

STUDIES ON THE PATHOGENESIS OF STAPHYLOCOCCAL INFECTION*

I. THE EFFECT OF REPEATED SKIN INFECTIONS

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PLATE 40

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The extent to which acquired immunity participates in protecting against staphylococcal infection is not clear. Considerable evidence would suggest that antibody to the staphylococcus and its products plays only a secondary role in modifying the nature of the lesion produced by the organism, and that non-specific factors may be more important in determining host susceptibility (1). It has been shown, for example, that host susceptibility to staphylococcal infection may be markedly influenced by nutritional factors and other metabolic disturbances, as well as by toxemias and certain allergic reactions (2). However, the role of specific hypersensitivity to the staphylococcus and its products remains obscure. It has been stated that the understanding of acquired immunity and the utilization of this knowledge in the prevention and treatment of staphylococcus infections will probably await identification of the factors enabling the organism to establish infection and bring about damage to host tissues (2). To date, success in elucidating the pathogenesis of staphylococcal infection has been quite limited.

Following the identification by Ogston of the staphylococcus as the probable etiologic agent in a variety of abscesses (3), a number of workers turned to the investigation of immunological problems related to staphylococcal infection (4-7). It was soon evident that vaccination with the organism failed to provide protection against infection in the same manner as was the case with a number of other bacterial diseases, and attention was thereafter focused upon the question of antitoxic immunity which, by analogy with diphtheria, was felt to be a more important mechanism of host resistance. The characterization of diffusible toxins (in particular, the alpha toxin) by

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early workers (8, 9) seemed to furnish the orientation toward which immunization might successfully be directed. The importance of antibacterial immunity was minimized. Forssman, on the other hand, felt that antihemolysins were of little importance since his vaccinated "immune" animals did not develop significant antihemolysin titers (10). Others also failed to correlate immunity with antihemolysin levels (11), and assumed that some other toxin (such as the leukocidin) was the more important agent against which studies of immunity should be directed. A comparison of the relative importance of heat-killed vaccines and toxoid was carried out by Downie (12) who found that animals immunized with vaccine did not develop antitoxin titers and that skin lesions in such animals did not differ significantly from those in the control animals. In animals immunized with culture filtrates, antitoxin developed, and the skin lesions produced by live cocci in such animals appeared less extensive and showed more active phagocytosis of the bacteria. He concluded that in rabbits (which normally possess little or no detectable alpha hemolysin antitoxin) resistance to infection is increased by immunization which results in antitoxin production. He pointed out, however, that in man, staphylococcal infections more closely resemble the lesions in toxin-immunized animals since the majority of normal adult humans have at least low levels of circulating antitoxin. In chronic recurrent staphylococcal infections in man, therefore, other factors must be considered. Despite the fact that a number of observers had claimed that sufficiently high antitoxin levels produced immunity in rabbits (13), it remained apparent that the development of local metastatic lesions is not prevented by antitoxin, and actually the only consistent protection afforded toxin-immune animals was that of slightly longer survival. Indeed, many experimental results have obviously been influenced by the presence of free toxin in the challenging dose. Favorable results thus have indicated antitoxic immunity under the conditions of the experiment, but not necessarily any significant protection against natural infection.

The possible role of hypersensitivity in staphylococcal infection was suggested by Panton and Valentine who in 1929 studied the effect of repeated skin infections in rabbits (14). The animals were challenged at weekly intervals with a series of 4 tenfold dilutions of staphylococci and the nature of the resulting lesions observed. Interestingly, animals so challenged began to show a diminution in size of lesions produced by large doses but at the same time developed increased susceptibility to production of purulent lesions by doses 100 times less than the minimal infective dose for a normal rabbit. They concluded that the animals responded to repeated infection with "partial immunity" to a large dose and a lessened resistance to a small dose.

Cutaneous reactions to staphylococcal vaccines in man were studied by a number of workers. Coggi found that such reactions were not correlated with circulating antibody levels (15). Zironi believed that it was the allergic state which permitted staphylococcal infection to occur despite the presence of large amounts of circulating antitoxin (16). Some have felt that non-specific inflammation is of greater importance than specific immunologic reactions in the establishment of staphylococcal infection (1).

