THE EFFECT OF INFLAMMATORY REACTIONS ON TISSUE IMMUNITY

By FRANKLIN M. HANGER, M.D.

With the Technical Assistance of Mr. Michael Bauer

(From the Department of Medicine of the College of Physicians and Surgeons of Columbia University, and the Presbyterian Hospital, New York)

Plates 18 and 19

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The problems of immunity in the past have been approached chiefly by noting alterations in the blood plasma. Although it has long been recognized that the invaded tissues also play a significant rôle in resistance, the difficulties encountered in studying this mechanism have greatly retarded a complete understanding of the problem. Most of the studies on cellular immunity have been devoted to various aspects of tuberculosis, for in this disease true humoral immunity is strikingly absent. Only recently has it been adequately appreciated that most "normal individuals" harbor a variety of pathogenic organisms in chronic foci in the naso-pharynx or elsewhere in the body and derive from them a generalized alteration of tissue response quite analogous to that found in tuberculosis (1). This bacterial allergy is usually demonstrated by injecting small amounts of broth filtrates of the organism intradermally and noting the degree of redness and infiltration that has developed 24 hours later. The skin reactions are not type specific and may be demonstrated for many organisms biologically unrelated. The relationship of tissue reactivity to bacterial invasion and the development of disease is obviously difficult to study in the human being. Consequently we have transferred our investigations to rabbits using as our infecting agent *B. lepisepticum* which is harbored in the naso-pharynx and is the cause of the majority of acute and chronic lesions of the respiratory tract in this convenient laboratory animal (2, 3, and 4). Infections of the skin chiefly have been employed because here it is easiest to observe the progress of the lesion.

Before undertaking any experiments it has been our routine procedure to skin-test the animals with 0.2 cc. of a 48-hour broth filtrate of *B. lepisepticum*. Readings were made 24 hours later and the animals classified, according to the size, redness and induration of the injected skin, into the following groups: (1) "negative reactors," (2) "weak reactors," (3) "moderate reactors" and (4) "strong reactors." This classification has proved important because the strong reactors show a definite tendency to localize the infection, even when the serum in such animals (in those animals tested) shows no agglutinins or demonstrable protective substances against the organism used.

TABLE I

Comparative Infections in Strong Reactors and Weak Reactors

	Total infection mortality	Died		Survived	
		Large lesion	Small lesion	Large lesion	Small lesion
Strong reactors.....	9	7	2	5	13
Total number, 27.....	(33.3%)	(25.8%)	(7.4%)	(18.5%)	(48.1%)
Weak reactors.....	40	34	6	3	10
Total number, 53.....	(75.5%)	(64.1%)	(11.3%)	(5.6%)	(18.8%)

Contrasted Infections in Weak and Strong Reactors

The negative and weak reactors, unless serological immunity is present, show but slight ability to limit the spread of the local lesion. After the injection of about 0.1 cc. of a light suspension of a virulent strain of *B. lepisepticum* (R. D.) into the skin of the flank, the characteristic infection begins as a small, pale papule at the site of inoculation and is well developed in 24 hours. Hemorrhagic necrosis appears quickly and from its intensity may be predicted the severity of the infection which will subsequently develop. Within from 5 to 10 days the lesion spreads downward over the abdomen forming a large, black oedematous mass. The animal becomes feverish and frequently dies. Post mortem examination usually reveals a positive blood culture but seldom metastatic lesions.

The strong reactors may occasionally develop similar large lesions

and succumb to the infection; in such cases they quickly lose all skin reactivity, hemorrhagic necrosis appears and the progress of the disease is in every way identical to that in the poor reactors. More frequently, however, the site of the infection is larger and redder after the first 24 hours and spreads but little thereafter. Histologically, the typical lesions of the strong reactors show more leucocytes, both of the polymorphonuclear and monocytic variety, and much less cellular disintegration than those of the weak reactors.

In Table I is shown the outcome of comparable infections in a series of eighty rabbits. It is obvious that there is a higher degree of resistance among the strongly reacting groups, this immunity being manifested chiefly by lesions more limited in extent. It might be argued that the presence of allergy presupposes a past "infection experience" from which a generalized immunity might also be derived. Evidence for this cannot be demonstrated in the serum of these animals. It therefore appears that the immunity furnished is *cellular* in nature and the present work is reported in an attempt to analyze some of the factors in such a type of immunity.

Infection of Skin Areas Previously Infiltrated with Bacterial Filtrates

Much study has been devoted to the fate of various pathogenic bacteria when injected into inflamed tissues. The results reported depend upon the organisms used, the irritant employed, and the duration and intensity of the inflammation before the infecting agent is introduced. An excellent summary of the important work on this subject has recently been made by Opie (5).

