Part I. General Discussion of Immunology: Systemic and Local Factors

TISSUE RESPONSE IN IMMUNITY

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Introduction

A vital defensive response of the body is its ability to localize attacking microorganisms and wall them off in the area in which they gain entry. This response is often life-saving because it prevents the microorganisms from invading the blood stream and reaching the other tissues of the body. The response is not rare or occasional, but is a commonplace manifestation in immunity. Should a localized infection in a given tissue break down, causing the escape of microorganisms into the blood stream, the localizing mechanism is then manifested in other tissues of the body. The localizing capacities of different tissues and, particularly, the localizing capacity of the skin investigated in this laboratory some years ago,[1] are briefly summarized in PART I of this article. The correlation of the results with some other experimental findings on tissue reactions in immunity, including reactions which are generally interpreted on the basis of tissue hypersensitiveness, is considered in PART II of the article.

Few persons go through life without some readily visible localized infections, such as a streptococcic sore throat or a staphylococcic skin infection. What is the basis of the localizations of these microorganisms? Why do they not become widespread throughout the body? The answer generally given by students of immunity is that antibodies and phagocytes are responsible for these localizations. The studies herewith considered indicate that the initial localization of the microorganisms is a tissue response and is a result of the capability of the throat tissues, in the one case, and of the skin in the other, to detect these microorganisms and enter into specific physicochemical union with them. This union is believed to be akin to the union between phagocytes and microorganisms. The union between tissue cells and microorganisms results in injury to the cells. The injury calls forth a defensive response in the form of an inflammatory reaction. Antibodies and phagocytes play an important part in this reaction and, under favorable conditions, the local destruction of the microorganisms and the subsequent healing of the injured tissues take place.

When we lack immunity toward microorganisms, we lack the capacity to localize them. For example, human beings lack immunity to the *Treponema pallidum*. The result is that when these microorganisms gain entry into the body, they are soon found in the blood stream. Similarly, when a protein mixture, such as horse serum, is injected into the skin of a normal rabbit, it soon reaches the blood stream and no inflammatory reaction is noted in the area of injection. But when the horse serum is injected into the skin of a specificially immunized rabbit, it is localized in the injected area and is thereby prevented from entering the blood stream. Within several hours after the

injection, evidence of local inflammation is noted, reaching its height about 24 to 48 hours after the injection.

Also, in the interpretation of this experiment, the general belief is that the localization is the result of the union *in vivo* of the injected antigen (horse serum) with the circulating antibodies that have reached the area, a concept proposed by Opie.[2] Our view is that the cutaneous tissue of the immunized rabbit possesses the specific capability to detect the injected horse serum, to differentiate it from other antigens, and to enter into union with it. This union interferes with the normal life of the cutaneous cells, and the resulting injury calls forth an inflammatory reaction which, under favorable conditions, leads to the destruction of the horse serum, presumably by proteolysis, and to the healing of the local area. Inflammatory tissue is known to possess a marked localizing capacity, especially for specific antigen. Therefore, in local infections and local injections of antigen in an immunized host, the oncoming inflammatory response plays an important part in holding the microorganisms or antigen within the confines of the local area.

The tissue response to protein and microbial antigens which is herein considered does not include the response to endotoxins and exotoxins. As will be seen below, horse serum diphtheria antitoxin was used as a gauge in the study of the localizing capacities of different tissues for horse serum proteins, which also necessitated the use of diphtheria toxin. But the tissue response to the toxin was not investigated. Neither were histologic reports of the tissue response included in these studies. The aim was to present the results of a group of immunologic experiments believed to enlarge the knowledge of the role of the tissues in immunity, then to determine the extent to which the interpretation of the experimental results was applicable to some other tissue reactions in infection and immunity.*

Part I. The Localizing Capacities of Different Tissues in Immunity

Quantitative determination of the localizing capacities of different tissues for specific antigen. The method employed consisted of the immunization of rabbits to horse serum in accordance with a uniform plan, and of the determination of the localizing capacities of different tissues of the animals for the horse serum by employing, instead, horse serum diphtheria antitoxin standardized in toxin-neutralizing units. In determining the localizing capacity of a tissue for a given number of units of the horse serum antitoxin, a standard dose of diphtheria toxin was simultaneously injected under uniform conditions into the skin. The toxin dose, which readily reached the blood stream, was lethal to rabbits and, to neutralize it, the antitoxin injected into a tissue similarly had to reach the blood stream. If the antitoxin remained localized, the animal succumbed to the toxin. Obviously, the greater the localizing capacity of a given tissue for the horse serum antitoxin, the greater the number of antitoxin units that was localized in the tissue and prevented from

* Definition of terms used in this article: the term "tissue response" is used to differentiate this response from that of circulating antibodies and phagocytes, and is employed because visibly it is just what the term suggests—a tissue response. This definition does not preclude the possibility that the tissue response in the absence of circulating antibodies may not be attributable to tissue-retained antibodies. By the term "antibodies" is meant circulating antibodies. The term "tissue hypersensitiveness" is used only in accordance with the classical definition which is the opposite of defense, or "against defense". It should be added that detailed experiments upon which the views expressed in this article are based are presented elsewhere.[1]

reaching the blood stream and neutralizing the toxin; and when attempting to protect the animal from toxic death in these experiments, the number of antitoxin units injected into a given tissue had to exceed the localizing capacity of that tissue. Accordingly, the number of antitoxin units injected into a tissue that was necessary to neutralize the toxin was the gauge of the localizing capacity of that tissue.

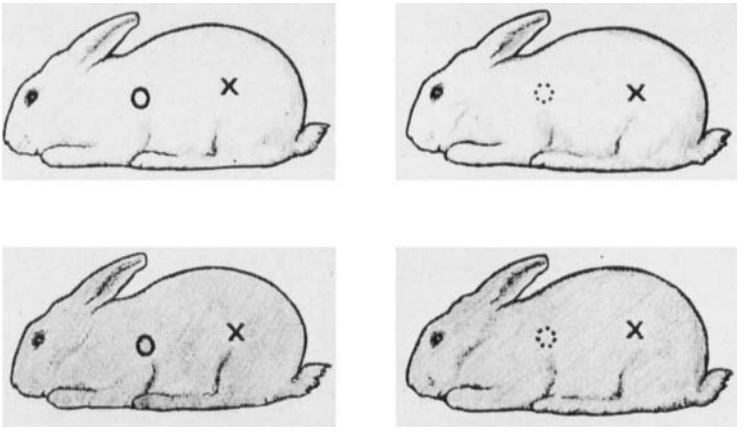
The localizing capacity of the skin of normal rabbits was first determined, and the experiments employed are briefly outlined as follows:

Localizing capacity of skin of normal rabbits. Six rabbits were inoculated intracutaneously with a standard dose, 50 MLD, for a guinea pig weighing 250 grams, of diphtheria toxin, under uniform conditions. Simultaneously, and in another area of the skin, the rabbits were inoculated with 1, 2.5, 5, 10, 15, and 20 units, respectively, of antitoxin. Results: only the rabbit inoculated with 20 units survived, while the others succumbed to the toxin. This experiment indicated that the skin of a normal rabbit was capable of localizing nonspecifically at least 15 units of antitoxin, thereby preventing a sufficient amount from reaching the blood stream and neutralizing the toxin.