By the intracutaneous injection of rabbits with formalin-killed staphylococci, Boe (17), was able to elicit a state of "delayed" hypersensitivity since the skin reactivity induced was maximal at 24 hours, not influenced by the presence of immune sera,

and not passively transferable with serum. In such animals minimal doses of live organisms produced abscesses larger and more severe than in control rabbits. In addition, these animals could be "desensitized" by intravenous injection of culture filtrates. Similar results were obtained by Forney who injected guinea pigs with killed staphylococci plus acid-fast "wax" (18).

More recently the effect of the allergic state to a heterologous antigen upon susceptibility to staphylococcal infection was studied by Prigal and Dubos (19) who found that mice sensitized against bovine serum, when challenged concomitantly with staphylococci and bovine serum, had many more organisms present in the kidneys, liver, and lungs at 24 hours than did non-sensitized controls.

Finally, Johanson (20) showed that mild staphylococcal infection in rabbits produced both partial "immunity" as evidenced by increased bactericidal activity of leukocytes and increased clearing of injected staphylococci from blood and organs, as well as a state of cutaneous and systemic hypersensitivity which was associated with lowered resistance to staphylococcal reinfection. The state of delayed cutaneous hypersensitivity and lowered resistance could be transferred with living spleen or peritoneal exudate cells but not with killed cells or those from non-sensitized animals. This work gives considerable weight to the postulate that specific hypersensitivity to the staphylococcus is associated with lowered resistance to staphylococcal infection, possibly due to the tissue changes of inflammation and cell injury accompanying the allergic reaction.

The present study represents an attempt to investigate the effect of previous experience with staphylococcal infection upon the response of the host to subsequent challenge by the organism. It was hoped that information could be obtained concerning the relative importance of acquired antibody (both antitoxic and antibacterial) on the one hand and of hypersensitivity on the other, in the initiation and subsequent evolution of staphylococcal infection.

Subsequent reports will deal with the question of whether or not the effect of hypersensitivity is attributable simply to the inflammation which accompanies the allergic reaction, and consequently whether or not similar effect may be obtained by other inflammatory stimuli.

Materials and Methods

The strain of *Staphylococcus aureus* used in these experiments (Lafferty) was coagulase-positive, bacteriophage type 52/42B/80/81. It fermented mannitol, produced alpha hemolysin, and fibrinolysin. It was inhibited by 250 units of penicillin per ml. but not killed by 500 units per ml. It was also resistant to the tetracyclines but moderately sensitive to chloramphenicol and erythromycin.

A frozen stock culture was prepared after a single passage in broth, following isolation from a patient with septicemia. Prior to use in the experiments the organism was grown in trypticase soy broth for 18 hours.

By plate counts of appropriate dilutions, it was determined that the 18 hour culture quite consistently contained approximately 10^9 organisms per ml.

The virulence of the organism was assayed by determining the minimal infective dose for the skin, anterior chamber of the eye, and knee joint of the rabbit (Table I).

The anterior chamber of the rabbit eye was infected by inoculation of 0.1 ml. of diluted culture after withdrawal of an equal amount of aqueous humor. Control injections of broth or saline produced no detectable ocular lesions. The knee joint of the rabbit was infected by inoculation with 0.2 ml. of diluted bacterial culture. The severity of infection in the joint was estimated by measuring the intra-articular pressure with a strain gauge manometer (21). Injection of 10^9 organisms intraperitoneally was found to be lethal for mice.

Prior to injection into the skin the staphylococcus culture was either serially diluted in broth (1:10, 1:100, 1:1000) or centrifuged, washed with saline and diluted in 0.85 per cent NaCl in the same manner. Grown in this way the culture filtrate was quite low in alpha hemolysin (toxin) content and the results were essentially the same whether the cells were suspended in broth or washed and diluted in saline.