Besredka (6) observed that filtrates of certain bacteria applied to the skin rendered the skin immune to these bacteria and suggested the use of these filtrates therapeutically. Gay (7) and his co-workers have shown that a non-specific sterile inflammation which caused an increase of clasmatocytes protected the pleura of rabbits against streptococcus. Rivers and Tillett (8) and Mallory and Marble (9) have demonstrated a local protection of the skin to streptococcus and staphylococcus by infiltrating the dermis 24 hours previously with bacterial filtrates, or even plain broth, which suggests very strongly that there is no specificity to the phenomenon.

We have been able to demonstrate a local protection of the skin to virulent *B. lepisepticum* by infiltrating 24 hours previously with

filtrates of various strains of this organism. Equal protection, however, was obtained with filtrates of *B. coli*, *Streptococcus hemolyticus* and to a less extent with plain broth. When filtrates and organisms are injected into the tissues simultaneously, there is no demonstrable protection.

Normal rabbits showing weak skin reactions, and presumably susceptible to infection, were shaved over the flanks at least 48 hours before the experiments. An area of skin about 4 cm. in diameter was infiltrated with one of the above filtrates by injecting small amounts at adjacent points. The total quantity employed was usually 0.7 cc. 24 hours later slight redness and oedema were present depending upon the reactivity of the skin of the individual animal. Skin thus prepared was injected with 0.1 cc. of standard virulent strain of *B. lepisepticum* (R. D.). In some animals a similar injection was made in the opposite flank.

TABLE II

Effect of Infiltrating Skin with Bacterial Filtrates 24 Hours before Inoculation with Virulent B. lepisepticum

Total number of rabbits, 26	No necrosis Infection localized to filtrate treated skin	9	34.7%
	No necrosis in filtrate treated area Infection spread beyond filtrate area with usual necrosis	12	45.7%
	Necrosis present in filtrate treated skin	5	19.2%

In others no control injection was made in order to avoid the possible inhibiting effect of another lesion. Many of this group were infected intracutaneously several days later to demonstrate absence of generalized immunity.

The results summarized in Table II indicate roughly three different types of response:

1. A complete localization of the lesion to the infiltrated skin.

2. Spreading of the lesion with development of the usual large necrotic area over the abdomen, *but in which the skin showed no necrosis in the filtrate-treated portion.*

3. Lesions similar to the above (2) except for necrosis in the filtrate-treated portion. This necrosis was usually very slight except in one case. This animal received only broth infiltration before being injected.

Infection in the prepared skin often produces, within a few hours, a diffuse, red lesion in which the absence of necrosis is very striking and in which healing takes place more promptly than in control infections. This absence of necrosis was also noted by Mallory and Marble and appears to the writer to be of great significance, for we can assume that the inflammation surrounding a lesion is due to the diffusion of bacterial products resembling those found in filtrates. A non-specific mechanism for localizing certain infections might be assumed to exist if this zone of preparation antedates the bacterial invasion by sufficient time or if the animal possesses cells capable of rapid stimulation by these substances, as is the case in the allergic group.

Infection of Skin Areas into Which B. lepisepticum Immune Serum Has Been Infiltrated

The type of resistance just described must not be confused with that conferred upon an area of skin by injecting previously or simultaneously with the organisms a small amount of immune serum. We have employed for our experiments a stock mixture of sera obtained from rabbits convalescing from a large cutaneous infection caused by *B. lepisepticum.* This serum contains practically no agglutinins for the organism, but protests in minute amounts an area of skin infiltrated with it several hours before infection. In such cases the site of inoculation is hardly visible. When organisms and immune serum are injected simultaneously there is often a redness and induration at the site of injection which tends to heal rapidly and never develops into a formidable lesion, even in the most susceptible animal. In the last analysis the efficacy of immune serum in *B. lepisepticum* infection is its ability to protect the tissue cells of all varieties from bacteria and their injurious products.

Infection of Skin Areas Reacting to Various Chemical Irritants

Before analyzing the various factors involved in the localization of infection in animals showing no serum immune substances, it is important to contrast the effect of filtrates described above with that of various chemical irritants such as dilute acetic acid, croton oil and xylol.

Normal weak reactors were injected intradermally with small amounts of one of these substances. Within 24 hours a large red lesion developed with varying degrees of necrosis at the point of injection and often with puffy oedema over the dependent portions. Bacteria injected into such an area spread rapidly, produc-