Localizing capacity of skin of rabbits previously injected with horse serum. Twelve rabbits were given two injections of horse serum. The first, given intravenously, consisted of 0.2 cc. per kilogram of body weight. The second, given subcutaneously a week later, consisted of 0.1 cc. per kilogram of body weight. Eight days after the second injection, each of the rabbits was given 50 MLD of diphtheria toxin as above. Simultaneously, 0, 5, 10, 20, 50, 100, 200, 300, 500, 750, 1000, and 1500 units of antitoxin, respectively, were injected into the skin of the rabbits. The amounts of antitoxin employed were determined by previous experimental trial. Results: all of the rabbits succumbed to the diphtheria toxin, except the one inoculated with 1500 units of antitoxin. This experiment indicated that the skin of an immunized rabbit was capable of localizing at least 1000 units, thereby preventing the antitoxin from reaching the blood stream and neutralizing the toxin.

Evidently, two injections of horse serum into rabbits, which produced far from a strong immunity to this antigen, were sufficient to raise the localizing capacity of the skin for horse serum antitoxin from about 15 to 20 units to about 1000 to 1500 units. This experiment is illustrated diagramatically in FIGURE 1.

Localizing capacities of different tissues of nonimmunized, actively and passively immunized rabbits. The same experimental plan as above was employed in testing the localizing capacities of the subcutaneous tissues, peritoneal tissues, lung, spleen, uterine wall, popliteal gland, testis, liver, joint space, skeletal muscle, brain and blood stream of normal rabbits, and of animals previously given two immunizing injections of horse serum, employing the method indicated above. When necessary, the injections of antitoxin were made in tissues of anesthesized animals under operative procedures. Thus, when it was desired to determine the localizing capacity of the spleen for injected horse serum antitoxin, four horse serum immunized rabbits were anesthesized, the abdomen opened, and the spleen was exposed under surgical conditions. Diphtheria toxin (50 MLD) was then injected into the skin and, simultaneously, horse serum antitoxin was injected into the spleen. One of the rabbits was injected



O INJECTED ANTITOXIN VERY NEARLY COMPLETELY LOCALIZED

⁘ INJECTED ANTITOXIN INCOMPLETELY LOCALIZED

X INJECTION OF 50 MLD DIPHTHERIA TOXIN

FIGURE 1

THE QUANTITATIVE DETERMINATION OF THE LOCALIZING CAPACITY OF THE SKIN, EXPRESSED IN UNITS

Rabbits were first immunized to horse serum and the specific localizing capacity was then tested by the injection of horse serum diphtheria antitoxin into the skin, simultaneously with a lethal dose (50 MLD) of diptheria toxin.

The greater the localizing capacity of the skin for the horse serum antitoxin, the greater was the number of antitoxin units necessary to exceed the localizing capacity, escape into the blood stream, and neutralize the toxin.

Normal Rabbits
(Nonimmunized controls)

15 units antitoxin localized in injected area animal succumbed	20 units of antitoxin incompletely localized animal survived

Immunized Rabbits
(Based on two immunizing injections of horse serum)

1000 units of antitoxin localized in injected areas animal succumbed	1500 units of antitoxin incompletely localized animal survived

with 100 units of antitoxin, another with 200 units, a third with 300 units, a fourth with 400 units of antitoxin. The first three succumbed, the last one survived.

The localizing capacities of 13 tissues of normal rabbits and of rabbits actively immunized with horse serum are presented in TABLE 1. The localizing capacities of four tissues of passively immunized rabbits are also presented in the table. The passive immunization was carried out as follows: 14 rabbits were immunized to horse serum by employing the same technique as that used in the active-immunization experiments; namely, by a first injection of 0.2 cc. per kilogram of body weight given intravenously, followed about a week later by a second injection of 0.1 cc. per kilogram, given subcutaneously. Eight days

TABLE 1

LOCALIZING CAPACITIES FOR SPECIFIC ANTIGEN OF DIFFERENT TISSUES OF NONIMMUNIZED, ACTIVELY IMMUNIZED AND PASSIVELY IMMUNIZED ANIMALS, EXPRESSED IN UNITS

	Nonimmunized animals	Actively immunized animals	Passively Immunized animals
		Localizing units	
Skin	15.0–20.0	1000–1500	75–100
Under skin	15.0–20.0	750–1000	
Peritoneal tissue	5.0– 7.5	750–1000	25– 50
Lung	2.5– 5.0	400– 500	
Spleen	2.5– 5.0	300– 400	
Uterine wall	2.5– 5.0	200– 300	
Popliteal gland	5.0–10.0	200– 300	
Testis	5.0–10.0	200– 300	
Liver	2.5– 5.0	150– 200	
Articular space	5.0–10.0	150– 200	
Skeletal muscle	5.0–10.0	75– 100	25– 50
Brain	5.0–10.0	75– 100	
Blood stream	2.5– 5.0	50– 75	10– 30

later, the rabbits were exsanguinated and the plasma pooled. The total amount, which was 800 cc., was employed for the passive immunization of 17 normal rabbits. These animals were injected intravenously with 20 cc. per kilogram, and two days later the rabbits were treated with toxin and antitoxin exactly as were the actively immunized rabbits.

The results (TABLE 1) indicate that the tissues of normal animals possess very weak but measurable nonspecific localizing capacities; that the skin possesses the greatest localizing capacity, ranging from 15 to 20 localizing units; and that such tissues as the spleen and liver possess localizing capacities ranging from 2.5 to 5 localizing units. The tissues of actively immunized animals (based on two immunizing injections) possess relatively marked localizing capacities. The skin again shows the greatest localizing capacity, ranging, as was seen above, from 1000 to 1500 units. The localizing capacity of the subcutaneous and peritoneal tissues ranges from 750 to 1000 units; of the lung, 400 to 500 units; of the spleen, 300 to 400 units; of the uterine wall, popliteal gland, and testis, 200 to 300 units. A stronger localizing capacity of the liver tissue, which was 150 to 200 units, was expected. Skeletal muscle and brain showed localizing capacities of 75 to 100 units and, when the horse serum antitoxin was injected into the blood stream, 50 to 75 units were sufficient for the neutralization of the toxin.

In the passively immunized rabbits, the localizing capacity of the four tissues studied showed a range of 75 to 100 units when the antitoxin was injected into the skin, a range of 25 to 50 units when injected into the peritoneal tissue and in skeletal muscle, and 10 to 30 units when the antitoxin was injected into the blood stream.

It is understandable why the localizing capacity of the skin of an immunized animal is greater than that of the other tissues of the body. Immunologically, the skin guards against the entry of microorganisms into the body. The same

guarding action is undoubtedly manifested by the mucous membranes. The extensive localizing capacity of the peritoneal tissue may be attributable to the fact that the cellular elements of that tissue are in a highly active state. It may be that, in view of the constant presence of microorganisms and their products in the intestinal tract, some microorganisms or their products escape from time to time into the peritoneal cavity, thus helping to keep the cells in an active state.

The lung is next in line in possessing a marked localizing capacity. This tissue, being in direct contact with the atmosphere through the bronchial tree, is essentially a surface tissue, and would accordingly be expected to possess marked localizing capability. The localizing capacities of the spleen, popliteal node, liver, testis, uterine wall, and joint space follow in that order. Finally, toward the bottom of the list, are the localizing capacities of skeletal muscle and brain tissue, with the blood plasma at the very end. The relationship between anatomic structure and the defensive function, as manifested by the localizing capacities of the different tissues for specific antigen, is yet to be investigated.

