STUDIES IN TISSUE-IMMUNITY *

CELLULAR REACTIONS OF THE SKIN OF THE GUINEA PIG AS INFLUENCED BY LOCAL ACTIVE IMMUNIZATION

PAUL R. CANNON, M.D., AND G. A. PACHECO, B.S.

(From the Department of Pathology and the Otho S. A. Sprague Memorial Institute, University of Chicago, Chicago, Ill.)

The relatively unsatisfactory results of many years emphasis upon humoral factors in the defense against infectious diseases have gradually led to a reëxamination of some of the underlying mechanisms involved, and as a result the cellular reactions of immunity are now receiving more attention. This is not surprizing in view of the many disappointments following attempts to secure preventive and curative serums, the transitory nature of passive immunity and the failure of protection at times in the presence of high concentrations of immune bodies in the serum. On the other hand, the permanency of active immunity, as after an attack of typhoid fever or small-pox, or from the administration of certain vaccines, offers possibilities of prevention far superior to those hitherto attained by the use of serums.

The emphasis upon cellular mechanisms of defense received renewed stimulus through the attention paid to the mesenchymal tissues by Aschoff [1] in the reticulo-endothelial system. The principal merit of Aschoff's work probably lies in its development of certain morphological aspects of defensive mechanisms; as a result, principles enunciated by Metschnikoff are now receiving more serious attention and are being revealed as fundamental in all considerations of the basic problems of immunology. Furthermore, accumulating evidence points increasingly to the close relationship between phagocytic cells, particularly macrophages, and antibody-formation, so that at present it is no radical concept that antibodies may be merely excess products resulting from the ingestion of antigenic substances by phagocytes. If this view is correct, the attempts to obtain serums or solutions of antibodies may be concerned mainly with by-products, the fundamental reaction actually occurring within the

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phagocytic cells. This does not imply that antibodies *per se* are unimportant; it does suggest the need of directing more attention to the cells themselves.

A further impetus to the enlargement of our concepts of tissue-immunity was furnished by Besredka [2] in his studies of local immunity. Although Besredka's interpretations of the mechanism of local tissue-immunity are not accepted by many, his ideas have stimulated much investigation and have aided in the clarification of a difficult problem of immunology. We also owe much of this clarification to the work of Gay and his associates,[3, 4, 5] who have greatly extended our knowledge of tissue-immunity and have done the most to correlate immune processes with histological changes in the tissues, thus furnishing a basis for the development of a field of histological immunology. In an extensive investigation of the problem of experimental streptococcic empyema, Gay and his collaborators have shown clearly that in this condition increased resistance of pleural cavities to streptococci is due primarily to an actual increase in numbers of tissue-macrophages in the wall of the thorax. Previous irritation of the pleural surfaces by the injection of such substances as gum arabic broth leads to the development or mobilization of large numbers of macrophages beneath the parietal pleura and these actively phagocytic cells ensure protection against large dosages when streptococci are later injected into the pleural cavity.

Opie [6, 7, 8, 9] has also made observations of the greatest importance in his studies of anaphylactic inflammation. He has demonstrated that when rabbits are injected intradermally with foreign proteins such as horse serum or crystalline egg albumin, much of the material is quickly demonstrable in the blood stream by precipitin tests, but "with repeated injection of the antigen the quantity of foreign protein that enters the blood stream diminishes and finally with advanced immunization none enters unless massive doses have been employed." Furthermore, in the immunized animal the foreign protein is fixed at the site of injection, a fact of significance because this is where the anaphylactic inflammation occurs. This inflammation is characterized by a rapid infiltration of polymorphonuclear leucocytes at the site of the inoculation, with edema and the deposition of fibrin. The small blood and lymph vessels are injured and thrombosis frequently occurs, leading, especially in the rabbit, to necrosis (Arthus phenomenon). Since a similar reaction occurs when antigen

and antibody are simultaneously injected into the tissues of a rabbit, but not when antigen and normal serum are so injected, and since the same effect is observed when antiserum is injected into the tissues of a sensitized animal, Opie concludes that "anaphylactic inflammation occurs because antigen and antibody have met in the tissues." If we assume that at least part of this union occurs within tissue macrophages, the intracellular reaction may conceivably either give no effect or may give anaphylactic inflammation, depending upon the amount of antigen entering sensitized macrophages in a short period of time, or the relative concentration of intracellular antibodies available for the reaction with the antigen. In either case a too violent reaction could be followed by a certain degree of damage to the surrounding tissues with injury to capillaries, thus increasing their permeability for the plasma and cellular elements so prominent in inflammation, anaphylactic or otherwise. It is a reasonable hypothesis that at least some of the cutaneous reactions which we regard as anaphylactic or allergic may be due to an altered reactivity of these phagocytic cells, both as to rate of phagocytosis and rate of intracellular digestion of antigenic substances, as determined by the number of phagocytes available, their state of reactivity and the amount of antigen utilized per unit of time. Where anaphylactic inflammation occurs, the extent of the cellular infiltration may depend upon the degree of the chemotactic stimulus; at times the very violence of the reaction may overshadow the beneficent action, giving the "two-edged sword" effect of dissemination of the antigen.

