

LOCAL PASSIVE IMMUNITY IN THE SKIN OF RABBITS TO
INFECTION WITH (1) A FILTERABLE VIRUS,
AND (2) HEMOLYTIC STREPTOCOCCI.

BY THOMAS M. RIVERS, M.D., AND WILLIAM S. TILLET, M.D.
(From the Hospital of The Rockefeller Institute for Medical Research.)

PLATES 10 AND 11.

(Received for publication, September, 10, 1924.)

Observations reported in a previous paper¹ concerning the neutralization of a filterable rabbit virus by immune serum have led us to experiments on the production of local passive immunity to this virus. The experiments have been instrumental in arousing our interest in the general subject of local passive immunity. A report on them will be followed in the present paper by another dealing with experiments on local passive immunity in the skin of rabbits against infections with hemolytic streptococci isolated from a human case of erysipelas.

The phenomena of local resistance and local immunity against infectious agents have interested a number of investigators for many years. Special attention has been directed recently towards the study of problems in this field by Besredka,² and by Gay,^{3,4} who has comprehensively reviewed the literature.⁵ In the treatment of certain infectious diseases the advantages pertaining to the local administration of the antisera are well known. These advantages have been enumerated and emphasized by Flexner.⁶

Significant observations, not included in Gay's⁵ review, concerning local passive immunity in human skin have been recorded by Blake⁷ and Debré.⁸ Blake states that the intradermal injection of Dochez's antiscarlet serum blanches the rash of

¹ Rivers, T. M., and Tillett, W. S., *J. Exp. Med.*, 1924, xxxix, 777.

² Besredka, A., *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1923-24, xvii, 346.

³ Gay, F. P., and Rhodes, B., *J. Infect. Dis.*, 1922, xxxi, 101.

⁴ Gay, F. P., and Morrison, L. F., *J. Infect. Dis.*, 1923, xxxiii, 338.

⁵ Gay, F. P., *Physiol. Rev.*, 1924, iv, 191.

⁶ Flexner, S., *J. Am. Med. Assn.*, 1913, lxi, 447, 1872.

⁷ Blake, F. G., Trask, J. D., Jr., and Lynch, J. F., *J. Am. Med. Assn.*, 1924, lxxxii, 712.

⁸ Debré, R., Bonnet, H., and Broca, R., *Compt. rend. Soc. biol.*, 1923, lxxxix, 70,

scarlet fever patients locally and that the blanching persists. Furthermore, he states that when the serum is given intradermally during the first 24 hours of the disease, in addition to the persistent local blanching of the rash, the skin does not later become pigmented over this area nor does it desquamate. Normal horse serum, on the other hand, fails to blanch the rash. Debré reports the following observations upon the use of convalescent serum in the prevention of measles. When the serum is given soon after exposure, the disease is prevented, but when given 5 or 6 days after exposure a modified measles eruption results. Serum administered subcutaneously at the beginning of the invasion inhibits the eruption locally over the area of injection. A rash already present is not blanched by convalescent serum.

Methods.

Rabbits rendered immune to the virus above referred to were anesthetized and bled from the heart. The sera were collected, pooled, inactivated, and stored on ice. No preservative was added to the serum. The virucidal properties of the serum were tested in the manner described in a previous report.¹ Several normal rabbits were also bled from the heart. The sera were collected, inactivated, and stored on ice without the addition of a preservative. The sera from normal rabbits were tested individually for virucidal properties before they were pooled. This was necessary since it has been shown that the serum of about 20 per cent of stock rabbits neutralizes the virus.¹

The source of the hemolytic streptococcus, the preparation of suspensions of the streptococci, and the preparation of the normal and the immune sera employed in these experiments were described in a preceding paper.⁹

Extensive areas of skin over both sides of the chest and abdomen of rabbits were shaved. By means of tuberculin syringes and 22 gauge needles areas of shaved skin about 2 cm. in diameter were infiltrated with sera, broth, or other substances as desired. Care was taken to make the infiltrations intracutaneous, yet it is not unlikely that a portion of the infiltrated substances reached the subcutaneous tissues.

Local Protection against the Action of the Rabbit Virus as Result of Injection of Immune Serum.

To determine whether the skin of rabbits can be locally protected against the action of the virus the following experiments were performed.