The fact that the localizing response is manifested in passive immunity is proof that antibodies are capable of calling forth this response. However, the differences in the localizing capacities of the different tissues in passive immunity and in the absence of immunity are similar, and these differences are not nearly as marked as in active immunity. Thus: (1) in the absence of immunity, the localizing capacity of the skin was 15 to 20 units; of skeletal muscle, 5 to 10 units; (2) in passive immunity, the localizing capacity of the skin was 75 to 100 units; of skeletal muscle 30 to 50 units; (3) in active immunity, the localizing capacity of the skin was 1000 to 1500 units; and of skeletal muscle, 75 to 100 units. If, then, the localizing response in active immunity were attributable only to antibodies, the difference in localizing units of the skin and skeletal muscle should have been similar in active and in passive immunity. The unusually marked localizing capacity of the skin in active immunity would suggest that the intracutaneous tissues possess certain defensive capabilities over and above those due to circulating antibodies. If the localizing capacity of the skin were attributable to stored antibodies, it would have to be assumed that the body stores antibodies in the skin to a far greater extent than in other tissues.

Additional Data on the Localizing Capacity of the Skin

Development of localizing capacity. Experimental indications are that, 48 hours after a single immunizing injection of an appropriate amount of horse serum into a rabbit, an increase in the localizing capacity of the skin over the normal capacity becomes measurable. Accordingly, on the injection of 1 cc. of horse serum, previously diluted 1:1000 with physiologic salt solution, the localizing capacity of the skin for horse serum antitoxin is increased from 15 to 20 units to 25 to 30 units.

TABLE 2 gives the results of another experiment. Eighteen rabbits were given a subcutaneous immunizing injection of 0.2 cc. horse serum per kilogram of body weight. At specified intervals after the injection, a given num-

TABLE 2

DEVELOPMENT OF LOCALIZING CAPACITY OF THE SKIN FOLLOWING A SINGLE INJECTION OF HORSE SERUM

Days after immunizing injection	Localizing capacity of skin in units
0	15– 20
3	40– 75
7	150–200
10	200–300

ber of rabbits was tested for the localizing capacity of the skin. As is evident from the table, the localizing capacity on the day of the immunizing injection was 15 to 20 units; three days later it was 40 to 75 units; seven days later, 150 to 200 units; and, ten days later, 200 to 300 units.

These immunologic changes in the skin, beginning about 48 hours after the injection of protein, suggest that the period of incubation in immunity is not a dormant but an active period. It is possible that, during an incubation period in an infection, a subclinical struggle goes on between the host and microorganisms. If the host succeeds in destroying the invaders by the development of adequate defensive mechanisms, he will not succumb to the infection. Furthermore, as a result of this successful subclinicial struggle, he will have acquired active immunity to the microorganisms. If the host does not succeed in destroying the invaders during the incubation period, he will succumb to the infection and the struggle will be on a much larger scale. The tissues will then have developed relatively marked localizing capacities for the microorganisms, and a sufficient concentration of antibodies and phagocytes will have become available that are specifically directed against the microorganisms.

Subsidence of localizing capacity. TABLE 3 gives the results of an experiment in which the subsidence of the localizing capacity was measured. Twenty-seven rabbits were used in this experiment. They were all injected intravenously with 0.2 cc. of horse serum per kilogram of body weight and, about a week later, with 0.1 cc. per kilogram of body weight, given subcutaneously. It is evident from the table that 20 days after the second injection, the localizing capacity of the skin was 200 to 250 units; 31 and 50 days after, 50 to 100 units, and, 72 days after, 20 to 30 units. In another experiment to be described later, it was shown that 189 days after an immunizing injection of human

TABLE 3

SUBSIDENCE OF LOCALIZING CAPACITY OF SKIN FOLLOWING TWO IMMUNIZING INJECTIONS OF HORSE SERUM

Days after second immunizing injection	Localizing capacity of skin in units
20	200–250
31	50–100
50	50–100
72	20– 30

TABLE 4

INCREASE IN LOCALIZING CAPACITY OF SKIN AFTER REPEATED IMMUNIZING INJECTIONS OF HORSE SERUM

Number of immunizing injections	Localizing capacity of skin in units
0	15– 20
One	200– 300
Two	500–1000
Three	1750–2000
Four	2000–2500
Five	2000–2500

serum, rabbits still manifested the localizing capacity, shown by an inflammatory reaction in the skin to the injection of specific antigen.

Increase in localizing capacity. In another experiment, in which 29 rabbits were employed, it was desired to determine the extent to which the localizing capacity of a tissue for specific antigen may be increased by repeated immunizing injections of the antigen. The method consisted of repeated subcutaneous injections of 0.2 cc. of horse serum per kilogram of body weight, given at 10-day intervals, followed by the determination of the localizing capacity of the skin 10 days after the last injection. The results of this experiment (TABLE 4) in which five immunizing injections in rabbits were employed, and of another which consisted of eight immunizing injections, showed that after four or five immunizing injections, given under the conditions of this experiment, localizing powers of 2000 to 2500 units were reached. Beyond four or five immunizing injections, no further increase in localizing units was observed.

Localizing capacity in the young. In a series of experiments on the localizing capacities of the tissues of young rabbits, it was observed that these possessed but weak localizing capacities following immunizing injections. The results of one experiment are presented in TABLE 5. In the case of immature rabbits, six animals, seven days old, were injected subcutaneously with 0.25 cc. of horse serum, followed by a similar injection of 0.2 cc. of horse serum 10 days later.

TABLE 5

DIFFERENCES IN LOCALIZING CAPACITIES OF THE SUBCUTANEOUS TISSUES OF IMMATURE (26 DAYS OLD) AND MATURE RABBITS

Type of rabbits	Localizing capacity of skin in units
Normal controls	
Mature	15–20
Immature*	2.5– 5
Nine days after two subcutaneous injections of horse serum	
Mature	700–1000
Immature†	50– 75

* 9 to 11 days old
† 26 days old

In so far as the adult rabbits were concerned, these were given subcutaneously 0.2 cc. of horse serum per kilogram of body weight, followed 10 days later with 0.1 cc. of horse serum per kilogram, also subcutaneously. The methods of immunization were thus similar but not identical in the mature and immature rabbits.

The results of this experiment indicate that the subcutaneous tissue of normal, immature rabbits possesses a localizing capacity for foreign protein about one third of that possessed by the subcutaneous tissue of adult rabbits. After immunization, the localizing capacity of the subcutaneous tissue of the immature rabbits approximates one tenth of that manifested by the same tissue of the mature animals. The basis for the weaker localizing capability of young rabbits lies, very likely, in the immaturity of the defensive function.

Nature of the localizing response. It should be emphasized that we are not dealing here with antigens that are toxic, but with antigens that will call forth a local inflammatory reaction when injected into a tissue of a specifically immunized animal and will not call forth an inflammatory reaction when injected under similar conditions into a nonimmunized animal.

It is believed that a tissue cannot localize specific antigen unless the tissue cells possess an attractive force for the antigen and a capability to enter into some physicochemical union with it. This union involves also the capability of the tissue cells to detect specific antigen and to differentiate it from nonspecific substances. The union leading to the localization of the antigen is not considered unique in immunity. For the union between antibody and specific antigen may be said to be the localization of the antigen by the antibody, and the union between phagocyte and specific bacteria to be the localization of the bacteria by the phagocyte. It would appear, therefore, that a primary response in immunity is the development of the capability of the body tissues, the antibodies, and the phagocytes, to enter into specific combination with the antigen. It is possible, of course, that the capability of the body tissues as well as of the phagocytes to combine specifically with antigen is attributable to retained antibodies.