TABLE I
Staphylococcus Virulence in Normal Rabbits

Site of Inoculation	Strain	MID*	Nature of Lesion
Skin	52/42B/80/81	10^7	Pustule (abscess)
Joint (knee)	52/42B/80/81	10^4	Pyoarthrosis (limp, fever, swelling, limitation of motion, pus, pressure in joint)
Eye (anterior chamber)	52/42B/80/81	10^8 (600 organisms)	Hypopyon
	VA ₄ (penicillin-sensitive)	10^8 (1000 organisms)	Hypopyon

* Minimal infective dose.

Antibody Assays.—*Anti-alpha hemolysin.* Alpha hemolysin antitoxin was assayed by a modification of the NIH standard staphylococcus antitoxin method (22). A potent alpha toxin was prepared by growing the Lafferty strain at 37°C. in brain-heart infusion broth through which was constantly bubbled a mixture of 30 per cent CO₂ and 70 per cent oxygen. The culture was periodically enriched with the addition of glucose and the pH adjusted to 6-7 (using phenol red as an indicator) by the addition of 0.1 N sodium hydroxide at 6 to 10 hour intervals.

The LH₅₀ dose of toxin was determined using NIH standard staphylococcal antitoxin as a reference. The end point (50 per cent hemolysis of a 2 per cent suspension of rabbit red blood cells) was measured colorimetrically using the Coleman Jr. spectrophotometer (readings at 550 lambda). Anti-alpha hemolysin titer was then determined on the test sera as a measure of its ability to neutralize an LH₅₀ dose of toxin.

Precipitin Titers.—Precipitin titers of sera were determined by means of the agar gel diffusion technique. The antigens used included a culture filtrate high in alpha hemolysin toxin content, and "soluble cellular protoplasm" obtained by sonic disruption of washed staphylococci (23).

Skin Tests.—Skin tests were performed intradermally using 0.1 ml. of a heat-killed vaccine prepared from the Lafferty strain by subjecting a washed culture of cells to 56°C. for 1 hour.

Animals.—2 to 3 kg. albino male rabbits were used throughout. They were prepared for skin injection by the use of an electric shaver.

EXPERIMENTAL RESULTS

Skin Infections.—The studies of Panton and Valentine (14) had suggested that repeated skin infections in rabbits were associated with development of

“immunity” to a large dose and at the same time “hypersensitivity” to a previously subinfective dose. To clarify this point it was decided to perform a similar study utilizing immunological and histopathological techniques.

Three albino rabbits were bled *via* the ear vein and the serum separated and stored at 4°C. The backs of these animals were then shaved and each rabbit injected intradermally with a series of four 0.2 ml. doses of staphylococci representing tenfold dilutions of an 18 hour culture suspended in saline (see Materials and Methods). The undiluted dose contained approximately 2×10^8 organisms and the remaining doses 2×10^7 , 2×10^6 , and 2×10^5 , respectively. The character and extent of the resulting lesions were observed and photographed at 24, 48, and 72 hours. At the end of 1 week, at a time when the lesions were undergoing healing, the animals were again bled and challenged with a similar series of 4 doses of staphylococci given on the opposite side of the back. At the same time 3 additional rabbits, previously uninfected, were added to the study receiving their initial injections simultaneously with the second challenge of the previous group. In this way, rabbits receiving their first, second, third, etc. challenge on the same day could be compared with each other; and, in addition, the results of a particular rabbit could be followed by the photographic record from week to week. The animals were bled at weekly intervals throughout the experiment and the serum stored at 4°C. An effort was made to assure that the injections were made in previously uninfected skin. The experiment was continued for 7 weeks so that a total of 21 rabbits was used. (These animals were then challenged with eye and knee joint infections to be described subsequently). A second experiment was then performed in exactly the same manner and at the end of 8 weeks the animals were sacrificed and autopsied.