Two normal rabbits were shaved on both sides of the body. On the left side of each animal an area 2 cm. in diameter was infiltrated intracutaneously with 0.5 to 0.75 cc. of immune serum. On the right side of each rabbit a similar area was

⁹ Rivers, T. M., *J. Exp. Med.*, 1925, xli, 179.

infiltrated with normal serum. 24 hours later 0.2 cc. of a fresh testicular emulsion containing active virus was injected intracutaneously in each of the infiltrated areas. In addition to these inoculations a normal portion of the skin on each side, 2 inches away from the infiltrated areas, was inoculated with the virus. Two other rabbits were treated in a similar manner with the exception that 48 hours elapsed between the infiltrations with the sera and the inoculations with the virus. The animals were kept under observation during the 10 days following the inoculations.

The virus did not produce visible lesions in areas of skin infiltrated with immune serum 24 to 48 hours previously. Lesions were pro-

TABLE I.

Summary of Experiments Demonstrating in the Skin of Rabbits a Local Passive Immunity to Virus III.

Rabbit No.	Left side of rabbit.		Right side of rabbit.	
	Area of skin infiltrated with immune serum.	Uninfiltrated area of skin.	Area of skin infiltrated with normal serum.	Uninfiltrated area of skin.
Virus injected 24 hrs. after the skin was infiltrated with the sera.				
1	—	+	+	+
2	—	+	+	+
Virus injected 48 hrs. after the skin was infiltrated with the sera.				
3	—	+	+	+
4	—	+	+	+

+ indicates the presence of a visible lesion usually produced in the skin of rabbits by the virus. — indicates absence of a visible lesion.

duced by the virus, however, in areas of skin not so infiltrated and essentially similar ones also in the areas of skin infiltrated with normal serum. No titrations of the virus were made to determine whether, despite these findings, a slight degree of protection had resulted from the infiltrations of normal serum. The results of the experiments are summarized in Table I.

Since intracutaneous infiltrations of normal serum did not and intracutaneous infiltrations of immune serum did inhibit the action of the virus in the same animals, it seems reasonable to conclude that a local passive immunity against the action of the virus was produced

in the skin of rabbits by the infiltrations of immune serum. This local protection persisted for at least 48 hours.

Local Protection against the Action of Hemolytic Streptococci as Result of the Injection of Broth, Normal Serum, or Immune Serum.

A review of the literature on hemolytic streptococci seemed to indicate that meat infusion broth and possibly normal serum infiltrated into the skin might produce some local protection against these organisms injected 24 hours later. It was necessary to determine the truth of the matter before attempting to induce a local passive immunity. The experiments with broth and with normal serum will be described first and then the experiments with immune serum will be reported.

Broth.—The following experiment was performed to determine whether intracutaneous infiltrations of broth give rise to local protection in the skin of rabbits against the action of hemolytic streptococci.

Two rabbits were shaved on both sides of the body. In four areas on the right side of each animal, 0.5 cc. of meat infusion broth was infiltrated intracutaneously. The left side was merely shaved. 24 hours after the infiltrations, various dilutions of a streptococcus suspension were injected intracutaneously to the amount of 0.1 cc. in four areas on each side of both rabbits. The inoculations on the right side were made in the center of each area previously infiltrated with broth. The animals were examined each day for 10 days.

No lesions developed in the areas of skin infiltrated with broth 24 hours prior to inoculation with streptococci. A definite lesion developed at the point of each inoculation on the uninfiltrated side (Figs. 1 and 2). The experiment demonstrated conclusively that meat infusion broth infiltrated into the skin of a rabbit protected it 24 hours later against doses of hemolytic streptococci that produced visible lesions in the uninfiltrated skin of the same animal. The results of the experiment are summarized in Table II.

It was found absolutely necessary to infiltrate the skin before the streptococci were inoculated if protection was desired because broth was found to afford no protection when mixed with the suspensions of streptococci and then injected into normal skin. Data are not available at the moment with regard to how soon the protection

appears after the infiltrations or how long it persists. 24 hours was chosen arbitrarily as the period to elapse between the broth infiltrations and the streptococcus inoculations.

Normal Rabbit Serum.—The details of the experiments with normal rabbit serum will not be given because normal serum was used as a control in all of the experiments performed with immune serum and abundant opportunities will be presented in these to observe its effects. It is sufficient to say that infiltrations of 0.5 cc. of normal rabbit serum into areas of skin were found to afford considerable local protection against infection with hemolytic streptococci inoculated 24 hours later. No experiments were made to compare the effectiveness of broth with that of normal serum. The impression was

TABLE II.