TABLE III

Infection of Areas of Skin Inflamed by Chemical Irritants 24 Hours Previously

Animal number	Chemical irritant used	Amount of irritant	Severity of local inflammation	Result of infection of local inflamed area after 24 hours	Result of infection of a control area of normal skin after 24 hours
		cc.			
3-42	Acetic acid 2%	0.5	Severe	Very large, necrotic lesion	Not done
3-43	Xylol	0.5	Severe localized	Large, necrotic, spreading	Not done
3-53	Croton oil	0.1	Severe oedema	Very large, necrotic, spreading	Not done
4-72	Acetic acid 5%	0.1	Very slight	Moderate 3.5 x 3 cm., spreading	1 x 1 cm.
4-73	Acetic acid 0.25%	0.1	Very slight	Moderate 3 x 2.5 cm., spreading	1 x 1.5 cm.
	Infection of Similar Areas in Immune Animals				
2-45	Acetic acid 2%	0.5	Moderate oedema	Large 5.5 x 5 cm. No spread	Moderate 4 x 4 cm.
2-68	Acetic acid 2%	0.5	Very slight	Small 2 x 2 cm. No spread	Small 1.5 x 1.5 cm.
2-78	Croton oil	0.1	Severe oedema, moderate necrosis	Large 7 x 3 cm. No spread	Small 1 x 1 cm.
2-81	Croton oil	0.1	Moderate oedema, slight necrosis	Large 8 x 3 cm. No spread	Small 1.5 x 1.5 cm.
2-99	Xylol	0.2	Moderate lesion red, localized	Moderate 4 x 4 cm. No spread	2 x 2 cm.
3-02	Xylol	0.2	Moderate lesion red, localized	4.5 x 4 cm. No spread	Small 1.5 x 1 cm.

ing a large necrotic lesion which usually proved fatal within a few days. Even when the amount of irritant was very small the dissemination of bacteria seemed to be increased. 24 hours later the infected irritated site was redder and larger than a control infection made elsewhere in normal skin. However, like a filtrate-treated area, no necrosis appeared at the areas receiving small amounts and healing

began much sooner. This confirms the work of Gay and others that the intensity of the previous injury often determines the outcome of local infection. (See Table III.)

Animals immunized to *B. lepisepticum* were treated similarly. Twenty-four hours after injecting an area with one of the above-mentioned irritants, the site was inoculated with *B. lepisepticum.* The infection spread throughout the inflamed area producing a lesion proportional in severity to that of the preliminary reaction. The immune animals never became ill, the lesions did not spread beyond the limits of the chemical irritation, necrosis was not so extensive, the discharge from the lesion was more purulent and, most striking of all, healing began early and developed more rapidly at the site previously inflamed than in a control infection in the same animal.

Immune serum injected mixed with the irritant is quite effective in controlling an infection induced the next day in the irritated area. A small series of normal rabbits received 0.4 cc. immune serum mixed with 0.1 cc. croton oil or 0.5 cc. of 2 per cent acetic acid, and 24 hours later, the lesions were inoculated with organisms but showed only slight infiltration which healed rapidly in the portion receiving the immune serum, but outside this protected area showed the characteristic necrotic spread. This observation is of some interest when contrasted with the fate of immune serum injected with a protein antigen to be described in the next experiments.

Infection of Skin Areas Reacting to a Coagulable Protein to Which the Animal Has Been Previously Sensitized

When organisms are introduced into a wheal of reacting skin of an animal sensitized to a coagulable protein, a decreased tissue resistance is observed similar to that obtained when tissues are inflamed by chemical irritants.

Normal animals were sensitized to egg albumin by injecting 1 cc. of egg solution subcutaneously at 5-day intervals for 15 days. When 0.2 cc. of egg solution was then injected intradermally the familiar pink, oedematous lesion of hypersensitiveness developed in 24 hours. Organisms injected into it spread with amazing rapidity throughout the whole reacting area, so that cultures made 18 hours later were strongly positive 10 cm. or further from the point of inoculation. Rapid necrosis appeared and the animals practically always succumbed. (See Fig. 1.)

The extent of the lesion in the first 24 hours was in proportion to the degree of hypersensitiveness shown by the animal.

We have studied the effect of immune serum on this type of infection and have obtained results which may throw some light on the nature of the underlying processes:

I. Infection of Egg-Reacting Sites in Animals Immune to the Infecting Organism

Rabbits were selected which had survived an infection with *B. lepisepticum* and which had protective substances demonstrable in the serum in such concentration that 0.2 cc. injected into the skin of susceptible rabbits protected locally against a large number of *B. lepisepticum* injected into the same site.

TABLE IV

Results of Infecting Areas of Egg Reacting Skin in Animals Immune to B. lepisepticum and Sensitized to Egg Protein

Rabbit number	Intensity of skin reactivity to egg protein	Extent of infection in egg reacting site after 24 hours		Extent of infection in control site after 24 hours	
3-98	Marked oedema Sl. hemorrhagic	Large	8 x 4 cm.	Small	0.7 x 0.7 cm.
4-00	Marked oedema Mod. hemorrhage	Large	7 x 4 cm.	Small	1 x 0.7 cm.
2-72	Marked oedema	Moderate	6.5 x 5 cm.	Small	2 x 2 cm.
2-68	Moderate oedema Sl. hemorrhagic	Moderate	5 x 4.5 cm.	Small	1 x 1 cm.
2-66	Moderate oedema	Moderate	4.5 x 3.5 cm.	Small	3 x 2 cm.
3-87	Slight	Small	2.5 x 2 cm.	Small	1.5 x 1.5 cm.
3-88	Very slight	Small	1.3 x 1.2 cm.	Moderate	2.3 x 3 cm.
3-89	Very slight	Small	1.2 x 1.5 cm.	Small	1.5 x 1.3 cm.
3-99	Very slight	Small	1.7 x 1.7 cm.	Small	2 x 2 cm.