The skin shows a greater localizing capacity than that shown by other tissues, presumably because it is a surface tissue. Having been exposed to the outer world through evolutionary ages, it has built up a marked capacity to localize microorganisms, to prevent thereby their entry into the blood stream. Apparently, the same localizing capacity is manifested against other antigens, such as proteins, since the latter represent a major antigenic constituent of microorganisms.

It is assumed that the tissues of an immunized animal are capable of manifesting only an initial localizing response for the specific antigen. This initial response, while protective to the body as a whole, is injurious to the local tissue, just as phagocytosis is protective to the body as a whole, but injurious to the phagocytes. The local tissue injury calls forth an inflammatory reaction which greatly strengthens the localization, undoubtedly because inflammatory tissue, being rich in phagocytes and having a high blood volume containing antibodies, possesses a marked affinity for the specific antigen. The localization may also be strengthened by the fine network of fibrin formed within the

inflammatory area which provides a mechanical barrier against the escape of the antigen.

What becomes of the specific antigen within an inflammatory area? It can be demonstrated experimentally that, on the injection of a given amount of protein into the skin of a specifically immunized animal, the antigen in the form of precipitinogen is not found in the blood stream. It can also be demonstrated that extracts pressed from the local inflammatory tissue yield very nearly the total amount of antigen injected if obtained soon after the injection. But from one to five days after the injection, the amounts found in the extracts are gradually reduced, until none can be detected. Based on these findings it is assumed that the disappearance of the antigen from the injected area is the result of local proteolysis.

Although the studies herewith presented indicate that the tissues in immunity possess the initial capacity to localize specific antigen, it is not desired to stress the fact that this capacity may not be based on undetected antibodies. But until such antibodies are detected, it seems best to associate the initial localization with the tissues, since localization is visibly a tissue reaction. What we wish to stress is the defensive nature of the localizing response. This response is apparently so vital to the defense of the host that it becomes measurable as early as 48 hours after an immunizing injection of foreign protein, some days before circulating antibodies can be detected, and is still measurable about six months after an injection of antigen, weeks after such antibodies have disappeared.

Part II. Correlation of Localizing Response with Other Tissue Responses in Immunity

In this section, an attempt will be made to correlate the defensive role of the tissues, as manifested by the localizing response, with reactions generally classed as hypersensitive, such as the Arthus reaction,[3] anaphylactic shock,[4] and certain other tissue reactions in immunity. These reactions will be considered very briefly, since the aim is merely to determine whether they are hypersensitive or defensive responses.

Defensive inadequacy of localizing response—Arthus reaction. The basic aim of the localizing response is presumably the local destruction of the microorganism or antigen. This aim is not always fulfilled because a direct quantitative relationship exists between the degree of immunity of an animal and its capacity to localize and destroy specific antigen by means of an inflammatory reaction.

This quantitative relationship between the degree of immunity of rabbits to a protein antigen (horse serum) and the capacity of the animals to localize the antigen when injected into the skin was studied in this laboratory. It was found that, as is generally known, a rabbit that is weakly immunized to horse serum will manifest a slight localizing capacity to a subcutaneous injection of this antigen, followed by a mild inflammatory reaction in the injected area. If, in such a rabbit, an amount of horse serum is injected subcutaneously which exceeds the specific localizing capacity of the subcutaneous tissue, the excess will escape from the area of injection, enter the blood stream, and become dissem-

inated throughout the body. A rabbit that is strongly immunized to horse serum will, of course, localize a comparatively larger amount of injected antigen. The resulting inflammatory reaction will be more marked, and the injected horse serum will disappear from the local area and will not be found in the blood stream, indicating that it is destroyed locally. After a given number of days the local area will revert to normal.

Suppose, however, an amount of horse serum is injected subcutaneously in a strongly immunized animal in excess of its capacity to destroy the antigen by means of the inflammatory response. The localizing capacity of such an animal is generally so marked that it will be able to retain the excessive amount of injected antigen, but the inflammatory reaction may not be strong enough to destroy it completely. The inflammatory area will then become black and necrotic. Instead of increased blood volume, which is part of the defensive inflammatory response, the blood supply especially within the central inflammatory area is cut off. The blood vessels become filled with thrombi, and death of tissue is the result.

Indications are that the presence of free antigen in a given area within a specific inflammatory reaction will cause that area to become necrotic. Tissue necrosis as part of the specific inflammatory response to injected antigen, referred to as the Arthus reaction, is believed to be associated with the inability of an immune animal to destroy all of the antigen. That the animal is in a defensive state, and not in a state of local anaphylaxis or "against defense," is shown by the fact that it will readily localize and, by means of an inflammatory response, completely destroy nonexcessive amounts of injected antigen. Only when the amount injected exceeds the defensive capacity of the animal is tissue necrosis within the local inflammatory reaction noted.

That the presence of free antigen within a specific inflammatory reaction will lead to tissue necrosis can also be demonstrated by the following simple experiment. A specific inflammatory reaction is elicited in the skin of a rabbit that had been previously immunized by several injections of horse serum. A minute amount, such as 0.03 cc. of horse serum, is injected within the raised inflammatory area. The injected focus will then become black and necrotic. A similar injection of nonspecific antigen, such as human serum, may cause the injected focus to become slightly bluish temporarily, but no necrosis results and the discoloration soon disappears.

Still another way in which an inflammatory area can be experimentally changed into a necrotic area is by first injecting a small amount of the antigen into the skin of an immunized animal—enough to call forth a mild inflammatory reaction—and then to inject an excess of the same antigen intravenously. When the amount injected is sufficiently large, the local inflammatory reaction will very rapidly undergo necrotic changes, owing undoubtedly to the accumulation of the circulating antigen within the inflammatory area. This aspect will be discussed in the pages that follow.

Direct interference with localizing response—anaphylactic shock. Recognizing that the localizing response of an immune animal to infectious microorganisms is a vital defensive mechanism in preventing the microorganisms from entering the blood stream and becoming disseminated throughout the body, the

animal might be expected to show some untoward effects when this mechanism is disrupted by the injection of microorganisms directly into the blood stream. Recognizing also that an animal reacts to proteins in essentially the same way as it does to infectious microorganisms, the basis for anaphylactic shock becomes understandable. Accordingly, a first injection of antigen causes an animal to become immunized and to develop the capacity to localize the specific antigen and prevent its entry into the blood stream. Then, when this defensive response is antagonized by injecting the antigen directly into the blood stream, shock is the result. It is as if an ancient city fortified itself against an enemy attack by building an almost impregnable wall, and then suddenly found the enemy practically everywhere parachuting from the sky. Confusion, havoc, and the death of many citizens would be bound to follow.

In the study of differences in the specific localizing response of different tissues of rabbits, more than 1000 of these animals were uniformly immunized to horse serum by an intravenous injection of 0.2 cc. per kilogram of body weight followed 8 to 10 days later by 0.1 cc. per kilogram of body weight, injected subcutaneously. Not a single rabbit showed any untoward effect following the subcutaneous injection. Had the procedure been reversed, and had the animals been given a subcutaneous injection followed by an intravenous injection, a number of them would undoubtedly have manifested symptoms of shock, and would have been considered in an anaphylactic state. Evidently the intravenous injection would have directly interfered with the localizing mechanisms brought into play by the first injection.