Summary of an Experiment Showing that Meat Infusion Broth Infiltrated in the Skin of Rabbits Affords Local Protection against Infection with Hemolytic Streptococci Inoculated 24 Hours Later.

Rabbit No.	Right side of rabbit infiltrated in each of four areas with 0.5 cc. of meat infusion broth 24 hrs. previous to inoculation with the streptococci.				Left side of rabbit unhandled except for shaving.			
	Dilutions of streptococcus suspension.				Dilutions of streptococcus suspension.			
	1:4	1:8	1:16	1:32	1:4	1:8	1:16	1:32
1	—	—	—	—	+	+	+	+
2	—	—	—	—	+	+	+	+

+ indicates presence of visible lesion at site of inoculation of the streptococci.
— indicates absence of visible lesion.

obtained, however, that infiltrations of the broth afforded more protection than did infiltrations of similar amounts of normal serum. As with broth, the normal serum had to be infiltrated into the skin some hours before the streptococci were inoculated if protection was to be obtained.

Immune Serum.—No attempt has been made to analyze the mechanism of the protection afforded by normal serum and broth which, for convenience, will be spoken of as a local non-specific protection. It now seemed desirable to determine if a specific local passive immunity against hemolytic streptococcus infections could be demonstrated in areas of skin infiltrated with a homologous immune serum 24 hours

prior to inoculation. Therefore experiments were performed to ascertain if more protection was afforded by infiltrations of immune serum than by infiltrations of normal serum. In order to do this it was necessary that each experiment should be complete in one animal and the doses of streptococci had to be so graded that an infection would occur in the areas of skin infiltrated with normal serum but not in those infiltrated with immune serum. Furthermore, one had to be sure the results obtained with immune serum were not merely the consequence of variations in non-specific protection. For this reason it was necessary to employ immune serum and normal serum from many rabbits. In the majority of instances the immune serum and the normal serum were pooled specimens from at least six or seven rabbits. Of the many experiments performed, only two will be described in detail as they furnish completely the desired information. The results all attested to the same facts.

Rabbit 1 was shaved on both sides of the body. Three areas of skin 2 cm. in diameter on the right side were infiltrated with 0.5 cc. each of serum, the front and rear areas with normal serum, the middle one with immune serum. 24 hours later the three areas were inoculated with 0.1 cc. each of the same streptococcus suspension. The left side was untouched except for shaving until 24 hours later, when it was inoculated in two places, the front one with 0.1 cc. of a mixture of equal amounts of immune serum and streptococcus suspension, the rear one with 0.1 cc. of a mixture of equal amounts of normal serum and streptococcus suspension. Portions of the same immune and normal sera were used for the tests on the two sides and the same suspension of streptococci was used throughout. The inoculations were nearly simultaneous.

It was found that immune serum and streptococci mixed and immediately injected into the skin produced no lesion. The mixture of normal serum and streptococci, however, gave rise to a large phlegmon around which an erysipelas-like lesion developed later. No lesion developed in the area of skin that was infiltrated with immune serum and inoculated with streptococci 24 hours later, whereas in those areas that had been infiltrated with normal serum definite lesions developed. They were much smaller, however, than the phlegmon that developed on the other side of the animal where the mixture of streptococci and normal serum was injected.

Rabbit 2 was shaved on both sides of the body. Four areas of skin 2 cm. in diameter on each side were infiltrated with serum in the usual manner. Pooled normal sera were used on the left, pooled immune sera on the right. 24 hours after the infiltrations the four areas on each side were inoculated respectively from back to front with decreasing doses of streptococci. The animal was examined daily for a fortnight.

No visible lesions developed following the inoculations of streptococci in the areas of skin previously prepared with immune serum.

TABLE III.

Summary of Two Experiments Showing that Immune Serum Affords More Protection than Normal Serum When Infiltrated into the Skin 24 Hours Prior to Inoculations of Hemolytic Streptococci.