These immune animals were sensitized to egg albumin as outlined above, skin-tested with egg, and the reacting skin inoculated with virulent *B. lepisepticum* 24 hours later. A control injection with the organism was made in the opposite flank.

In many animals which showed a strong reaction to egg protein, there appeared a large red lesion which spread to the margins of the reacting area but never beyond. The control sites were always small red papules or pustules which never attained a comparable size and healed rapidly. (See Table IV.)

The importance of this is obvious in explaining the development and persistence of local lesions in individuals having a high humoral im-

munity. On the one hand the cells locally may become so altered that the immune bodies of the blood do not penetrate into the lesion or, on the other hand, the cells are so injured that the immune bodies no longer exert the usual protecting mechanism. In animals which did not react strongly to the egg there was quite the opposite effect. The lesions were actually smaller than in the controls and healed more rapidly.

TABLE V

Infection by B. lepisepticum of Areas of Skin Reacting to Egg into Which Bacterial Immune Serum Was Injected Mixed with the Egg Antigen

Rabbit number	Amount of egg protein in skin	Amount of serum mixed with egg	Intensity of skin reactivity to egg serum mixture (after 24 hours)	Infection in egg reacting site (after 24 hours)		Control infection into normal skin of anima (after 24 hours)	
	cc.	*cc.*					
4-28	0.3	1	Marked	Mod.	6.5 x 5.5 cm.	Small	2.5 x 2.5 cm.
4-30	0.3	0.2	Mod. oedema	Mod.	4 x 3.5 cm.	Small	1 x 1 cm.
4-22	0.3	2	Marked oedema	Large necrotic	7 x 5 cm.	Small	2.5 x 1.5 cm.
4-33	0.3	0.2	Slight	Small	1 x 1 cm.	Small	1.5 x 1.5 cm.
4-25	0.3	0.4	Marked oedema	Large	9.5 x 5 cm.	Small	2.5 x 2.5 cm.
4-44	0.3	4	Marked	Large	8 x 5 cm.	Mod.	3 x 2.5 cm.
			Controls Using Normal Serum Mixed with Egg				
4-29	0.3	0.1	Marked	Large necrotic	7.5 x 5 cm.	Small	2.5 x 2 cm.
4-35	0.3	0.2	Slight	Small	2.5 x 2.5 cm.	Small	1 x 1 cm.
4-24	0.3	0.2	Moderate	Large	8 x 5.5 cm.	Small	2.5 x 2.5 cm.
4-26	0.3	0.4	Moderate	Large	6.5 x 5.5 cm.	Small	1.5 x 1 cm.

II. Infection of Egg-Reacting Sites into Which Immune Serum Is Injected Simultaneously with the Egg Antigen

Normal rabbits sensitized to egg were given intradermally 0.3 cc. of egg solution mixed with varying amounts of immune serum of recognized potency. 24 hours later a large reacting area was found which was identical to that produced by egg alone, though perhaps somewhat larger and redder. When such an area was injected with *B. lepisepticum* the spread was just as rapid and necrosis just as extensive as in the animals receiving egg alone, or in the controls receiving normal serum mixed with the egg. (See Fig. 2 and Table V.)

From this experiment it is obvious that either, (1) the immune serum has diffused out of the lesion or, (2) has been denatured in some way or, (3) the cells have been so injured that they no longer profit by its presence. The latter hypothesis can be excluded by the next experiment.

III. Infection of Egg-Reacting Sites When Immune Serum and Organisms Are Injected Simultaneously

Normal rabbits were sensitized to egg as outlined above. Reacting areas were produced by injecting 0.3 cc. of egg intradermally. These wheals were injected with *B. lepisepticum* with which was mixed 0.1 or 0.2 cc. of immune serum. The local protection was striking. No lesion developed in the region of the injection, but when the dose of serum was small and the infecting dose of organisms large, the infection appeared at the periphery of the egg-reacting site where there were necrosis and other manifestations of infection. (See Fig. 3.)

It is evident that immune serum, if present, is a potent protective agent, even in the presence of injured cells. This serum seems more accessible to the injured cells of this type of lesion when injected into the tissues than when supplied through the general circulation. Normal serum has no effect when injected with the bacteria.