If we were able to undertake studies of the localizing response of the tissues of guinea pigs to a protein antigen, we should simply avoid those experimental conditions that favor the production of anaphylactic shock. For example, we should avoid administering the protein in the blood stream, not only as a second injection but also as a first injection; the reason being that some guinea pigs show untoward effects even to a first injection. Instead, we should give several subcutaneous injections about five days apart, and thus eliminate also the 10-day incubation period after the first injection, which may lead to anaphylactic shock.

Why only a single injection of protein in some tissue is needed to sensitize a guinea pig to anaphylactic shock, and why the amount injected can be very small, we are not prepared to say. Nor do we understand why the second injection suddenly leads, among other things, to the contraction of smooth muscle, and causes so severe a spasm of the relatively rich bronchial musculature as to result in the asphyxiation of the animal.

But it is understandable why the second injection has to be given intravenously and in a larger dose than the first. However small the first dose might have been, it was apparently sufficient to develop in the animal defensive mechanisms against the entrance of the antigen in the blood stream: mechanisms developed through the ages for self-preservation in the struggle against microorganisms. The sudden breakdown of these mechanisms by the intravenous injection is apparently the forerunner of shock. A subcutaneous injection of the antigen may also produce shock in certain instances, but the

dose has to be many times larger than the intravenous dose, so as to permit the rapid entry of the antigen into the blood stream.

That passively immunized animals are sensitive to shock is also understandable. Since such animals manifest the tissue-localizing response, it would be expected that they would show shock when this response is interfered with by an intravenous injection. The dependence of anaphylactic shock on a specific antibody makes it difficult to explain why rabbits, which are far better antibody producers than guinea pigs, are less prone to manifest shock to an intravenous injection of the antigen.

As is well known, anaphylactic shock occurs only under conditions of injection. The presence of microorganisms or protein antigen in the blood stream under natural conditions produces no symptoms resembling shock. The study of any and all untoward effects associated with methods of injection is thus of the greatest importance. Medical practice has come to depend a great deal on injection methods. It is therefore essential to understand all effects resulting from the employment of these methods, aside from the substances injected.

Strange as it may seem, the classical concept of tissue hypersensitiveness, originally applied to a tissue response resulting from a particular injection, is commonly being applied to tissue responses in immunity under natural conditions. This application is so general that tissue responses in immunity have become almost synonomous with tissue hypersensitiveness. Thus is the student of infection and immunity constantly faced with the incongruity of correlating tissue hypersensitiveness with humoral and phagocytic defense. It is to overcome this difficulty that emphasis is given to the concept that anaphylactic shock is not attributable to a state of hypersensitiveness or "against defense" but to the interference with the defensive function by the intravenous injection.

In accordance with this concept, an infected or injected animal is always and invariably in a defensive state, and the defense is manifested jointly by the tissues, the antibodies, and the phagocytes. However, the defensive function may be inadequate in meeting a given situation; it may be interfered with by a particular method of injection; and, above all, it may manifest abnormalities just as other biologic functions manifest them. Abnormalities in the defensive function take us to allergic disturbances, the discussion of which is in adequate hands in this monograph.

Spontaneous intensification of localizing response—flare-up reactions. The spontaneous intensification of a preformed inflammatory response to specific antigen is a common tissue reaction in immunity. In order to bring about flare-up reactions to protein antigens in rabbits, it is necessary to have the animals in a highly immune state. For example, rabbits which were given but two injections of horse serum did not manifest the flare-up reaction. But rabbits given five or more injections, five to seven days apart, readily manifested this reaction.

A very small amount of horse serum, such as 0.1 cc. of 1:100 dilution with salt solution, was injected into the skin of such rabbits. A mild inflammatory reaction resulted. Then, by injecting a relative excess, such as 20 cc. of horse

serum intravenously, the inflammatory area was spontaneously intensified and became black in color centrally. A similar intravenous injection the next day resulted in ulcer formation within the inflammatory area. If the amount of horse serum originally injected into the skin was too small to call forth a visible inflammatory response, an appropriate intravenous injection of the same antigen caused the injected area to flare up and become visible.

At least three conditions are necessary to elicit specific flare-up reactions to protein or microbial antigens: (a) a given degree of immunity; (b) an inflammatory focus, visible or invisible, associated with the localization of specific antigen in a tissue; and (c) the presence of a given concentration of the same antigen in the blood stream. Apparently, the specific attraction of the inflammatory area for the circulating antigen leads to the accumulation of the antigen within that area and to the intensification of the inflammatory response. Actually, as the antigen accumulates in the inflammatory area, its concentration often exceeds the local antigen-destructive capacity. The presence of free antigen within the inflammatory area then results in tissue necrosis.

The defensive nature of spontaneous flare-up reactions is best illustrated by means of microorganisms. Suppose, in an immune animal, a localized streptococcic infection in the skin leads to an inflammatory focus which, at first, is too mild to prevent the escape of microorganisms. The result is the presence of the microorganisms in the blood stream which, of course, is of great danger to the life of the animal. The continued presence of the microorganisms in the blood stream indicates inadequate facilities for their destruction by phagocytosis and bacteriolysis, and may lead to multiple localizations in the body. It may lead to miliary localizations and death. But as the specific immunity of the animal is increased from day to day, the inflammatory focus is strengthened by the accumulation of phagocytes, the increase in fluid volume containing antibodies, and by other factors. The result is that the specific attractive force of the inflammatory area for the circulating microorganisms becomes increased. The microorganisms are then drawn from the blood stream, where facilities for their destruction are apparently inadequate, to the inflammatory focus where there exists more extensive facilities for their destruction. Actually, as the microorganisms accumulate in the inflammatory focus, their concentration may become so high as to overcome the defensive facilities of the inflammatory response, with the result that the flare-up reaction may include considerable destruction of the local tissue, leading to ulcer formation.

Other tissue reactions associated with the presence of antigen in the blood stream of a specifically immunized animal will be considered later. First, flare-up reactions in different situations in infection and immunity will be briefly summarized.

Spontaneous flare-up reaction in primary syphilis. A typical flare-up reaction is seen in primary syphilis. When the spirochetes first gain entry in, let us say, the skin, there exists no specific immunity to cause them to become so strongly localized as to prevent their reaching the blood stream. The nonspecific localizing response of the skin is apparently sufficient to establish a slight localization of the microorganisms, and the resulting tissue injury undoubtedly

leads to an inflammatory reaction so weak as to be invisible. Meanwhile, the microorganisms soon reach the adjoining lymph glands and the blood stream.

In about one month, the three factors may have developed which favor a flare-up reaction, namely: a high enough degree of immunity, a specific inflammatory focus where the microorganisms became localized, and the presence of a given concentration of the microorganisms in the circulation. Then, the specific attractive force of the inflammatory focus for the microorganisms draws them out of the blood stream into the focus, resulting in its intensification. As the microorganisms continue to accumulate in the focus, their concentration may become so high as to render the defensive inflammatory reaction inadequate for their destruction, the result then is necrosis of the local tissue and ulcer formation.

The question might arise as to why the assumption that the spontaneous flare-up of the primary lesion in syphilis is the result of the accumulation of microorganisms from the blood stream. Could not the microorganisms accumulate in the primary lesion without regard to the presence of the microorganisms in the circulation? The answer is, first, that the primary lesion follows the principles of experimental spontaneous flare-up reactions and, second, that the primary lesion occurs with the development of immunity and of spirocheticidal powers, and it is not likely that, at that time, the microorganisms would be growing freely within the primary focus to such an extent as to neutralize the defensive inflammatory reaction and lead to ulcer formation.