To what degree can we regard this reaction as a mechanism of defense? The answer will be determined by the behavior of properly sensitized tissues to the entrance of pathogenic microörganisms. Probably the essential difference between the effects of infectious agents and of inert antigens is in the ability of the former to multiply and to invade tissues. If both multiplication and invasion could be prevented to a large extent because of a local union of antigen and antibody through an agglomerating reaction, such as agglutination or precipitation of the microörganisms or their products, the tendency to dissemination of the germs would be greatly lessened. If, in addition, an increased number of macrophages, especially ones sensitized by previous experiences with the antigen, were present in the area of invasion, phagocytosis should be quantitatively increased. Finally, if the reaction to injury through this local meeting

of antigen and antibody were followed by a secondary infiltration of cells of inflammation into this area, the infectious agents should be even more effectively localized. This entire mechanism should then be considered as cellular and a type of tissue-immunity. Even in the presence of marked necrosis at the site of inoculation, if such a local reaction prevents the dissemination of the infectious antigen, the effect nevertheless is protective and an evidence of immunity. As Opie says, "the apparent susceptibility of the protected animal to local injury is a paradox explained by changes which serve to protect the organism as a whole." In other words, a scar from a furuncle is a small price to pay for the prevention of pyemia.

A phase of tissue-immunity which has received much attention within recent years is the problem of local immunity. In regard to this, we feel that too much emphasis has probably been placed on the localized nature of the immunity and on the rôle of antibodies in the phenomenon. Besredka defines local immunity as an "immunity without the obligatory participation of antibodies." Such a conception, if correct, would materially modify the usual conceptions as to the relative importance of cellular and humoral factors in immunity. Besredka's ideas are based, principally, on the evidence of an acquired immunity of local tissues, such as the skin and mucous membranes, in the absence of any significant degree of antibody-concentration in the blood serum. This, however, does not necessarily exclude the possibility of antibodies being within the cells or around them in the area of localized tissue-immunity; indeed, there is evidence that local concentration of antibodies may play a considerable part in the local tissue-immunity. Gay's definition of local immunity as an immunity "due to a locally superior mechanism for the disposal of a particular microörganism," seems to be much broader and more in conformity with the facts. This mechanism may or may not be associated with the action of antibodies, but until we know what function antibodies have within phagocytic cells and how quickly they may appear there, we cannot arbitrarily exclude them from participation in immune processes merely because they may seem to be of little significance as shown by the usual serological tests, especially in view of the increasing evidence that the usual site of antibody formation is in the individual cells of the mesenchymal tissues.

The demonstration of a localized type of immunity does not neces-

sarily imply the absence of protection elsewhere; resistance locally as well as generally is relative and a matter of degree. Nevertheless, by proper dosage, and by localized immunization, several investigators have shown that certain tissues may acquire an enhanced ability to resist invasion by microörganisms as compared with other tissues of the same individual. In such conditions of local immunity more evidence is needed as to the factors concerned, whether cellular, humoral, or both. Morphological evidence, particularly, is desirable, either of local dissolution or agglomeration of bacteria injected, or of an increased number of phagocytic cells in the area, or of increased metabolic activity of such cells. Curiously enough, although Besredka speaks of certain "receptive cells" which are dominant in localized areas of immunity, it seems that he has made little study of them from a histological viewpoint; nor have most of the other workers in this field given much consideration to this point.

Apparently the only histopathological study of the cellular reactions of the skin of the guinea pig to staphylococcus infection according to the methods of Besredka, is that of Freedlander and Toomey.[10] These workers made a detailed examination of the inflammatory response in the subcutaneous tissues of normal guinea pigs, and of ones previously treated with broth compresses and staphylococcus filtrates prepared according to the methods of Besredka. A definite localized protection was observed following the application of broth compresses, which protection persisted longer than twenty-four hours and less than seven days. The protection seemed to be non-specific and was correlated with histological changes in the subcutis where there was a significant increase in the numbers of clasmatocytes, fibrocytes and lymphoid cells following the application of sterile broth compresses for forty-eight hours. The inflammatory response to the subcutaneous injections of staphylococcus cultures in such animals was characterized by an infiltration of cells of inflammation in the subcutis, much more marked in degree than in normal animals similarly infected. Also, although polymorphonuclear leucocytes were the predominant cells in each case, they tended to degenerate in the control animals, whereas they retained their normal appearance in the broth-protected ones. In addition, in the latter animals there was an increased infiltration of small mononuclear cells and a greater prominence of clasmatocytes, with fibroblasts tending to organize the process at an early

stage. The authors concluded that "the clasmatocytes in large numbers diminish the virulence of the bacterial attack and also, by ingesting the polymorphonuclear leucocytes which contain staphylococci, they prevent a recurrence of bacterial activity."

An important study of the inflammatory reactions of the subcutaneous tissues of normal and immunized mice, using streptococci and pneumococci, was also made by Tsuda [11] in Lubarsch's laboratory. In normal animals this response varied with the degree of virulence of the microörganisms injected, but with weakly virulent germs the organisms remained at the site of inoculation, were quickly surrounded and phagocytosed by leucocytes and then encapsulated by connective tissue cells. There was thus no dissemination of the microörganisms through the adjacent tissues. With highly virulent microörganisms, however, there was an early injury to the tissues at the site of inoculation with very little phagocytosis of the germs, and with a consequent rapid dissemination of the latter throughout the surrounding tissues. In immunized animals both virulent and avirulent bacteria were quickly injured, as shown by evidences of degenerative changes such as swollen and poorly stained forms, inequality of size, etc. There was also a marked tendency to agglutination followed by active phagocytosis by the leucocytes and macrophages. This observation of agglutination *in vivo*, while not emphasized by Tsuda, is obviously of great significance. Tsuda states that "if the immunity is strong enough, the injected cocci show at the site of injection agglutination phenomena in the form of floccular clumps and aggregations of microörganisms."

More recently Imschenetzky [12] has shown that the application to rabbits of dressings saturated with isotonic salt solution or with staphylococcus antivirus solutions leads to a distinct inflammation in the subcutaneous tissues, with hyperemia, edema and an increased prominence of histiocytes and increased numbers of infiltrated leucocytes. Similar dressings saturated with a 1 per cent solution of trypan blue in salt solution led to the appearance of granules of dye in the histiocytes, but only after forty-eight-hour application of the dressings, and then only when there were evidences of slight injuries to the epidermis which had increased its permeability.