IV. Infection of Egg-Reacting Sites Which Are Induced by Injecting Bacterial Immune Serum with the Egg Antigen and in Which Normal Serum Is Injected with the Infecting Organisms

It seemed conceivable that the immune serum present during the antigen antibody reaction might be altered but not destroyed, and the following experiment has been devised to learn whether the immune serum could be reactivated by normal serum which itself has no protective value.

Normal rabbits were sensisized to egg and the usual hypersensitive reaction was induced by injecting intradermally 0.2 cc. of egg solution mixed with 0.5 cc. of immune serum on each side of the animal. 24 hours later the areas were injected with *B. lepisepticum* and, in addition, normal serum was injected into the reacting area, sometimes into the same site as the organisms, sometimes in another part of the wheal. The other side was infected with an equal dose of organisms but no normal serum was added. There was striking protection in the area receiving the normal serum, while the area receiving only bacteria showed the usual fulminating lesion.

TABLE VI

Wheals Induced in Both Flanks of Egg-Sensitized Rabbits by Injecting a Mixture of Egg and Bacterial Immune Serum. Infected 24 Hours Later, Injecting at Time of Infection Normal Serum in One Wheal, Heated Serum in Other

Number of animals	Amounts of egg and immune serum in mixture	Wheal receiving fresh normal rabbit serum at time of infection			Control wheal receiving only heated normal rabbit serum, or no serum at time of infection		
		Severity of preliminary wheal	Amount of normal serum injected	Result of infection	Severity of preliminary wheal	Substance injected with infection	Result of infection
			cc.				
4-48	0.3 cc. egg 0.4 cc. immune serum	Moderately severe	0.3	Small healing lesion, 2 x 2.5 cm.	Moderately severe	Nothing	Large spreading lesion, 8.5 x 6 cm. Necrosis at dependent part
4-57	0.3 cc. egg 0.6 cc. immune serum	Moderate reaction	0.4	Lesion moderate size, 7 x 3.5 cm. Healed promptly	Moderate reaction	Nothing	Large spreading necrotic lesion, 8.5 x 7 cm.
4-55	0.2 cc. egg 0.4 cc. immune serum	Moderate reaction	0.4	Moderate healing lesion, 8 x 4 cm.	Moderate reaction	Nothing	Large spreading necrotic lesion, 4 x 7 cm.
4-58	0.3 cc. egg 0.6 cc. immune serum	Moderate reaction	0.4	Small healing lesion, 3 x 3 cm.	Moderate reaction	Nothing	Large spreading necrotic lesion, 8.5 x 6.5 cm.
4-71	0.2 cc. egg 0.4 cc. immune serum	Moderate reaction	0.2	Moderate size, flat, sl. necrosis, healing, 5.5 x 5 cm.	Moderate reaction	0.2 cc. heated rabbit serum	Large spreading necrotic lesion, 9 x 6 cm.
4-74	0.2 cc. egg 0.4 cc. immune serum	Moderate reaction	0.4	Pale, healing, 4 x 4 cm.	Moderate reaction	0.4 cc. heated rabbit serum	Large spreading necrotic lesion, 9.5 x 5 cm.
4-67	0.2 cc. egg 0.4 cc. immune serum	Severe hemorrhagic	0.4 (Guinea-pig serum)	Small, pale, healing, 3.5 x 3.5 cm.	Moderate reaction	0.4 cc. heated guinea-pig serum	Large spreading necrotic lesion, 11.5 x 4 cm.

This experiment indicates the reactivation of the inert immune serum by normal serum. Experiments in a small series of animals indicate that the restoration of activity to the immune serum is due to the addition of *complement* or some other thermolabile substance.

Animals sensitized to egg were injected with 0.2 cc. of egg and 0.4 cc. of immune serum into the skin of both flanks. 24 hours later the wheals on each side were infected with equal doses of *B. lepisepticum*. At the same time 0.2 cc. to 0.4 cc. of fresh *normal* rabbit or guinea-pig serum was injected into one wheal while a similar amount of the same serum *heated* at 60° for 20 minutes was injected into the opposite wheal. Within 24 hours the wheal receiving the heated normal serum showed an extensive necrotic infection, while the wheal receiving the unheated normal serum showed definite evidence of healing. (See Table VI and Fig. 4.)

Further study is being made of the nature of this phenomenon and the indications are that some complement-like substance is used up in the egg-antibody reaction. Such a substance is probably necessary for the effective utilization by the cells of the immune serum which is present in the lesion but is inert.

DISCUSSION

It has been demonstrated in the preceding experiments that extensive tissue injury, due either to chemicals or to an intense antigen-antibody reaction allows pathogenic bacteria to disseminate with remarkable rapidity. This is observed even in animals with high humoral immunity though in such animals infection does not usually extend beyond the injured tissues. Such a mechanism of previous injury may be of importance in explaining the rapid development and persistence of large, local infections even in the presence of a high degree of humoral immunity.