Since primary lesions are dependent upon three different quantitative factors, it is understandable why such lesions are not alway seen in early syphilis. For example, if the concentration of spirochetes in the blood stream is low, due to high spirocheticidal powers possessed by the infected individual, the numbers accumulating in the initial focus will be insufficient to cause a flare-up reaction, and no primary lesion will be visible. High immunity might also mean the successful destruction of the spirochetes within the initial localized focus and the prevention of the flare-up reaction.

Flare-up reaction in tularemia. This reaction is considered because, although the *P. tularensis* is explosively virulent, the basic immunologic cycle is the same as in early syphilis. Thus, the *P. tularensis* might become inoculated in the skin of the hand through an abrasion while skinning an infected rabbit. Due to the lack of immunity of the host, no inflammatory reaction is visible. The microorganisms soon reach the lymph glands, as in syphilis, and find their way into the blood stream. Associated with the rapid growth of the microorganisms in the blood stream, immunity begins to develop in the course of days (instead of in the course of weeks as in syphilis). Soon conditions begin to be favorable for the accumulation of microorganisms in the area of primary inoculation and for the flare-up of that area, and the result is ulcer formation.

Flare-up reaction in typhoid fever. In this disease, important disseminating foci of the typhoid bacilli are Peyer's patches and the solitary lymph lodes. The degree of the inflammatory response in the areas of initial localizations of the microorganisms is dependent on a number of factors, which include the size

of the infecting dose and the extent of natural immunity to typhoid bacilli possessed by the infected individual. It is assumed that the inflammatory response is inadequate to destroy the microorganisms locally, with the result that they escape into the blood stream where they circulate for the first week or 10 days. Under conditions of low bactericidal powers, large numbers of typhoid bacilli will undoubtedly be drawn from the blood stream to the initial inflammatory foci until the bactericidal powers may become strong enough to destroy the microorganisms. If, however, the microorganisms should continue to accumulate within the inflammatory foci in the intestinal wall, due to insufficient defensive powers of the host, the flare-up reaction may lead to such marked tissue necrosis as to result in the perforation of the intestine.

Flare-up reaction in experimental tuberculosis. If living tubercle bacilli are injected into the skin of a guinea pig, no significant local inflammatory reaction is evident. The guinea pig, lacking immunity to these bacilli, possesses no capability to localize them and to prevent or delay their entry into the blood stream. Only a weak, nonspecific localizing response comes into play. Meanwhile, the bacilli soon reach the regional lymph glands and the general circulation. In due time, the animal will have passed from a nonimmune into the immune state. The circulating bacilli will be drawn from the blood stream to the local area of injection and call forth a spontaneous inflammatory reaction and, as sufficient numbers of the tubercle bacilli accumulate in that area, ulcer formation will result.

Flare-up reaction in serum sickness. A given amount of horse serum antitoxin is injected into a patient. About 10 days later, the site of injection may flare up. The factors which govern this flare-up reaction are, in our opinion, the same as those which determine the flare-up reactions considered above. During the 10-day interval following the injection, the patient has developed a given degree of immunity and the horse serum antitoxin begins to disappear, presumably by proteolysis. In those patients, however, in whom the concentration of horse serum in the blood stream remains sufficiently high, the serum may accumulate in the area of initial injection so as to cause the weak or perhaps invisible inflammatory reaction to flare up.

Flare-up reactions in natural immunity. By the use of killed suspensions of staphylococci, streptococci, or the colon-typhoid group of microorganisms, we have been able to produce flare-up reactions in normal rabbits. All that is necessary is to inject a small amount of a given suspension into the skin. An inflammatory reaction will appear in 24 to 48 hours. This reaction is attributable to the natural immunity of rabbits to these microorganisms, since in the absence of immunity, no local inflammatory reaction would occur. Then, by injecting intravenously relatively large numbers, such as several cubic centimeters each containing a billion microorganisms, the inflammatory area will change from red to purple in color. With repeated intravenous injections of the microorganisms, the local area will ulcerate.

Exaggerated flare-up reaction—Shwartzman phenomenon.[5] This phenomenon is a flare-up reaction having certain distinctive features. To elicit a typical Shwartzman phenomenon, the following factors should be noted: (1) non-

immunized rabbits are employed; (2) the preparatory injection in the skin is made with a filtrate of bacterial origin; (3) the provocative injection may be made with some nonspecific agent; (4) the provocative injection must be made intravenously to call forth necrosis in the skin area; (5) it must be injected close to 24 hours after the preparatory skin injection; (6) if the provocative agent, whether specific or nonspecific, is injected directly in the preparatory skin area, no necrotic changes are noted in the area.

First, it will be recalled that rabbits possess a relatively high degree of natural immunity to practically all common microorganisms, especially to those of the colon-typhoid group. It is then conceivable that the preparatory injection of the bacterial filtrate activates the natural immunity sufficiently to stimulate the localizing capacity of the animals and the sensitivity to intravenous injections. Whatever the explanation of the Shwartzman phenomenon may be, the animals apparently are in an immune state, and a local injection, followed by an intravenous injection, fulfills the three conditions necessary to call forth flare-up reactions.

Temporary loss of localizing response. As already noted, a given concentration of antigen in the blood stream of an immunized animal causes a specific preformed inflammatory reaction to flare up. Actually, several other immunologic changes are noted. One of these occurs when the concentration of the specific antigen in the blood stream is sufficiently high. Then there is a temporary loss of the localizing response to the injection of the antigen into a tissue, such as the skin, accompanied also by the temporary loss of serum precipitins.

Thus, the intravenous injection into a specifically immunized rabbit, of 5 cc. of human serum per kilogram of body weight, will result in the loss of capability of the localizing response to an injection of human serum in the skin for a period of about six hours, after which the response begins to return. Serum precipitins also practically disappear during that time. But about five days later the titer is likely to be higher than before the injection. By that time, the specific antigen (precipitinogen) is generally no longer detectable in the blood stream. The explanation for the temporary loss of the skin response to the local injection of the antigen is that the tissues very likely have become saturated with the circulating antigen. This explanation is believed to be particularly true of the cutaneous tissue which has so marked an affinity for the antigen. It is therefore understandable why this tissue would not be capable of localizing additional antigen.

An intravenous injection of a killed suspension of microorganisms in a specifically immunized rabbit will result in no change in agglutination titer, but in a temporary loss in the capability to localize the same microorganisms when injected into the skin. For example, a rabbit showing a skin response to an injection of 200,000 killed typhoid bacilli was injected intravenously with 4 cc. per kilogram of body weight of a suspension of three billion microorganisms per cubic centimeter. One hour after the intravenous injection, no skin response was noted to the local injection of as many as two billion microorganisms per cubic centimeter. At the time of the loss of the skin response, the agglutination titer of the rabbit was not affected. As in the case of the protein antigen, the

loss of the skin response is believed to be due to the saturation of the tissues with the circulating microorganisms or their antigenic constituents. The lack of effect on the agglutination reaction is not understood.

Skin eruptions. It was reported in PART I that, in the presence of immunity, the cutaneous tissue possesses more than ten times the localizing capacity for injected antigen that some of the other tissues possess. Such a marked capacity must be based on a similarly marked affinity of the cutaneous tissue for specific antigen and, when this affinity is manifested at a time when the antigen is circulating in the blood stream, it would be expected that the antigen would be drawn to the cutaneous tissue.