Experimental Procedures

Our studies have been concerned with the cellular reactions of defense in the skin of normal guinea pigs and of others previously immunized by the intracutaneous injection of a staphylococcus vaccine. More than 100 different animals have been observed during the course of the investigation. A strain of staphylococcus aureus freshly isolated from a furuncle was used. For immunization, a twenty-four-hour growth on agar slants was suspended in 1 cc. of sterile 0.9 per cent salt solution and heated at 60° C for one hour. Two-tenths of a cubic centimeter of this vaccine was injected intradermally at daily intervals for ten days into the anterior abdominal wall of guinea pigs weighing from 200 to 300 grams, thus infiltrating an area of skin of approximately four square centimeters. The animals were then allowed to rest for twenty-five days in order to permit the skin to return to approximately normal conditions in so far as external appearances were concerned. Then these animals, and normal ones of the same size, were injected intradermally with 0.2 cc. of a living virulent culture of the organism, the growth from one agar slant again having been suspended in 1 cc. of sterile salt solution.

At intervals the guinea pigs were anesthetized and the areas of inflammation were excised after attaching the peritoneal surface to a cork frame by means of bamboo pegs. The tissues, usually averaging from one to one and a half centimeters in width, were immediately fixed in formol-Zenker fluid and subsequently embedded in celloidin and sectioned at 10 microns. The sections were stained routinely with Maximow's hematoxylin-eosin-azur II as well as with special stains such as Mallory's connective tissue stain, Goldmann's carmine stain and Gram's stain.

Effects of the Intradermal Injection of a Staphylococcus Vaccine upon the Skin

Sections of skin taken twenty-five days subsequent to the ten intradermal inoculations of killed staphylococcus vaccine show the principal effect in the subreticular zone of the subcutis. Here there is an extremely marked increase in macrophages. In the looser areas many sizes and types of non-granular cells may be seen, varying from typical lymphocytes and monocytoid forms to typical macrophages.

In the denser areas the latter are elongated and compressed and at times resemble fibroblasts. Mallory's connective tissue stain, however, shows but slight increase in the amount of collagen, although there is a definite increase of collagen in the densest areas of macrophages. Mitotic figures are not seen in the regions of thickening, and the multiplicity of mononuclear types, from lymphocytes to typical macrophages, suggests that, as maintained by Maximow, the latter may be differentiated forms of cells of hematogenous origin, particularly lymphocytes or monocytes (see Fig. 1).

The Behavior of India Ink Injected into Normal Skin and into Skin Previously Immunized against Staphylococci

The intradermal injection of 0.2 cc. of a 4 per cent suspension of India ink in sterile isotonic salt solution into a normal guinea pig was followed by a diffuse dispersion of the particulate material through the subcutis. Sections showed much of the ink caught along the collagenic fibrils, although some of it was engulfed by tissue macrophages. A moderate infiltration of polymorphonuclear leucocytes occurred at the end of twenty-four hours, but these did not engulf the particles of ink to any extent. When the same quantity of ink was injected into an area of skin of a guinea pig which had previously been given ten intracutaneous injections of plain peptone broth, the effects were not noticeably different from those observed in the normal animal. When a similar quantity was injected, however, into the skin of a guinea pig which had previously been immunized by ten intradermal injections of the killed staphylococcus vaccine, there was a distinct tendency for the ink to remain localized near the site of inoculation rather than to be dispersed through the subcutis. Fig. 9 shows the distribution of the ink in the skins of the three animals and Fig. 2 illustrates the mode of disposal of the ink particles by the macrophages of the subcutis. It is interesting that monocytoid cells, lymphocytes and polymorphonuclear leucocytes show little tendency to engulf the particles of ink. These observations suggest that merely the presence of increased numbers of macrophages increases quantitatively the engulfment of particulate material and thus effectively aids the localization of such material after its inoculation. It is also possible that local hindrances to lymph flow may further prevent, to some extent, the dissemination of the particulate material.

General Results

The inflammatory response to the living staphylococci was markedly different in the skins of the two groups of animals. In the normal guinea pigs the intradermal inoculation led to a serosanguineous inflammation which spread as a diffuse cellulitis through the subcutaneous tissues and frequently led to the death of the animal in from eighteen to twenty-four hours. In the previously immunized ones, however, the intradermal injection was followed by a localized small area of suppuration which tended to ulcerate and heal with no serious consequences to the host. It is evident from these differences in the reactivity that the intradermal injections of the killed culture of staphylococcus led to an increased resistance of the skin to the later injection of the living virulent organisms. Examination of the tissues confirmed the above observations and in addition suggested an explanation for the increased resistance of the skin of the immunized animals.

Effects of Intradermal Injections with Living Staphylococcus Aureus Suspension upon Normal Guinea Pigs

The inflammatory response is well developed within six hours, as shown in Fig. 5*a*. The principal finding in the skin of the normal animal at this stage is edema of the subcutis with separation of the collagenic fibrils and a beginning infiltration of cells of inflammation. Polymorphonuclear leucocytes comprise the vast majority of the incoming cells and these are actively phagocytosing the staphylococci as shown in Fig. 3. In spite of this fact, however, the staphylococci are diffusely spread along the subcuticular tissue in the form of a developing cellulitis. There is no evidence of any localizing tendency of the microörganisms, as shown in Fig. 10, nor are there evidences of injury to the bacteria, such as numerous swollen or distorted forms or frequent Gram-negative cocci. The infection is predominantly dispersive and generalizing. In the later stages, twelve, eighteen, and twenty-two hours, the picture is similar except for the greater infiltration of cells of inflammation, principally polymorphonuclear leucocytes (Figs. 6*a*, 7*a* and 8*a*). In spite of the activity of the microphages in ingesting many staphylococci, the infection progresses; in other words, the natural resistance is inadequate as a defensive mechanism.

