 1:250	3	0	3	0
" " 3	1 " " 1:600	3	0	3	0
" " 2	1 " " 1:25	3	2	1	0
" " 2	1 " " 1:100	3	1	2	0
" " 2	1 " " 1:250	3	1	2	0
" " 2	1 " " 1:600	3	0	3	0
" " 2	1 " " 1:1000	3	0	3	0
" " 1	1 " " 1:25	3	3	0	0
" " 1	1 " " 1:100	3	1	2	0
" " 1	1 " " 1:250	3	1	2	0
" " 1	1 " " 1:1000	3	1	2	0
" " 1	1 " " 1:1200	3	0	3	0
<i>B. typhosus</i> Besredka disintegrate	1 " undiluted	3	2	1	0
" " " "	1 " diluted 1:10	3	1	2	0
" " " "	1 " " 1:50	3	0	3	0
" " " "	1 " " 1:100	3	0	3	0
" " " "	1 " " 1:200	3	0	3	0
" " Washings 1	1 " " 1:10	3	3	0	0
" " " 1	1 " " 1:100	3	3	0	0
" " " 1	1 " " 1:200	3	1	2	0
" " " 1	1 " " 1:300	3	1	2	0
" " " 1	1 " " 1:500	3	0	3	0
" " " 2	1 " undiluted	3	1	2	0
" " " 2	1 " diluted 1:10	3	0	3	0
" " " 2	1 " " 1:25	3	0	3	0
" " " 2	1 " " 1:50	3	0	3	0
" " " 2	1 " " 1:100	3	0	3	0
" " " 3	1 " undiluted	3	0	3	0
" " " 3	1 " diluted 1:10	3	0	3	0
" " " 3	1 " " 1:25	3	0	3	0
" " " 3	1 " " 1:50	3	0	3	0

Washings 1, 2, 3—first, second and third washings of bacterial agar cultures.

in the autoclave at 250°F. for 15 minutes and dried over calcium chloride. The mixture was dried in a vacuum desiccator overnight, ground to a fine powder in an agate mortar and then suspended in 40 cc. of sterile distilled water, containing 0.4 per cent phenol. This was left overnight in the incubator and then centrifuged for 1 hour. The supernatant fluid was called "Besredka *B. typhosus* disintegrate" (6).

Rabbits were prepared by single intradermal injections of 0.25 cc. of undiluted material. Various dilutions of each of "agar washings" and disintegrates were used for intravenous injections 24 hours later. Each dilution was tested in a group of 3 rabbits.

As is evident from Table I, accurate determinations of the potencies of various preparations can be made only by titrations to the end-point, not by comparing the percentage of positive results obtained with given dilutions in small groups of rabbits. Thus, if one should compare, for instance, meningococcus Washings 1 and 2 in dilution 1:100 by the percentage of positive rabbits, both preparations would appear of the same strength. Titrations to the end-point show that one preparation is ten times stronger than the other. The fact is due to natural resistance shown by a certain percentage of rabbits to moderate doses of toxic substances, which, however is not displayed with large doses.

As is also seen from Table I, the concentration of toxic factors in the first washing of a given number of meningococcus cells is approximately 1000 times stronger than in the disintegrate of the same number of cells dissolved in the same volume of NaCl solution. With *B. typhosus*, the first washing is about 30 times stronger than the disintegrate prepared according to Besredka's method. Evidently, then, the major portion of the factors is obtained in the first washing and only an insignificant one is found in disintegrates and the further washings. The process of washing probably causes very little disintegration since in these experiments young bacterial cells on solid media are centrifuged as soon as they are suspended in NaCl solution and the supernatant fluid is separated from the sediment shortly after completion of centrifugalization. It can be concluded, therefore, that the toxic substances necessary for the phenomenon are extracellular.

DISCUSSION

On the basis of the observations reported in this paper, it becomes necessary to differentiate between active specific and non-specific

immunity to the phenomenon of local skin reactivity to bacterial filtrates. The active specific immunity can be induced by immunization of highly susceptible rabbits with the toxic material. The non-specific immunity is elicited in rabbits already showing partial spontaneous immunity. The question of active acquired immunity to the phenomenon under discussion has been studied by various authors. It seems of interest to discuss their findings in connection with the observations reported here.