Whether or not the antigen could permeate the cutaneous tissue and reach the outside, would probably depend largely on the molecular size of the antigen. Viruses would undoubtedly be more successful than bacteria in permeating the cutaneous tissue. Meanwhile, the cutaneous tissue cells would undergo some injury, however slight, by this close contact with the antigen, leading to redness which represents a partial inflammatory response to the injury. Mucous membranes also may be the sites of rashes because they too undoubtedly possess a marked affinity for circulating antigen.

Let us consider the secondary rash in syphilis. When the rash appears, the infected individual will have developed a considerable degree of immunity, shown by the fact that the spirochetes in the primary lesions have been or are being destroyed by spirocheticidal activity. But this spirocheticidal activity is apparently inadequate in ridding the circulation of the microorganisms. If this activity were adequate, the circulation would have become free of the microorganisms and there would be no secondary rash. Meanwhile, even in the face of weak spirocheticidal powers, the immune response of the tissues continues to increase and, in due time, the attractive force of the cutaneous tissues for the circulating microorganisms may become so pronounced as to draw them from the circulation outwardly. It is as though the body, finding that its antibody mechanism is inadequate in destroying the enemy in the blood stream, makes an effort to eliminate them through the surface tissues. Apparently, the first line of defense in syphilis is the development of spirocheticidal powers and, when this defensive mechanism is fully developed, most of the microorganisms are killed off and no secondary rash results.

Undoubtedly, marked differences exist in the capacities of the cutaneous tissues of different infected individuals to attract the spirochetes and draw them outwardly. It should be added that, while the rash is evidently a defensive mechanism in enabling the infected individual to eliminate vast numbers of microorganisms, it is also helpful to the parasite inasmuch as it is thereby enabled to continue its own survival by establishing itself in other individuals. The rash indeed illustrates a common characteristic in parasitism; namely, that a defensive reaction of the host may be of some advantage also to the parasite.

The severe skin eruption in smallpox might be taken as another illustration. The eruption generally makes its appearance by the third or the fourth day after the onset of the disease. The infected person is not yet able to destroy the virus. Still, the fact that, as the rash comes out the temperature falls, the

general symptoms subside and the patient feels comfortable would suggest that the rash is a defensive mechanism by which the body attempts to eliminate the virus. By the fifth day of the infection, the eruption becomes pustular. Having seen the extent to which tissue necrosis takes place in specific inflammatory areas in which the antigen accumulates, the likelihood is that the tissue necrosis within the pustules is due to continued growth of the virus within them.

In measles, the skin eruption is accompanied by Koplik spots in the mucous membrane in the mouth. Immunologically these spots must be the result of an attempt to draw the virus outwardly, based on an attractive force existing between the mucous membrane and the virus similar to that existing between the skin and the virus.

Rash formation may also be noted in human beings following injection of protein solutions, such as horse serum antitoxin. If, as immunity develops, the horse serum proteins are readily destroyed by proteolysis, the development of the marked attractive force of the cutaneous tissue for the proteins exerts no untoward effect on the host, because they have already disappeared. If, however, as immunity develops, the horse serum proteins are destroyed slowly, they may be present in high enough concentration in the blood stream some ten days after the injection, at the time when the cutaneous tissue is beginning to manifest a marked attractive force for them. The horse serum proteins will then be drawn to that tissue. Apparently, the body lacks the capacity to eliminate the proteins outwardly, as in the case of the outward elimination of the *T. pallidum* in the secondary rash in syphilis and of the virus during the eruption in smallpox. The result is edema and other symptoms clinically known as "serum sickness."

The question arises why, in an immunized host, when microorganisms circulate in the blood stream, they are drawn to the skin, resulting in rash formation only in certain infections, especially those associated with virus diseases and with spirochetes, but not commonly in infections of bacterial origin. The answer may be that rashes, as a sequel to the presence of microorganisms in the blood stream, are very likely governed by a number of factors, including the size of the microorganisms, especially their capacity to pass through membranes, their mobility, and the defensive capacity of the host to destroy them. When circulating spirochetes in syphilis are eliminated through the skin, resulting in rash formation, the indications are that the host has lacked the capacity to destroy them, and their elimination is in the nature of a last attempt on the part of the body to free itself of them. The same may be true of certain viruses circulating in the blood stream. In the case of circulating bacteria, however, phagocytosis may generally be successful in dealing with the microorganisms. Thus, Cannon, Sullivan, and Neckermann[7] injected living bacteria, such as staphylococci and paratyphoid bacilli, into the blood stream of rabbits and followed their disappearance from the circulation. In immunized animals, the bacilli were rapidly removed from the blood stream by various organs, particularly the liver and the spleen, where they were phagocytized by the macrophages and leucocytes.

Localizing response and nonspecific immunization. Rabbits immunized with human serum have been found to show a more marked localizing response than

normal rabbits to the subcutaneous injection of horse serum. When this response was measured by the unit scale described in PART I of this report, it was found that, whereas the skin of normal rabbits localized between 15 to 20 units of horse serum antitoxin, the skin of human serum-immunized rabbits localized between 30 to 40 units. This nonspecific increase in the localizing capacity of the skin was obtained after five immunizing injections of human serum, but not after two injections. The human serum had been heated for 30 minutes at 56° C. before injecting, and the amounts injected were 0.2 cc. followed by 0.1 cc. amounts per kilogram of body weight of the rabbits.

The subject of nonspecific immunity is a field in itself and cannot be discussed here comprehensively. It is in this area, however, that an answer may ultimately be found to the question as to why a febrile attack often leads to the production of nonspecific antibodies, such as typhoid agglutinins. It is as though the febrile condition places the antibody-producing mechanism of the body on the alert, awakening this mechanism to increased activity.

Localizing response to incomplete, modified, and split proteins. The incomplete protein employed was purified gelatin. The gelatin molecule lacks three important amino acids: cystine, tyrosine, and tryptophane. It is known that this incomplete protein does not call forth antibody formation, and it was desired to find whether it would elicit a localizing tissue response. It was found that normal rabbits gave no reaction to an intracutaneous injection of 0.1 cc. of a solution containing 0.1 gram of gelatin. Approximately a month after three immunizing subcutaneous injections of 0.1, 0.1, and 0.5 grams of gelatin, respectively, local inflammatory reactions were obtained to the intracutaneous injection of 0.01 gram of gelatin. Ten days after seven immunizing injections, as little as 0.0001 gram of gelatin called forth a positive skin reaction. No serum precipitins could be demonstrated at any time throughout the experiment.

The localizing skin response to racemized protein was then tried. Racemized proteins are prepared by digesting protein in large quantities of normal sodium hydroxide and permitting the mixture to stand for a long interval, during which time the plane of polarization of the protein is changed. The experimental plan consisted in giving rabbits five subcutaneous injections of racemized egg albumin in 0.05 gram amounts. Before the immunizing injections, and after the first and second injections, no skin reactions were observed. But after the third injection, a positive skin reaction was obtained to the intracutaneous injection of 0.01 gram of the racemized protein. After the fourth and fifth injections, positive skin reactions were obtained to the intracutaneous injection of 0.0001 gram. No serum precipitins were observed at any time during the experiment. Similar results were obtained when employing the soluble residue of the preparation of Vaughan's crude soluble poison from casein, also when employing three different proteoses. The results indicate that immunizing injections of incomplete, modified, or split protein, while incapable of calling forth detectable precipitins, are capable of eliciting the localizing tissue response.