Reactions in Skin Previously Immunized by Intradermal Injections of a Killed Staphylococcus Vaccine

The inflammatory response to the injection of 0.2 cc. of the same suspension of living staphylococci into the skins previously immunized is markedly different. As may be seen in Figs. 5*b*, 6*b*, 7*b* and 8*b* the principal difference is one of degree. At the six-hour stage there is an enormous infiltration of cells of inflammation in the subcutis of the immunized skin, much more abundant than in the normal animal at this stage. Furthermore, there is a qualitative difference in that there are many more lymphocytes and monocytoid cells present. These cells tend to become massed around a region of marked bacterial concentration and here there are many evidences of necrosis of the cells of inflammation, with accompanying hemorrhage and even thrombosis of the capillaries. Outside this area there are very few staphylococci to be seen; the infection is definitely localized and non-dispersive. A point of interest and probably of great importance is that in the area where the staphylococci are massed they are not present as individual organisms, but occur extracellularly in clumps and clusters, large and small, even in the six-hour stage. The appearance is that of agglutination *in vivo* (Figs. 11 and 12). It is around these clumps of staphylococci that the infiltration of leucocytes is densest, with the microphages nearest to the bacteria containing large masses of microörganisms. Here also the macrophages contain countless numbers of cocci, as shown in Fig. 4. It is obvious that the infection is localized to the immediate vicinity of the site of inoculation and that the acquired resistance is adequate as a defensive mechanism.

Discussion

It is an interesting fact that Metschnikoff showed in 1884 [13] that the principal difference in the reaction to the subcutaneous injection of virulent anthrax bacilli in normal rabbits, and in others previously immunized, lay in the greater degree of phagocytosis in the latter. He noted that within a few hours after the injection of the organisms into the normal animal there was an exudation rich in fluid and poor in leucocytes, in spite of the fact that the blood vessels in the vicinity were distended with blood and therefore could not be considered as unable to bring the leucocytes to the infected area. In the

vaccinated rabbits, however, there was an exudate rich in leucocytes at the site of inoculation and these were actively phagocytosing the bacilli. Metschnikoff concluded that the essential difference depended upon the sensitiveness of the leucocytes which exhibited a negative chemotaxis in the normal rabbit, but a marked positive chemotaxis in the immunized ones.

The effects of the entrance of pathogenic bacteria into the skin will obviously depend upon their ability to gain a foothold, multiply and disseminate throughout the body. There is no doubt that microorganisms vary in their ability to adapt themselves in the animal's body, this probably being a property inherent in the microörganisms themselves. The growth energy of certain highly virulent strains may possibly be so pronounced at times that dissemination occurs before the body cells or fluids can mobilize to hinder this dissemination.

Under more usual conditions the ability to adapt, multiply and disseminate is prevented by the defensive forces of the body. These may be both cellular and humoral. The early mobilization of phagocytic cells at the site of bacterial infection may serve to restrain the rapid increase in numbers of bacteria and in most cases the rate of engulfment by the macrophages may exceed the rate of multiplication of the bacteria. This, plus probable mechanical hindrances from the accumulation of fibrin and cells around the region of infection, will effectually localize the latter. In any event, the infection is obviously localized and the dissemination of the microörganisms is prevented.

In this localization of the bacteria at the site of inoculation the exact mechanism is still somewhat obscure. Are the organisms localized because of the intense infiltration of cells and fluids which mechanically hinder the further spread of the bacteria in the sense of the allergic inflammation of tuberculosis as conceived by Krause,[14] or are the organisms first localized by a mechanism of immunity and secondarily encapsulated because of the infiltration of cells of inflammation? The work of Opie would suggest the latter explanation as better fitting the facts and our results indicate the same probability. For example, the demonstration in the immune guinea pig within six hours after the injection of distinct extracellular clumping of the staphylococci with an accompanying failure of dissemination of the microörganisms strongly suggests a primary localiza-

tion of the bacteria through their reaction with antibody. Furthermore, no such tendency at any stage was noticed in the normal animals so that it does not seem probable that the bacterial masses are colonies growing in the tissues. It is of course possible that the clumping of the bacteria in the immune animal may be due to mechanical interferences with lymph flow which, with the dense layer of macrophages surrounding them, may keep the staphylococci localized. This conception seems less probable, however, when one sees how easily the polymorphonuclear leucocytes infiltrate the area and surround the masses of bacteria. We suggest, rather, that there is an actual antigen-antibody reaction in the tissues; as a result of this reaction chemotactic substances are formed which quickly lead to a pronounced infiltration of cells of inflammation, which are both quantitatively and qualitatively different from those responding in the normal animal (anaphylactic inflammation). Added to this, also, is the evidence that phagocytosis by the histiocytes is more abundant quantitatively than in the normal animals, in addition to the greatly increased number of histiocytes available in the former. The experiments with India ink, described above, strongly suggest hat an increase in histiocytes alone tends to localize particulate materials, but whether this is the result of increased phagocytosis or of a mechanical barrier remains uncertain. In active infection, however, it is probably the summation of all of these forces, specific as well as non-specific, that ensures an effective resistance against extension of the infection.