On initial injection the rabbits consistently developed pustular lesions with the undiluted and 1:10 dilutions but essentially no reaction other than a faint erythema with the 1:100 and 1:1000 dilutions. The undiluted dose (2×10^8 organisms) uniformly produced an abscess 10 to 20 mm. in diameter and the 1:10 dose a smaller nodule. Reactions were maximal at 24 to 48 hours and thereafter declined in severity. The larger lesions frequently ulcerated and drained purulent material after a few days. Eventually all lesions healed (usually by 5 to 10 days). On subsequent challenge lesions were now produced by the last 2 dilutions and this was most evident on the 3rd to 5th weekly challenge when pustular lesions were consistently produced by the 1:100 and 1:1000 dilutions. At this time there was an increase in the extent of the inflammatory reaction with the 2 larger doses as well, plus what appeared to be more localization of the pustular reaction when compared to the diffuse and superficial distribution of pus on initial challenge. These results are summarized for the first, fourth, and seventh challenge in Table II.

Autopsies were performed on the second group of animals at the end of 8 weeks and included animals challenged successively one to eight times by weekly intradermal inoculation. In none of the group were metastatic abscesses found.

Histopathologic sections of the skin sites (obtained 48 hours after injection of the organism) showed the presence of abscess formation with 2×10^6 and 2×10^5 organisms in the previously infected animals but not in animals challenged for the first time (Fig. 1). That these lesions were the result of

bacterial multiplication was indicated by the presence of large numbers of organisms on Gram stain of the histopathological sections.

The results would seem to confirm the observations of Panton and Valentine that prior infection with the staphylococcus is associated with susceptibility to the production of a lesion by a previously subinfective dose. At the same time the lesion produced by a larger dose in the previously infected rabbit

TABLE II
Staphylococcus Skin Infection in Normal and Previously Infected Rabbits

Dose	1st infection			4th weekly infection				7th weekly infection			
	10 ⁸	10 ⁷	10 ⁶ 10 ⁵	10 ⁸	10 ⁷	10 ⁶	10 ⁵	10 ⁸	10 ⁷	10 ⁶	10 ⁵
Erythema, 48 hrs.	++	+	± 0	++++	+++	++	++	+++	+++	++	++
Induration, 48 hrs.	+	+	0 0	+++	++	+	+	+++	++	+	+
Abscess, 48 hrs.	+	+	0 0	+	+	+	±	+	+	+	±
Ulceration, 48 hrs.	+	0 (±)	0 0	+	+	0	0	+	+	0	0
Histopathology											
Abscess	+	+	0 0	+	+	+	±	+	+	+	±
Bacteria	+	+	0 0	+	+	+	±	+	+	+	±
Serum antialpha hemolysin titer	Negative			Negative				30 animals negative (<2 u) 2 animals minimal (2 u)			
Serum precipitin* (culture filtrate)	Negative			30 animals negative 2 animals positive				29 animals negative 3 animals positive			
Skin test											
Culture filtrate	Negative			±				±			
Vaccine	Negative			Positive (delayed)				Positive (delayed)			

* Precipitin by Ouchterlony gel diffusion: undiluted serum.

was modified in character but there would seem to be insufficient grounds to conclude that this represents "immunity."

Skin Tests.—To evaluate the nature of the altered host reactivity in the test animals described in the preceding experiments, intradermal tests were performed using a heat-killed vaccine.

Test rabbits consistently showed a delayed type of skin reaction with the formation of a nodule at 24 hours. Control rabbits occasionally showed a small nodule, always smaller, however, than the test animals.

The intradermal injection of sterile 24 hour culture filtrate produced a faint erythema of the skin appearing within 30 to 60 minutes after injection and

lasting only about 4 to 8 hours. This was not seen in control animals. Injection of broth never elicited a detectable reaction.

The results suggested that recurrent skin infections in rabbits were associated with the development of delayed hypersensitivity to some component of the staphylococcal cell. The results with the culture filtrate were more difficult to interpret since it was possible that small amounts of dermonecrotic toxin were present.

Antibody Studies.—The immune response was further studied by serial determination of the anti-alpha hemolysin titer of the rabbits during the course of the repeated skin infections and by assay of precipitating antibody.

Of the 32 rabbits from both experiments which were tested, 30 (94 per cent) had no detectable anti-alpha hemolysin titer during or at the end of the experiment. Two of the 32 (6 per cent) had minimal titers (2 units).