Certain of the actions of immune serum in focal infection seem clear from these experiments. When preliminary injury is intense the tissues involved seem to become segregated from the body as a whole, so that circulating immune substances do not reach the cells of this portion in spite of the marked hyperaemia. In areas thus isolated pathogenic organisms may thrive and destroy the unprotected tissues. However, the cells outside, abundantly supplied with circulating antibodies, withstand the processes of infection as do the wandering cells

entering the lesion. Hence, in the immune animal the lesion fails to spread, exudate from it is more purulent in character and repair proceeds more efficiently. On the other hand when the local damage is slight, no such tissue segregation occurs. The supply of immune substances may be actually increased by the irritant. In such cases healing begins almost at once.

In the type of injury produced by a local antigen-antibody reaction, a segregation of the area is also striking. But an additional factor is introduced which lowers resistance, for bacterial immune serum present during the reaction fails to exert a protective influence unless unheated complement is added. This using up of a complementary substance perhaps demonstrates a mechanism by which specific immune processes may be disturbed in disease. In addition, one would not expect the administration of therapeutic serum intravenously to be effective against such a segregated lesion.

In the absence of specific immune substances, it is important to the host to restrict the invading organisms to a localized area. It has long been assumed that the allergic animal is better equipped with such a localizing mechanism. In Table I it is seen that strong reactors withstand infection better than weak reactors. If, however, a large lesion develops the animal quickly loses its skin reactivity and the infection spreads just as actively as in the weak reactor. Furthermore, the allergic reaction may be so severe and the local damage so great that the inflammatory zone becomes analagous to the wheal of an antigen-antibody reaction and the infection instead of being restrained spreads with great rapidity.

It must also be emphasized that allergy is not the only tissue factor to be considered in localizing infections. Our observations on *B. lepisepticum* quite confirm Rich's (10) findings with the tubercle bacillus that localization of the lesion often depends upon an intrinsic immunity of the cells against the injurious effects of infection. This may be easily demonstrated by the fact that a temporarily desensitized allergic animal without demonstrable humoral immunity may possess the capacity to restrain the spread of an infecting organism.

In spite of these variations in the influence of allergy on the course of infection our observations lead us to the belief that allergy is more than a "concomitant phenomenon" of the tissue immune state, and a

number of facts regarding the tissue reactions in an allergic animal can be cited which tend to confirm this view.

1. It has been shown that mild irritants such as broth filtrates when injected several hours previous to infection tend to protect locally even in the absence of general immunity. This preliminary inflammation is seldom comparable in extent and intensity, or in the amount of oedema observed in the wheals produced by egg in the sensitized animal, or in the lesion produced by chemical irritants.

2. Within a few minutes after the introduction of a mild irritant into the skin of a strong reactor the endothelial cells of the area become highly permeable on the *vessel lumen side* as is illustrated by injecting India ink intravenously during the first few minutes of the reaction and noting the phagocytosis of ink by the vascular endothelium. Negative reactors practically never show this phenomenon (11).

3. A few hours after injection into the skin of a bacterial filtrate to which the animal responds, the endothelial cells undergo a peculiar alteration so that if homologous filtrates or certain heterologous bacterial filtrates are injected intravenously, a hemorrhagic necrosis occurs at the site of skin injection. After an animal develops humoral immunity this phenomenon no longer takes place (12, 13). If, however, the homologous filtrate is injected into the site of the skin test no hemorrhagic necrosis occurs. On the contrary, an accelerated skin reaction develops in which redness and swelling appears within a few hours and begins fading many hours before a control test in a normal area of skin.

These observations make us believe that the endothelial cells of a strong reactor are exceedingly sensitive to products of certain bacteria and that within a relatively short time they become, on the tissue side, impervious to these products, perhaps through some protoplasmic alteration. Such stimulated cells are not readily destroyed by the products of infection present in the tissues but may be seriously changed if such products are absorbed and reach them through the medium of the vascular system which would be analogous to the hemorrhagic necrosis following the intravenous injection of filtrate. Clinically such lesions are not uncommon during the course of severe infections.

CONCLUSIONS

1. Animals showing natural bacterial allergy to filtrates of *B. lepisepticum* survive infection by this organism more frequently than weak reactors. This increased resistance is manifested by better localization of infection.

2. Bacterial filtrates injected into skin 24 hours before infection exert a non-specific protection of that area against the organism, even

in susceptible animals. The cells of this protected area seldom undergo necrosis when infected.

3. Severe injury of tissues either by chemicals or an antigen-antibody reaction produces a loss of local resistance even in immune animals. Mild injuries have the opposite effect. It is believed that in cases of severe injury, the affected areas undergo a segregation from the circulating antibodies.