Additional support to the conception of the specific reaction is furnished by the experiments of Mudd, Lucké, McCutcheon and Strumia,[15, 16] which show the correlation between agglutination, cohesiveness, opsonization and phagocytosis of bacteria. If such correlations also obtain within the body, the evidences of agglutination and phagocytosis in our experiments may furnish a further clue to the function of immune bodies. Agglutination of the staphylococci may have furthered the tendency to their localization near the site of inoculation; their coalescence into small masses may also have increased the defensive efficiency of the phagocytes, both leucocytes and macrophages, since more microörganisms per phagocyte may be ingested following chance contacts than if the organisms were single and dispersed. Furthermore, if the agglutinating tendency and increased cohesiveness have an opsonizing effect, phagocytosis

will be further increased. In this connection it is interesting to recall the earlier conceptions of Bull [17] in his statement that "the degree of agglutination and opsonization of bacteria within the animal body is inversely parallel to the infectiousness of the bacteria for the host."

The relative importance of non-specific and specific agencies in localized tissue-immunity is difficult to evalute. Certainly non-specific factors may be sufficient to protect against many multiples of the lethal dose of an infectious agent, as was well shown in the experiments of Gay and his collaborators in the study of streptococcic empyema. Rivers and Tillett,[18] and Mallory and Marble,[19] found that the injection of plain meat infusion broth protected the skin of rabbits against later injections of streptococcus and staphylococcus. Miller [20] also demonstrated a definitely increased resistance of the skin of guinea pigs to staphylococcus, and of rabbits to streptococcus infections following previous treatments with dressings saturated with bouillon and peptone water. In all of these experiments, however, the results do not prove that such non-specifically increased resistance would have been adequate for larger infective doses of the microörganisms used, or that even better protection might not have been secured by the aid of specific modes of treatment.

The mechanism of the non-specifically increased resistance may be explained in at least two ways: first, in a local infiltration of leucocytes and bactericidal substances which may more effectively dispose of the infecting organisms later injected, or second, in a stimulation of the local fixed-tissue cells to increased functional activity. Evidence for the second possibility is suggested by Katsunuma and Sumi [21] in their observation that when rabbits were injected subcutaneously with a suspension of staphylococci on one side, and with a similar quantity of salt solution on the other side, followed a few hours later by an injection of an emulsion of staphylococci into both sides, the exudates collected showed much more active phagocytosis on the side previously injected with the staphylococci.

The evidence is quite convincing, however, that non-specific factors are not exclusively responsible for the increased resistance in localized tissue-immunity. For example, Gay and his associates found in testing the protection of the pleural cavity to streptococcus infection that "the degree of protection acquired by the repeated administration of living streptococci subsequent to broth or aleu-

ronat preparation of the cavity is markedly increased over the protection obtained by a single injection of the broth or aleuronat." More recently Clark [22] has shown the importance of specific mechanisms in experiments concerning tissue-immunity to pneumococcus infections of the pleural cavity of rabbits. With this organism no protection was obtained following the injection into pleural cavities of substances which had previously been shown to protect completely against infection with the streptococcus through the mobilization of macrophages. When the pneumococci, however, were treated with immune serum before being injected into the prepared pleural cavity, there was marked protection in cavities containing an exudate rich in mononuclear cells and with a pleural wall fortified by increased numbers of macrophages. These experiments clearly show the significance of specificity; the opsonization of the organisms is thus an important feature which favors increased phagocytosis by the macrophages.

The specific factor in our experiments seems to be mainly an agglomerating force which we believe is true agglutination *in vivo*. Certainly the microörganisms occur in clumps in the immune skins and are not thus seen in the normal skins. It is possible that the increased number of macrophages present in the immunized skins interferes with lymph flow in a mechanical fashion and thus encourages approximation of groups of cocci; experiments now in progress may throw further light on this phase of the problem. If we assume that the agglomeration of the staphylococci is specific agglutination *in vivo*, we have concrete evidence of a fundamental immunological rôle of such antibodies in aiding the local fixation of antigen in tissues. Opie's experiments clearly prove this for precipitins and he has shown that such precipitates are strongly chemotactic for polymorphonuclear leucocytes which probably destroy the injected antigen by intracellular digestion. We believe that this concept is of the utmost importance when applied to the fate of living antigen introduced into the tissues. In our experiments the facts are clear that the staphylococci disseminate diffusely throughout the subcutaneous tissues of the normal animals with no tendency to agglomerate or agglutinate. On the other hand, in the immune animals the tendency to agglomerate is noticed as early as six hours after injection of the microörganisms. Coincidently, there is a localization of the staphylococci near to the site of

inoculation, with a pronounced infiltration of cells of inflammation, both granulocytes and agranulocytes, around the bacteria. Local injury to this area occurs, but the animal itself is protected. In the words of Opie "vital organs are protected at the expense of local injury."

Summary and Conclusions

This paper describes histopathological studies of the skin and subcutaneous tissues of the abdominal wall of normal guinea pigs and of ones previously immunized by intracutaneous injections of a staphylococcus vaccine, all infected by the intracutaneous injection of a live virulent culture of staphylococcus aureus. The inflammatory responses were markedly different in the two groups. In the normal animals the inflammation was characterized mainly by an infiltration of polymorphonuclear leucocytes which actively phagocytosed the microörganisms. In spite of this the staphylococci showed no tendency to localize, but disseminated throughout the subcutaneous tissues in the form of a cellulitis.

In the previously immunized animals, however, the staphylococci tended to remain localized near the site of inoculation where they were seen agglomerated in bacterial masses of various sizes, presenting the picture of a genuine agglutination *in vivo*. Coincidently the infiltration of cells of inflammation led to further localization of the microörganisms so that only a localized area of necrosis resulted.

The previous immunization by intracutaneous injections of the staphylococcus vaccine was followed by a marked thickening of the subreticular layer of the subcutis, due mainly to increased numbers of tissue macrophages having been either activated or produced. Evidence is presented that many of these are derived from agranulocytes of the blood. These macrophages were actively phagocytic for the live staphylococci and furnished an effective barrier against extension of the infection.