The localizing response and skin tests in infection. Several decades ago, one encountered a considerable number of reports in the literature on the ty-

phoidin and luetin tests. Theoretically, as long as a host possesses sufficient immunity to hold infectious microorganisms localized and prevents them from circulating in the blood stream, he should localize microorganisms or their antigenic constituents when injected into the skin. But both the typhoidin and luetin tests gave weak or negative reactions in the early stages of typhoid fever and of syphilis. The reason becomes understandable when the relationship between the host and the microorganisms in the early stages of both diseases is considered.

It has already been shown that the introduction of protein antigen in the blood stream leads to a loss of the localizing capacity of the skin to injected antigen. The explanation was proposed that, in the presence of the antigen in the blood stream, the injection of the antigen into the cutaneous tissue does not lead to the physicochemical union between the tissue and the injected antigen necessary for the localizing response since that tissue may have already combined with all the antigen it could hold. It would therefore be expected that, at a time when the typhoid bacilli circulate in the blood stream, the typhoidin test would be weak or negative, and as long as the *T. pallidum* circulates in the blood stream, the luetin test would be weak or negative.

While the tuberculin reaction is generally classed as an allergic response, immunologically it follows the same principles as other skin reactions in infection and immunity. When a tuberculous patient possesses the capability of holding the tubercle bacilli localized, whether in an entire lobe of a lung or in a small tubercle, the skin will possess the capability of localizing injected tuberculin. In miliary tuberculosis and in situations when the tubercle bacilli circulate in the blood stream, tuberculin tests are likely to give weak or negative reactions.

A practical aspect of skin tests that must be kept in mind is the fact that the resulting reactions may have elements of nonspecificity. It has been seen that, measured by nonspecific localizing units, the normal skin possesses 15 to 20 localizing units while such tissues as the liver or the spleen possess 2.5 to 5 units. This high localizing capacity of the normal skin may lead to nonspecific positive skin reactions.

The localizing response of the skin apparently is lost before death. Prior to the development of modern therapeutic methods in septicemia, we had occasion to study the effect of injecting 0.1 cc. amounts of suspensions of microorganisms into the skin of patients from whose blood stream the microorganisms were isolated. The patients who did well gave positive reactions, but those who did not recover gave negative reactions. It may be mentioned in this connection that Francis[6] reported no skin reactions in patients with Type I pneumonia to the injection of the specific soluble substance of Type I pneumococcus when the patients did not recover, and positive skin reactions when the patients recovered.

Localizing response in latent immunity. The term "latent immunity" has been applied in this laboratory to the conditions in which, following an immunizing injection of antigen in an animal, the immunity is permitted to subside. If the immunizing injection consists of a protein, the precipitins will generally disappear in from one to two months. Increasing the immunizing

dose does not necessarily prolong the period in which precipitins will be circulating in the blood stream. Thus, in an experiment with six rabbits, numbers 1 and 2 were injected with 0.1 cc. horse serum subcutaneously; numbers 3 and 4 with 1 cc.; and numbers 5 and 6 with 10 cc. of horse serum. Precipitin titers were determined from time to time. It was found that rabbits 1 and 2 showed no precipitin titers 49 days after the immunizing injection. Sixty-three days after, rabbit 3 showed a titer of 1000, and 70 days after, rabbit 4 still showed a titer of 10,000. In the case of rabbits 5 and 6, however, injected with 10 cc. of horse serum each, the titer 63 days after the injection was only 100. All these rabbits, and one other immunized with 25 cc. of horse serum, were precipitin-free 189 days after the immunizing injection. Yet they continued to manifest a relatively marked localizing response to the intracutaneous injection of horse serum.

As is well known, an outstanding feature of the latent immune state is that an injection of a small amount of the specific antigen serves as a "booster" in rapidly reactivating the immunity. This fact would indicate that an injected animal, months after the disappearance of precipitins, continues to be specifically different immunologically from one which has never been immunized. Whether this difference is, in some way, associated with the existence of a residual capacity for the localizing response, is yet to be determined. The fact that the localizing response is manifested six months after an immunizing injection emphasizes the importance of this response to the life of the animal.

The defensive response defined. The defense against harmful microorganisms is so commonplace and vital to animal life that it is considered a physiologic function—a function that is believed to be manifested jointly by the antibodies, phagocytes, and the tissues. Since immunity to microorganisms means immunity to their antigenic constituents, it is to be expected that an animal will react to other antigens, such as proteins, by utilizing very much the same defensive mechanisms that are directed against the microorganisms.

Any physiologic function is likely to manifest disturbances, particularly of hypo- and hyperactivity. Thus, we may have hypo- and hyperthyroidism, or hypo- and hyperacidity, *etc.* A common disturbance of the defensive function is the specific hyperactivity of that function, as when the localizing response herein discussed manifests itself against some pollens or dust particles, or when a flare-up reaction results from the injection of traces of antigens or allergens.

While the defensive function may manifest a number of abnormalities, none of these, it seems to us, require for their explanation the existence of a biologic state in which a living being is against its own defense, implied by the classical definition of anaphylaxis. Such a state in infection is obviously equivalent to being lined up with the enemy. The struggle to hold on to life whether it be by an animal or by a cell, as emphasized by biologists, speaks against the existence of such a state.

That a physiologic function may break down, to the extent of doing great injury to the individual, is a common occurrence. When the function of growth undergoes abnormal changes and becomes markedly hyperactive, cancer and death may result. In the defense against microorganisms, serious functional errors might occur to the detriment of the individual; as, for example, when phagocytes which have attracted microorganisms for purposes of their destruc-

tion not only fail to destroy them but actually spread the infection. However, for the body to be against its own defense in a world in which parasitism is so commonplace raises the question as to how survival could have been possible through the evolutionary ages.

Our concern here is not with the nomenclature in common use in describing the response of the tissues in immunity. Our aim is to emphasize that this response is a defensive one. If this presentation accomplishes this aim, its purpose will have been fulfilled.

Summary

Immunologic tissue reactions to protein and microbial antigens are considered in this article. Tissue reactions are widely interpreted by the concept of tissue hypersensitiveness, the classical definition of which is "against defense." Based on this concept, the tissue response in infection and immunity has been difficult to correlate with humoral (antibody) and cellular (phagocyte) defense, for the implication is that the body is divided against itself. This difficulty led to the undertaking of tissue reaction studies in this laboratory a number of years ago.

A basic defensive reaction is the ability of the tissues to localize microorganisms or antigens and thus prevent their dissemination throughout the body. This localizing capacity was studied quantitatively in 13 different tissues. It was found that the skin possessed this capacity to a far greater degree than the other tissues. The onset, duration, and subsidence of the localizing response following immunization were also investigated. This response was then considered in relation to the Arthus reaction, anaphylactic shock, "flare-up" reactions, skin tests, skin rashes, and to other tissue responses associated with immunity.

The results herein presented make it difficult to interpret tissue responses in infection and immunity on the basis of tissue hypersensitiveness, but they lend themselves to ready interpretation on the basis of tissue defense. This interpretation assumes joint defensive action by the tissues, the phagocytes and the antibodies in infection and immunity and gives unity to the response of the body as a whole when called upon to react to antigens or to defend itself against attacks by microorganisms.

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