Rabbit No.	Right side.			Left side.				
	Front.	Middle.	Rear.	Front.		Rear.		
1	Infiltrated with 0.5 cc. normal serum and inoculated 24 hrs. later with streptococci.	Infiltrated with 0.5 cc. immune serum and inoculated 24 hrs. later with streptococci.	Infiltrated with 0.5 cc. normal serum and inoculated 24 hrs. later with streptococci.	Skin shaved and 24 hrs. later inoculated with a mixture of streptococci and immune serum.		Skin shaved and 24 hrs. later inoculated with a mixture of streptococci and normal serum.		
	+	-	+	-		++++		
2	The four places were infiltrated with 0.5 cc. each of immune serum and then 24 hrs. later were inoculated with 0.1 cc. of different dilutions of the streptococcus suspension.				The four places were infiltrated with 0.5 cc. each of normal serum and then 24 hrs. later were inoculated with 0.1 cc. of different dilutions of the streptococcus suspension.			
	1:8	1:4	1:2	Undiluted.	1:8	1:4	1:2	Undiluted.
	-	-	-	-	++	++	++	++

+ indicates a visible lesion. The number of pluses represent the size of the lesion, + + + + being the maximum. - indicates absence of a visible lesion.

Large lesions were produced by the streptococci inoculated in the skin previously prepared with normal serum. None of these lesions, however, became necrotic even though the doses of streptococci used in the experiment regularly produced large necrotic lesions in unprotected skin.

The two experiments are summarized in Table III and the results are pictured in Figs. 3 to 8. Areas of skin prepared with normal or immune sera were more refractory to infection with hemolytic

streptococci than was unprepared skin in the same animal. Since infiltrations with immune serum always afforded more protection than that produced by infiltrations with similar amounts of normal serum, one is justified in concluding that a local passive immunity was conferred by the immune serum.

DISCUSSION.

The experiments reported in the present paper were planned for the study of local passive immunity and its value as a prophylaxis against certain local infections in the skin. As the protocols show, a local passive immunity can be induced both to a filterable virus pathogenic for rabbits and to a hemolytic streptococcus.

Previous work by other authors has shown that a non-specific refractory state against infection with hemolytic streptococci can be induced by the injection of broth and probably of many other substances. One concludes from Gay's⁴ work that some of these must be administered in proper concentrations if a refractory rather than a more susceptible condition is to be obtained. Although in the present work infiltrations with normal serum produced a definite refractory state, a greater degree of local protection was conferred when immune instead of normal serum was employed. This difference in the amount of protection afforded may be thought of as representative of the degree of local passive immunity conferred by the immune serum. Further work will be required to show whether it is dependent upon the slow absorption of the serum and its protective bodies from the skin, upon a local fixation of the latter bodies altering the reactivity of the cells in the vicinity of the injected serum, or upon the mobilization of some special type of cell. Regardless of the mechanism underlying the phenomenon, it seems obvious from its occurrence that a local passive immunity can be induced and exist long enough to be of value in the prevention of certain local infections in the skin.

The question naturally arises as to whether the information obtained from the experiments here reported can be applied to the treatment of erysipelas. One might conceivably treat a local hemolytic streptococcus infection by infiltrations of the healthy tissues in the immediate vicinity with immune serum, on the assumption that when the infection spreads up to the prepared zone it will meet refrac-

tory tissues and be unable to progress further. This problem deserves and will receive further study.

SUMMARY.

Small areas of skin infiltrated with immune serum proved refractory to the injurious action of a filterable rabbit virus inoculated 24 to 48 hours later. Infiltrations of normal serum did not appreciably protect the skin against the virus. The local protection afforded by the infiltrations of immune serum appears to constitute an example of local passive immunity.

Small areas of skin infiltrated with either normal serum or meat infusion broth proved more refractory than normal skin to infection with hemolytic streptococci inoculated 24 hours later. The refractory state induced in the tissues was non-specific in character. A greater amount of local protection against hemolytic streptococci was afforded by infiltrations with a homologous immune serum than by infiltrations with normal serum. The difference in the amount of protection afforded by immune serum over that induced by normal serum may be thought of as representative of the degree of local passive immunity conferred.

EXPLANATION OF PLATES.

PLATE 10.

FIG. 1. Lesions produced by various dilutions of a suspension of hemolytic streptococci inoculated into the normal shaved skin of a rabbit. Photographed 7 days after the inoculations.

FIG. 2. Other side of rabbit of Fig. 1. Four areas of skin were infiltrated with 0.5 cc. each of meat infusion broth. 24 hours later these areas of skin were inoculated with portions of the same dilutions of the streptococcus suspension used on the side shown in Fig. 1. No lesions developed following the inoculations of streptococci into the infiltrated skin. Photographed 7 days after the inoculations.