The immunity secured by the above procedures is predominantly cellular in type with the tissue-macrophages playing the dominant part, due to increased numbers and also probably to increased metabolic activity. In addition, localization of the microörganisms by the action of agglutinating or opsonizing antibodies is suggested as of primary importance in preventing the dissemination of the infectious agent. The combination of humoral and cellular mechanisms ensures an adequate resistance against the bacterial invaders.

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DESCRIPTION OF PLATES

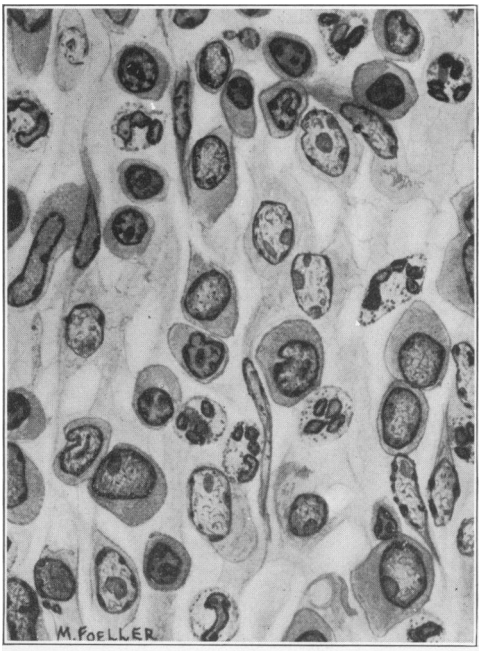
PLATE 140

FIG. 1. Drawing made at the level of the substage with the aid of the camera lucida, Leitz Oc. 4, Obj. 2 mm. apochromatic, showing the effects of intracutaneous injections of a staphylococcus vaccine upon the subcutis of a guinea pig. Note the types of agranulocytes, varying from typical lymphocytes and monocytoid forms to typical macrophages. The tissue was excised twenty-five days after the end of the period of immunization. Stained with hematoxylin-eosin-azur II.

FIG. 2. Oil immersion drawing made at the level of the substage with the aid of the camera lucida, Leitz Oc. 4, Obj. 2 mm. apochromatic, stained by Goldmann's carmine method. This illustrates the mode of disposal of particulate material by macrophages in the subcutis of the guinea pig after intracutaneous immunization by ten injections of staphylococcus vaccine. This animal was injected intradermally with 0.2 cc. of a 4 per cent suspension of India ink in 0.85 per cent sodium chloride solution, and the skin excised twenty-four hours later. Note the active ingestion of ink particles by the macrophages and the absence of such ingestion by the lymphocytes, polymorphonuclear leucocytes and monocytoid cells. Note also the adherence of small particles to fibrils of collagen.

FIG. 3. Oil immersion drawing, made at the level of the substage with the aid of the camera lucida, Leitz Oc. 4, Obj. 2 mm. apochromatic, stained with hematoxylin-eosin-azur II. This drawing is from the subcutis of a normal guinea pig six hours subsequent to the intracutaneous injection of 0.2 cc. of a living virulent suspension of staphylococcus aureus. Note the active ingestion of staphylococci by the polymorphonuclear leucocytes, which cells are almost the only ones responding at this stage of the inflammation. Note also the diffuse distribution of these cells and of the microörganisms.

FIG. 4. Oil immersion drawing, made at the level of the substage with the aid of the camera lucida, Leitz Oc. 4, Obj. 2 mm. apochromatic, from section stained with hematoxylin-eosin-azur II. From the subcutis of a guinea pig previously immunized intracutaneously by injections of staphylococcus vaccine, and infected intracutaneously with 0.2 cc. of a living virulent suspension of staphylococci. Tissue excised eighteen hours after the infection shows many macrophages actively phagocytosing staphylococci. The infection remained localized to the site of inoculation.



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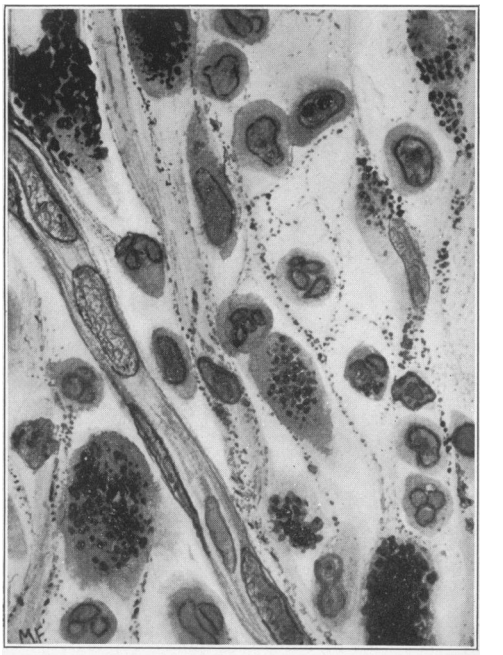

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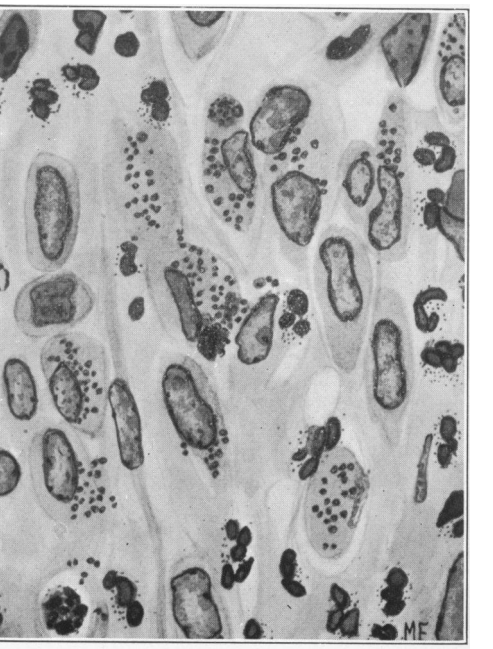