Similarly, precipitating antibodies against a culture filtrate with high alpha hemolysin content showed that of the 32 rabbits tested only 3 (9 per cent) had developed precipitins (as measured by the agar gel diffusion technique). Two of these three animals were the rabbits which had also shown minimal titers of anti-alpha hemolysin. No precipitins were found against "soluble cellular protoplasm" obtained by sonic disruption of staphylococcal cells.

Sensitization by Vaccination.—Because of the indication that increased susceptibility to a previously subinfective dose of organisms was related to the development of delayed hypersensitivity to a component of the staphylococcal cell, it was desirable to reproduce this effect in animals sensitized in some way other than by active infection.

Accordingly, 2 rabbits were inoculated with a washed heat-killed suspension of staphylococci. The initial injections consisted of 5×10^9 killed organisms suspended in 2 ml. mineral oil with mycobacteria ("complete" Freund's adjuvant-Difco) and emulsified. Thereafter 4 bi-weekly injections were made of a similar quantity of organisms emulsified in mineral oil without mycobacteria ("incomplete" Freund's adjuvant-Difco).

Skin tests of these rabbits before and after the course of inoculations indicated the development of delayed type hypersensitivity to the organism as elicited by dilutions of washed killed staphylococci. When these animals were challenged intradermally for the first time with live staphylococci in tenfold dilutions in the same way as the previous test group, they developed pustular lesions with all 4 dilutions—a response similar to that of animals which had experienced 3 or 4 previous intradermal infections and quite unlike that seen in animals with no previous infection.

These results afforded additional evidence that increased susceptibility to infection was associated with the development of delayed hypersensitivity to the staphylococcus.

Knee Joint Infections.—To evaluate the susceptibility of rabbits sensitized

by repeated skin lesions to infection by other routes the response to intra-articular inoculation was studied.

Fourteen rabbits which had previously experienced from 1 to 7 weekly skin challenges were given intra-articular injections of 10^4 staphylococci. Six control rabbits were injected in a similar fashion. The development of knee joint infection was followed by observations of the degree of swelling of the joint, resistance to extension, and the degree of limp which developed. Febrile response and white blood count were followed, and blood cultures were obtained. Finally, in an attempt to obtain an objective index of the severity of the infection, intra-articular pressure was measured.

Grading of the degree of swelling, limp, and resistance to extension revealed that the hypersensitive group developed more severe infections than did the control group. Confirmation of these observations was obtained by the knee

TABLE III
Staphylococcus Eye Infection in Normal and Previously Infected Rabbits

	Total animals	Minimal or no reactions	Infections (hypopyon)		
			Localized	Blindness and rupture	Total infections
Previously infected (skin)	16 (100 per cent)	7 (43 per cent)	5 (31 per cent)	4 (25 per cent)	9 (57 per cent)
Control (no previous infection)	12 (100 per cent)	4 (33 per cent)	1 (8 per cent)	7 (58 per cent)	8 (67 per cent)

joint pressures. The mean joint pressure in the hypersensitive group was 64 mm. H_2O (range 30 to 95 mm.) as contrasted to the mean of 29 mm. H_2O (range 3 to 48 mm.) in the control group. Febrile response and leukocytosis were approximately the same for both groups and there was no evidence of bacteriemia in either group. All animals showed evidence of pyoarthrosis and the staphylococcus was cultured from all injected joints.

The difficulties in grading severity of knee joint infection seemed to preclude comparison of the minimal infective dose for the knee joint in both groups. Instead a dose which was known to produce pyoarthrosis in normal controls (10^4 organisms) was utilized. The results paralleled those with skin infection in that hypersensitive animals developed more severe lesions with a given dose than did normal rabbits.

Eye Infections.—Infection of the anterior chamber of the eye in hypersensitive and control animals was studied.