FIG. 3. Three areas of skin were infiltrated with 0.5 cc. each of serum, the front and rear ones with normal rabbit serum, the middle one with immune serum. 24 hours later the infiltrated areas were inoculated with 0.1 cc. each of a suspension of hemolytic streptococci. No lesion developed in the skin infiltrated with immune serum. Small lesions developed in the skin infiltrated with normal serum. Compare with Fig. 4. Infiltrations of normal serum afforded some protection but not so much as that afforded by immune serum. Drawing 7 days after the inoculations.

FIG. 4. Other side of rabbit of Fig. 3. Equal parts of immune serum and a suspension of hemolytic streptococci were mixed and 0.1 cc. of this mixture was injected immediately into the normal skin. No lesion followed this inoculation. 0.1 cc. of a similar mixture of normal serum and streptococci was injected into the skin. A phlegmon followed this inoculation. Later an erysipelas-like lesion developed around the phlegmon. Immune serum protected, normal serum did not. Drawing 7 days after the inoculations.

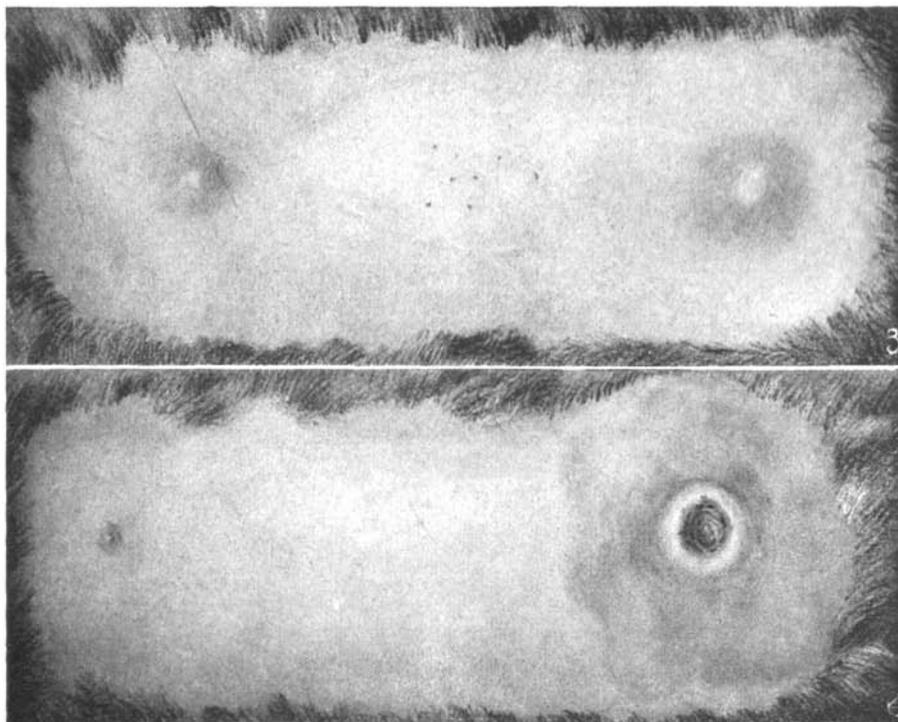
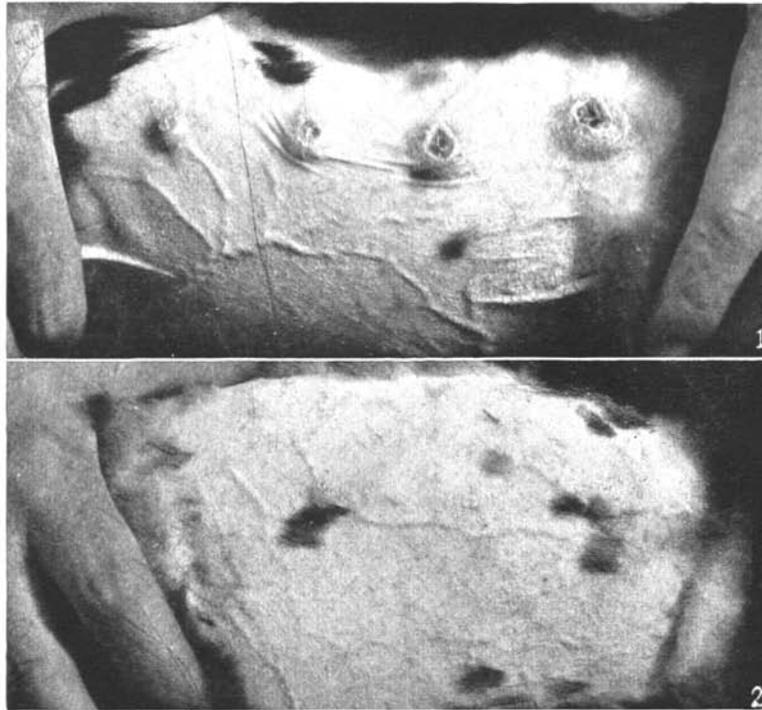
PLATE 11.