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PLATE 141

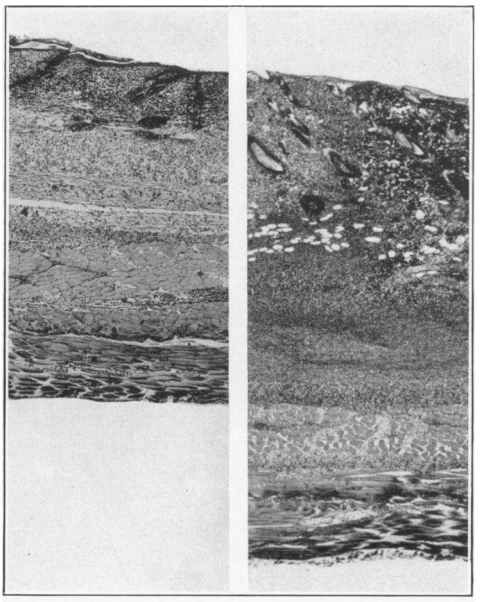
FIG. 5. Photomicrographs of sections through the skin and entire abdominal wall of (*a*) normal guinea pig and (*b*) intracutaneously immunized guinea pig. The tissues were excised six hours following the intracutaneous injection of 0.2 cc. of a living virulent suspension of staphylococci into each animal. In (*a*) there is slight edema of the subcutis with beginning infiltration of cells of inflammation. In (*b*) note the increased thickness of the subcutis and the more intense infiltration of cells of inflammation. × 25.

FIG. 6. Photomicrographs of sections through the skin and entire abdominal wall of two other guinea pigs treated as described in Fig. 5. The tissues were excised eleven to twelve hours following intracutaneous infection. × 25.

FIG. 7. Photomicrographs of sections through the skin and entire abdominal wall of two other guinea pigs treated as described in Fig. 5. The tissues were excised eighteen hours following intracutaneous infection. × 25.

FIG. 8. Photomicrographs of sections through the skin and entire abdominal wall of two other guinea pigs treated as described in Fig. 5. The tissues were excised twenty-two hours following intracutaneous infection. × 25.

Note in all instances the more intense inflammatory response in the subcutis of the immunized animals.



a b

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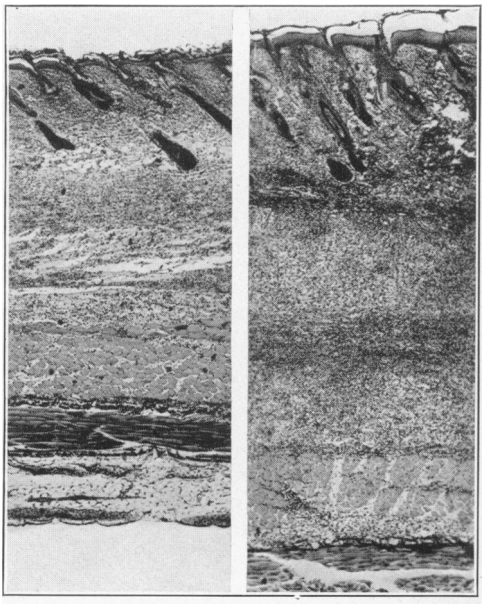

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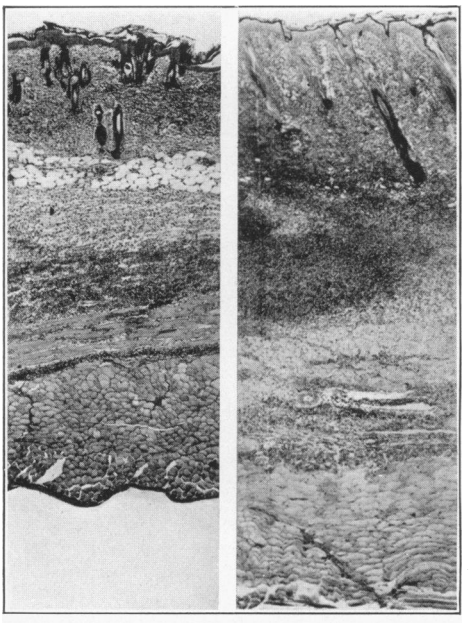

a b

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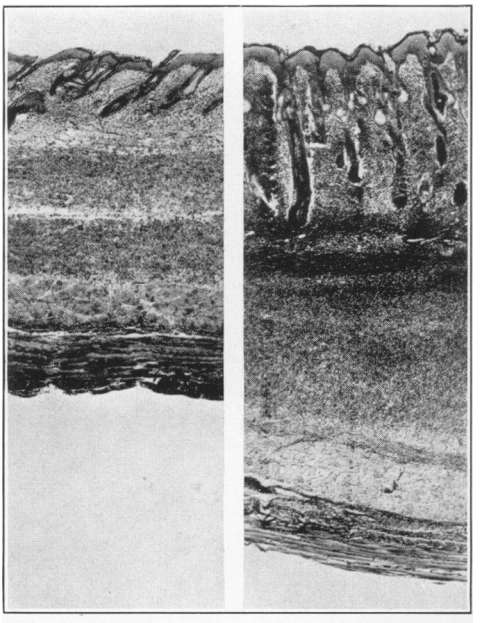

a b

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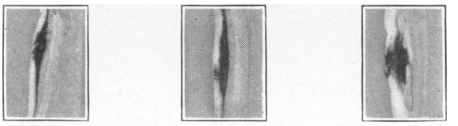
PLATE 142

FIG. 9. Photograph of celloidin-embedded tissues of the entire thickness of the abdominal wall from three guinea pigs, each having been injected intracutaneously twenty-four hours before with 0.2 cc. of a 4 per cent suspension of India ink in 0.85 per cent sodium chloride solution. (*a*) Normal. (*b*) Previously given ten intracutaneous injections of sterile peptone broth. (*c*) Previously immunized by ten intracutaneous injections of the staphylococcus vaccine, the last injection given eleven days before. Note the dissemination of the suspension of ink along the subcutis in Nos. 1 and 2 and the tendency to localization of the ink near the site of inoculation in No. 3.