4. When bacterial immune serum is injected with a protein antigen into the skin of a sensitized animal, a local alteration occurs in which substances necessary for the effective action of the immune serum are destroyed.

5. A protective action is restored to the altered immune serum by addition of complement to the lesion.

6. It is felt that allergy is not the chief mechanism in cellular resistance to infection, however data are advanced which suggest that allergy does exert local protection by acceleration of the immune processes and by rendering the cells locally refractory to further injury.

7. Chronic infection by a single strain of organism excites cellular reactivity to many strains of bacteria often unrelated biologically. Hence a non-specific mechanism for localizing infections throughout the body may be induced.

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EXPLANATION OF PLATES

PLATE 18

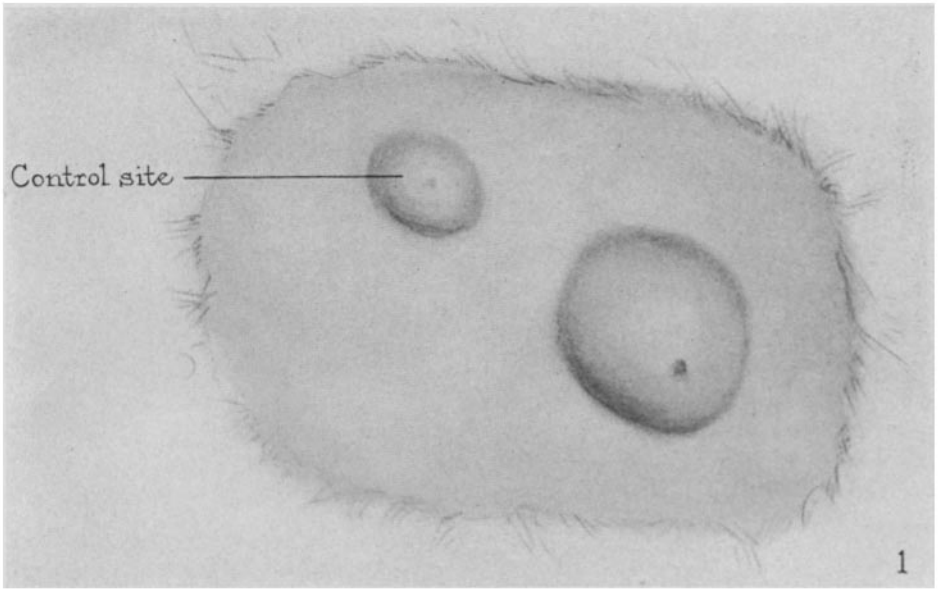
FIG. 1. No. 4–26. Animal sensitized to egg. Wheal induced with 0.3 cc. egg protein 24 hours before infection in it and in a control site of normal skin. Drawing, made 24 hours after infection, shows relative size of lesion.

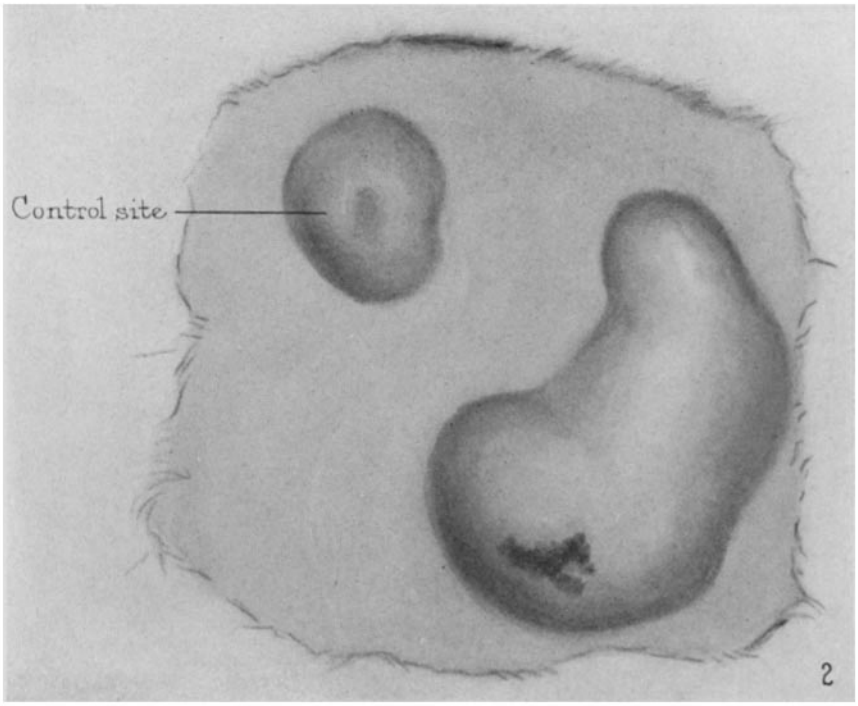
FIG. 2. No. 4-25. Animal sensitized to egg. Wheal induced by injecting a mixture composed of 0.3 cc. of egg protein and 0.4 cc. *B. lepisepticum* immune serum. Infected 24 hours later. Drawing, made 24 hours after infection, shows huge infection in spite of immune serum.