After withdrawal of 0.1 ml. of aqueous humor, the anterior chamber of the rabbit's eye was inoculated with 0.1 ml. of a saline suspension of approximately 600 staphylococci (a dose which produced infection in slightly more than half of control rabbits). Of 16 previously infected animals, 9 (57 per cent) developed a definite hypopyon, 4 (25 per cent) of which were severe leading to blindness

and rupture of the eyeball, while 5 (31 per cent) had infections which localized or eventually cleared. The remaining 7 animals (43 per cent) showed minimal or no reactions. Of 12 control animals, 8 (67 per cent) had a definite infection, 7 of which (58 per cent) were severe, leading to blindness, and 1 of which eventually cleared. Four (33 per cent) showed minimal or no reaction (Table III).

TABLE IV
Staphylococcus Susceptibility of Normal and Previously Infected Rabbits

Site of inoculation	Rabbit	80/81 inoculum		Uninfected/ infected	Severity of infection*	Mean joint pressure (4 days)
		Volume <i>ml.</i>	Dose			
Eye—anterior chamber	Normal	0.1	600 organisms	4/8	7—4+ 1—1+	
	Previously infected	0.1	600 organisms	7/9	4—4+ 5—1+	
Joint—knee	Normal	0.1	10 ⁴	0/6	1—3+ 3—2+ 2—±	29 mm. H ₂ O Range 3—48
	Previously infected	0.1	10 ⁴	0/14	3—3+ 8—2+ 3—1+	64 mm. H ₂ O Range 30—95

* Eye: 1+ = haziness of cornea, "fibrin" strands, or complete resolution.

4+ = corneal perforation, hypopyon, failure to resolve.

Joint: graded on basis of limp and swelling.

While susceptibility to infection in the anterior chamber of the eye by a small number of organisms seems approximately the same for the two groups (9/16 of the hypersensitive group and 8/12 of the control group) the control animals appeared to develop more severe infections (7/12) than did the hypersensitive group (4/16). These results stand in contrast to the more severe lesions seen in the skin and the knee joint in the previously infected animals. (Table IV) It is possible that local resistance factors in the eye differ significantly from those in other areas of the body.

DISCUSSION

In contrast to pneumococcal and streptococcal infections in which protection against reinfection is afforded by type-specific antibacterial immunity and to

diphtheria in which antitoxic immunity is effective, infections with the staphylococcus tend to recur repeatedly without evidence of the establishment of significant immunity by prior infection. Attempts to elicit immunity by the use of vaccines and toxoids have been of exceedingly limited, if any, success. Despite the unimpressive evidence of protection by specific antibody, vaccines and toxoids are still rather widely used in the treatment of chronic staphylococcal infections.

It is likely that clinical success in increasing host resistance to staphylococcal infection will await a more exact knowledge of the pathogenesis of these infections and of the mechanisms by which the organism is able to overcome host defenses and establish clinical disease. Early investigators, preoccupied with antitoxic immunity, were undoubtedly led somewhat astray by analogy with diphtheria. The staphylococcus, it would seem, may more closely resemble the tubercle bacillus in its ability to establish long term residence in the tissues and to survive within host phagocytic cells (24). The possibility arises that the staphylococcus may further resemble the tubercle bacillus with respect to the role of specific bacterial allergy in altering the nature of the host response to infection.

Observations of clinical staphylococcal infections in man have on occasion revealed a change in the character of the local lesions in the course of repeated infections. The initial furuncle or boil in a patient may be unassociated with significant cellulitis, lymphadenitis, or systemic symptoms such as fever. As repeated infections of the skin occur, these features often become more prominent. That re-infections are produced by the same organism can often be established by means of bacteriophage typing. No evidence of increased virulence of the organism has been obtained under such circumstances, and it is possible that the development of hypersensitivity to the staphylococcus is responsible for the heightened reactivity occurring with repeated infection.

An additional hint that a state of hypersensitivity even to a heterologous antigen may alter host resistance to staphylococcal infection is the clinical observation that recurrent furunculosis seems to occur in a number of patients only during the time when they are suffering from seasonal hay fever. Possible experimental confirmation of the effect of heterologous hypersensitivity is suggested by the findings of Prigal and Dubos (19), of the heightened ability of staphylococci to multiply in the organs of mice during anaphylactic reactions to bovine serum albumin.