Peck and Sobotka (7) reported that "the majority of rabbits receiving intradermal, interperitoneal or intravenous injections of moccasin snake venom became refractory to the development of the Shwartzman phenomenon. The refractory state was still present 44 days after the primary injection of snake venom. No circulating antibodies could be demonstrated to explain the refractory state. Antivenin had no effect on the course of the Shwartzman phenomenon." These findings of induced non-specific immunity are in line with those described in this paper. The question arises as to the mechanism of this immunity. Inasmuch as the *sine qua non* of the phenomenon is that the second injection be given *via* the blood stream, two explanations present themselves:—

One explanation is offered by Peck and Sobotka, as follows: "The Shwartzman phenomenon probably depends on the enhanced vulnerability of the capillaries, at the site of the primary injection to a subsequent intravenous injection of the bacterial filtrate. It is conceivable that by injections of a vascular poison such as snake venom a change is produced which raises the threshold for the elicitation of the phenomenon." Recently, however, certain facts were discovered which may lead to a different explanation:—

It was shown by Sickles (8) and the author (9) that agar which had no skin-preparatory effect, when injected intravenously elicited reactions at skin sites prepared with bacterial filtrates. It has also been shown by the present author (9) that whole blood sera have no reacting potency, whilst mixtures of precipitinogen containing sera with homologous precipitating antisera are of high reacting potency for areas prepared with bacterial filtrates. These observations, to be described in detail in a separate communication, demonstrate formation of reacting factors in blood serum through some disturbance in its col-

loidal state. In view of this, it is conceivable that the bacterial filtrates also have no primary reacting potency but that they act only as agents producing the disturbance in the colloidal state of the blood necessary for the formation of reacting factors in it. This hypothesis would explain the necessity of giving the second injection *via* the blood stream.

With these considerations in mind, it becomes possible to differentiate two defense mechanisms to the phenomenon of local skin reactivity to bacterial filtrates, one, depending on the specific antibody neutralization of the agents which induce the disturbance (*i.e.* bacterial filtrates), and another, depending on the presence in the blood stream of some factors which prevent the colloidal disturbance from taking place, or which prevent the formation of reacting factors in it. The first mechanism would bring about specific, and the second non-specific immunity to the phenomenon. Further experiments on this phase of the problem are under way.

Gratia and Linz (10) recently reported enhanced resistance to fatal doses of *B. anthracis* of rabbits in which the Shwartzman phenomenon had been previously elicited with *B. coli* toxins. These observations are of considerable interest in connection with a possible relationship between the above described non-specific immunity to the phenomenon and the non-specific resistance to bacterial infections. It would seem that the question of non-specific protein therapy should also receive consideration from this point of view.

Burnet (5) concluded from his studies that there was no evidence of a lasting active immunization against the phenomenon. According to him, repeated reactions could be elicited in rabbits without diminishing their reactivity. He recorded, however, experiments on only 4 rabbits and apparently used ungraded amounts of toxic material for testing their resistance. It had been shown in the present paper that rabbits display individual variations in their response to the process of active immunization. Some of them fail to acquire a state of immunity, whilst others show various grades of it. It becomes evident, therefore, that in order to demonstrate the existence of this immunity a considerable group of animals and graded amounts of toxic material should be employed. Moreover, in Burnet's experiments the immunizing material was Besredka's disintegrate which possibly is of lower

antigenicity than the "agar washings" employed in this work. The high antigenicity of the latter had been demonstrated by Ferry and Fisher (11) and Mishulow, Mowry and Scott (12).

The extracellular nature of the factors necessary for the phenomenon and their antigenicity, as demonstrated by elicitation of active specific acquired immunity, add further support to previously reported facts concerning the close similarity between these factors in bacterial filtrates, and true exotoxins (*i.e.* specific serum neutralization in multiple proportions *in vitro* and *in vivo*, various properties of the filtrates, etc. (1)). However, there remains a distinct difference between the two categories of bacterial substances in the mechanisms of their effects.

The classical exotoxins are capable of primary local injury, while those discussed here inflict injury *via* the blood stream on tissues of induced vulnerability.

CONCLUSIONS AND SUMMARY

Spontaneous active immunity to the phenomenon of local skin reactivity to bacterial filtrates has been demonstrated. In one experiment, the immunity was non-specific, while in others it appeared limited to one or two bacterial species.

Intradermal vaccination of rabbits with bacterial filtrates induced active immunity to the phenomenon. The specificity of this immunity was tested with graded amounts of toxic factors. The combined intradermal and intravenous immunization in these experiments elicited two types of response:—

1. Specific immunity, which was obtained in rabbits highly susceptible to the phenomenon (*i.e.*, showing reactions with 1 reacting unit).

2. Non-specific immunity which was elicited in rabbits with partial spontaneous immunity (*i.e.* showing no reactions with 1 reacting unit, but susceptible to 15 units 1 week later). Comparative studies on the reacting potency of "agar washings" and disintegrates (Besredka's method, freezing and thawing method) showed that the first washings of bacterial cells are considerably stronger than subsequent washings and disintegrates. These facts demonstrate once again the extracellular nature of the material necessary for the phenomenon.

Tentative explanations of the mechanisms involved in the specific and non-specific immunity to the phenomenon are discussed.

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