PLATE 19

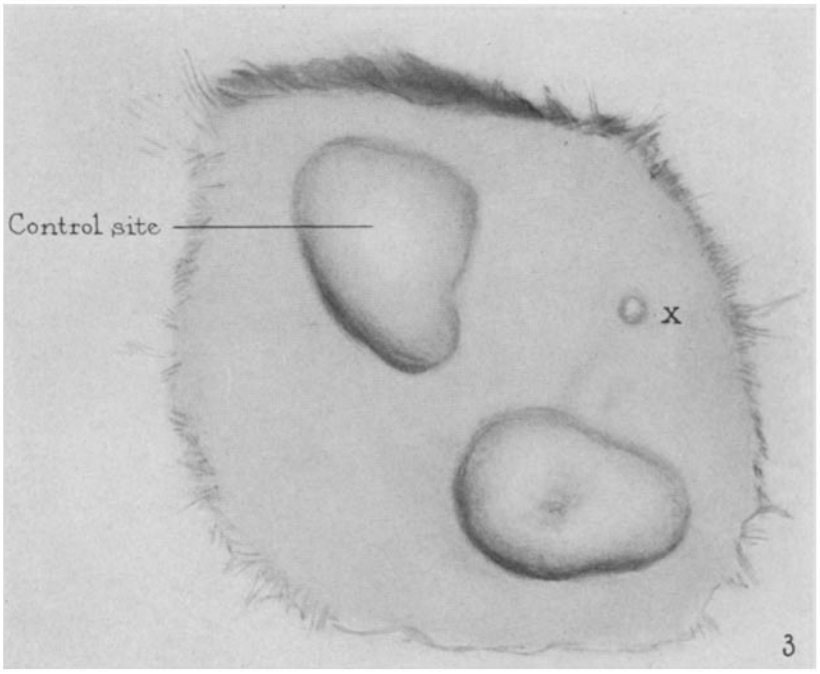
FIG. 3. No. 4-27. Wheal induced as in Fig. 1. *B. lepisepticum* immune serum 0.2 cc. injected into wheal at X with infecting bacteria. Drawing, made 24 hours after infection, shows striking protection of the portion of the wheal receiving immune serum.

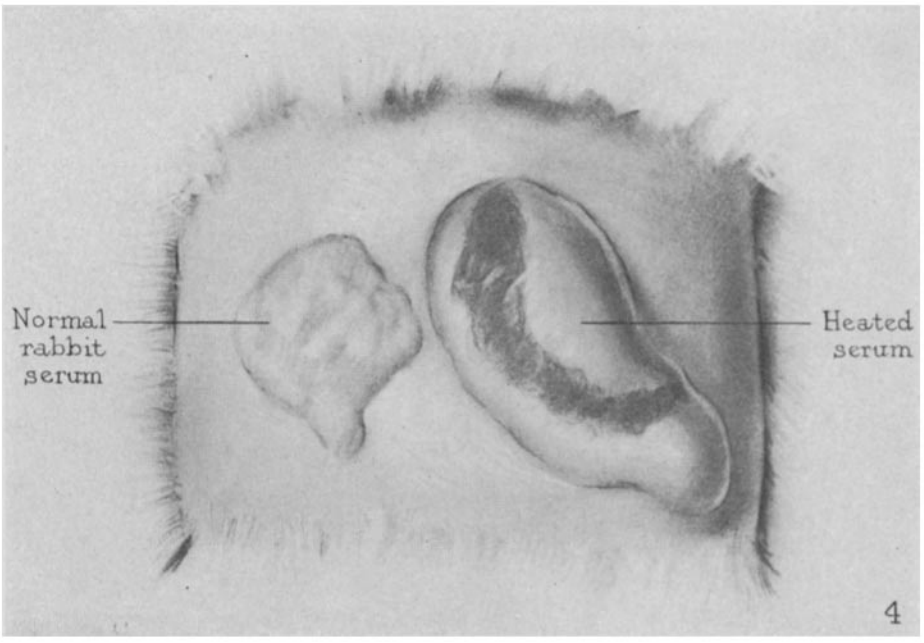
FIG. 4. No. 4-74, May 24, 930. Wheals induced in both flanks by injecting 0.2 cc. egg and 0.04 cc. bacterial immune serum in sensitized animal. Infected 24 hours later with equal dose of bacteria, but in one site 0.4 cc. normal rabbit serum was injected with organisms; in the other an equal amount of rabbit serum heated 60° for 20 minutes.





(Hanger: Inflammatory reactions and tissue immunity)





(Hanger: Inflammatory reactions and tissue immunity)