It therefore seemed of importance to attempt to answer the question of whether hypersensitivity to the staphylococcus is ordinarily a consequence of staphylococcal infection; and if so, whether it contributes to host resistance or whether the injurious effects of the hypersensitivity reaction actually increase host susceptibility. The present study was undertaken to investigate the effect of previous experience with staphylococcal infection upon the response of the

host to subsequent challenge, using a model similar to that employed by Panton and Valentine (14). Like man, rabbits possess a relatively high degree of resistance to infection by the staphylococcus, and upon initial skin challenge developed lesions only with 2×10^8 and 2×10^7 organisms. After repeated infections, however, pustular lesions containing increased numbers of organisms could be elicited with doses 10 and 100 times less than a previously minimal infective dose. Throughout these experiments the animals failed to develop significant antitoxin (alpha hemolysin) titers, presumably because the lesions remained localized to the skin without evidence of dissemination. Thus the effect of antitoxic immunity in these experiments was probably insignificant. Instead the animals developed a state of increased cutaneous reactivity of the delayed type to the killed bacterial cell. Under these circumstances specific bacterial hypersensitivity apparently increased the susceptibility of the host to cutaneous infection by the staphylococcus. Similarly, hypersensitive animals appeared to develop a more severe knee joint infection. In contrast, susceptibility to infection of the anterior chamber of the eye seemed little changed and indeed, the resulting lesions tended to be more severe in previously uninfected animals than in the hypersensitive group. It is of great interest that the minimal infective dose was much less for the eye than for the skin. Undoubtedly local factors play a significant role in determining resistance to staphylococcal infection and the apparent discrepancy in results may well be a reflection of local variation in resistance.

Confirmation of the impression that increased susceptibility to cutaneous infection was a manifestation of the hypersensitive state was obtained by rendering animals hypersensitive by injection of killed washed organisms, after which the response to cutaneous challenge with live staphylococci was found to resemble that in previously infected animals.

Impressive corroboration of the deleterious role of specific bacterial hypersensitivity may be found in the results of Johanovsky (20) who was able to transfer a state of increased host susceptibility to staphylococcal infection by means of lymphoid cells which concomitantly transferred delayed hypersensitivity.

The obvious question arises as to whether the effect of specific hypersensitivity is simply a result of the inflammatory reaction evoked. If so, it should be reproducible by other inflammatory stimuli. Experiments designed to answer this question are the subject of the following report.

SUMMARY

The influence of repeated staphylococcal infection of rabbit skin upon the characteristics of the experimentally induced lesion was studied. It was found that the repeated infection was associated with the development of delayed hypersensitivity unaccompanied by the appearance of demonstrable serum

antibody. The delayed hypersensitivity to the staphylococcus resulted in an increased infectivity of the organism in skin of the sensitized animal, characterized by intensification of the lesions seen with large bacterial inocula and the induction of abscesses with inocula incapable of producing any lesion in normal rabbit skin. Similarly, the severity of experimentally induced pyoarthrosis was greater in sensitized than in normal rabbits. Induction of delayed hypersensitivity by vaccination of rabbits with washed heat-killed staphylococci resulted in the same increased severity of the infection and an increase in infectivity of the microorganism.

In contrast to the observations of cutaneous and joint infection, the sensitized animals appeared to be less susceptible to severe infection of the anterior chamber of the eye.

The role of immunity and hypersensitivity in staphylococcal infection is discussed and the possibility that non-specific inflammation may influence staphylococcal infection in the same way as specific hypersensitivity is indicated. Studies to further elucidate this are presented in the following pages.

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EXPLANATION OF PLATE 40

FIG. 1. The left hand illustrations show the histopathological features of skin lesions at 48 hours in normal rabbits inoculated with 10^8 lower and 10^5 upper *Staphylococcus aureus*, showing abscess formation only with the larger dose of bacteria. The right hand illustrations show similar skin sites inoculated with 10^8 lower and 10^5 upper bacteria in rabbits who had been repeatedly infected in the same way at weekly intervals for 4 weeks, showing abscess formation at the sites injected with both doses of staphylococci.