FIG. 10. Photomicrograph of the subcutis of a normal guinea pig six hours following the intracutaneous injection of 0.2 cc. of a living virulent suspension of staphylococcus aureus. Note the diffuse distribution of the microörganisms with the tendency to spread along collagenic fibrils and to occur singly or in small clusters. × 1400.

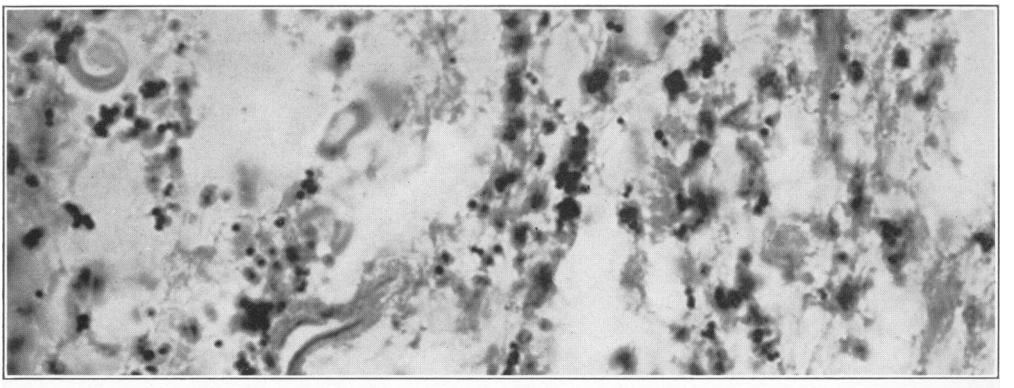
FIG. 11. Photomicrograph of the subcutis of a previously intracutaneously immunized guinea pig six hours following the intracutaneous injection of 0.2 cc. of the same suspension of staphylococci injected into the animal shown in Fig. 10. Note the greater tendency to concentration of the microorganisms, with the occurrence of the staphylococci extracellularly in coalescing clumps and clusters. The appearance is strongly suggestive of agglutination *in vivo*. × 1400.

FIG. 12. Photomicrograph of the subcutis of a guinea pig previously immunized intracutaneously, twenty-two hours following the intracutaneous injection of 0.2 cc. of a living virulent suspension of staphylococcus aureus. Note the large masses of extracellular staphylococci, in clusters large and small, suggesting agglutination *in vivo*. These masses were completely encircled by a dense accumulation of cells of inflammation, principally polymorphonuclear leucocytes and macrophages. There was no tendency for the infection to disseminate beyond this area of microörganisms. × 1400.



a b c

9



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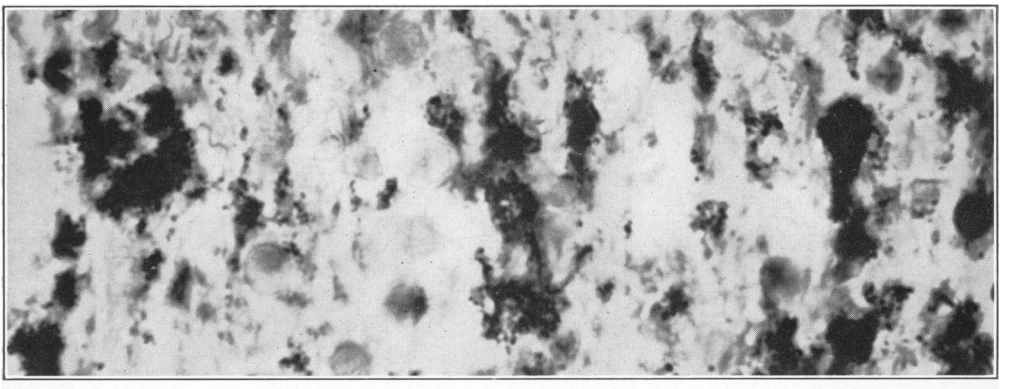

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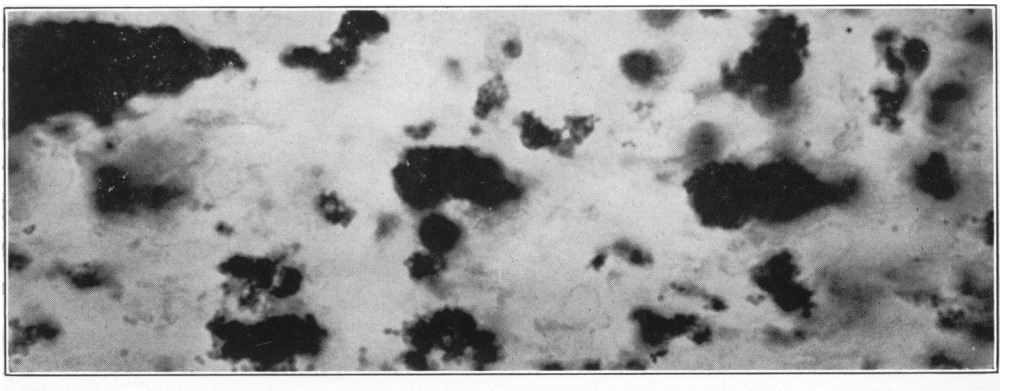

12