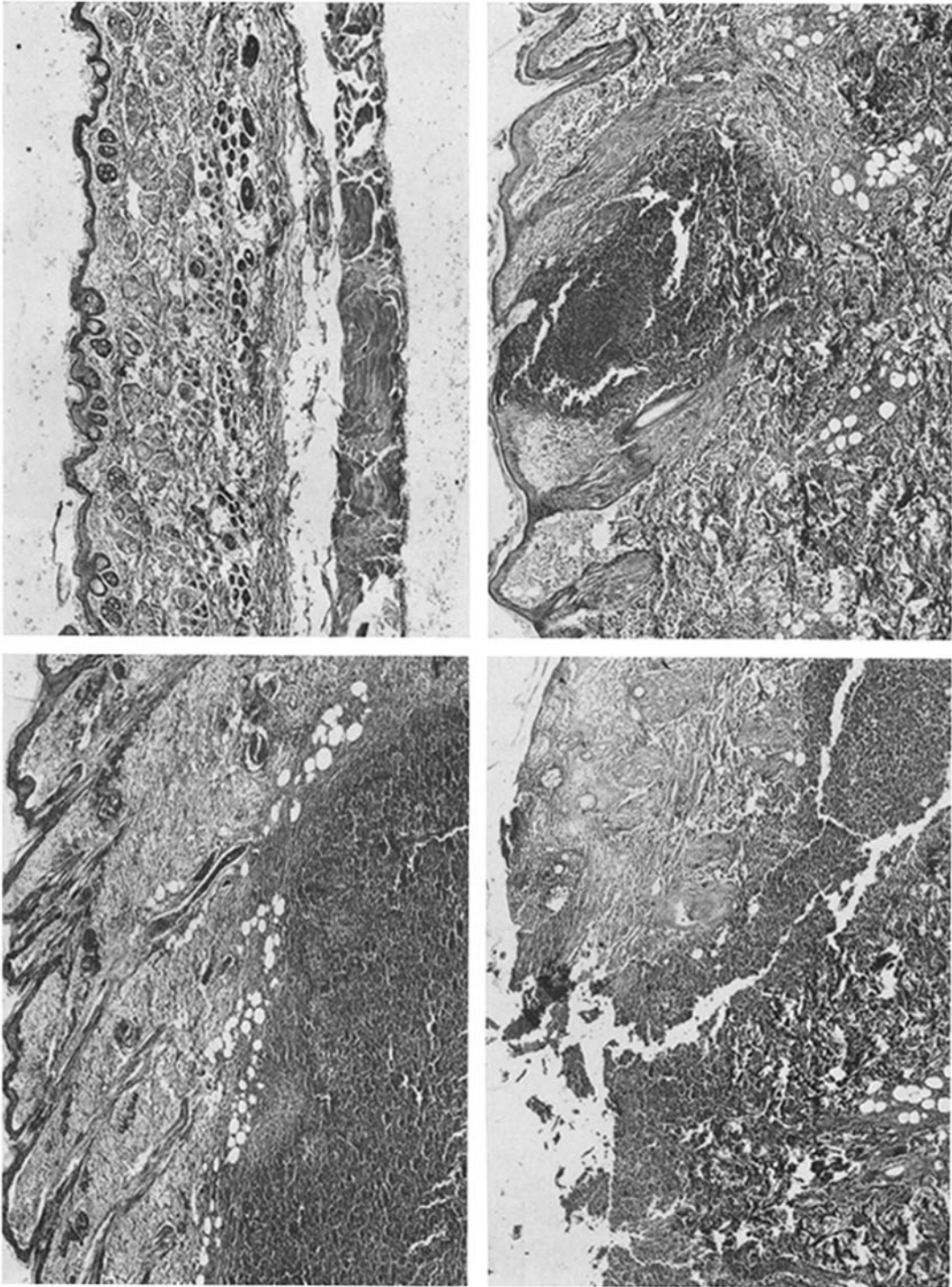


FIG. 1

(Johnson *et al.*: Pathogenesis of staphylococcal infection. I)