FIG. 5. Lesions produced by various dilutions of a hemolytic streptococcus suspension inoculated into four areas of skin 24 hours after they were infiltrated with 0.5 cc. of normal rabbit serum. Photographed 2 days after the inoculations.

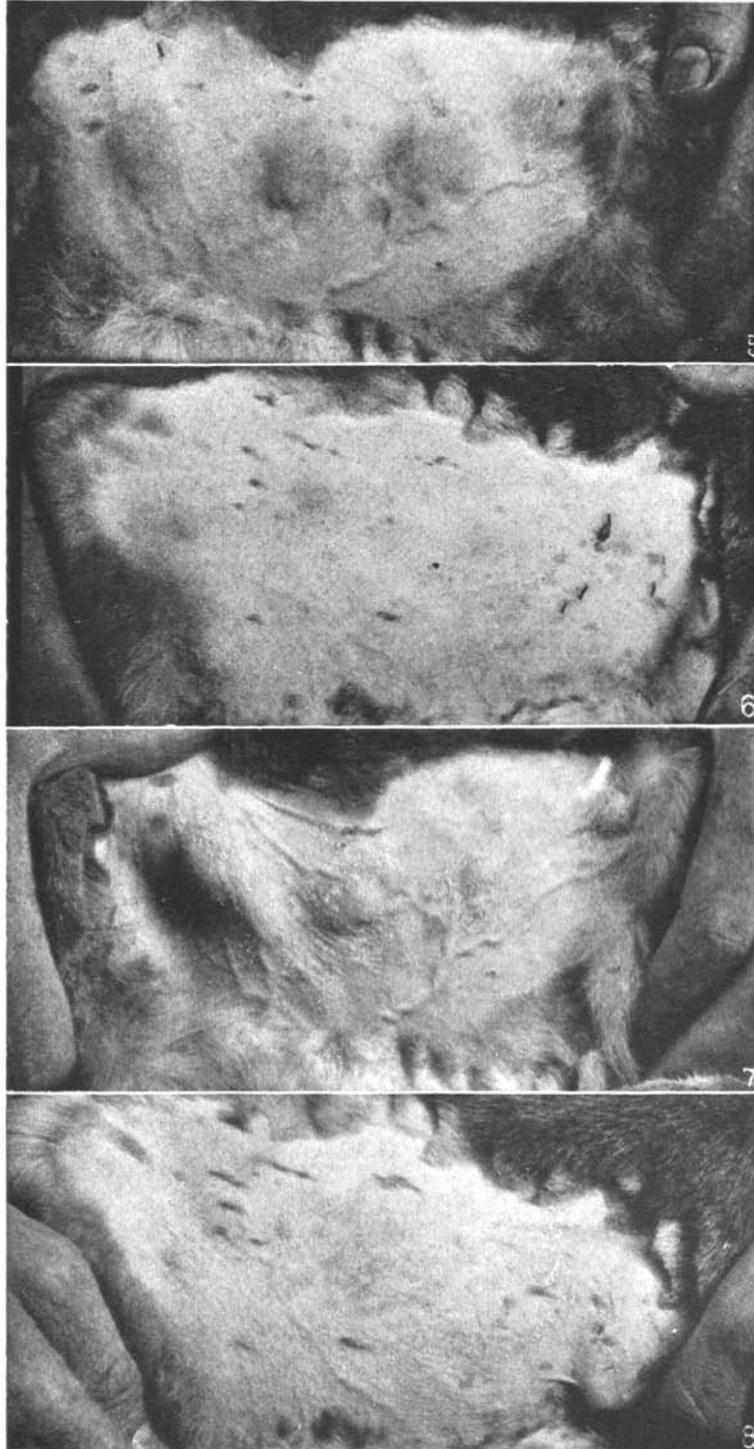
FIG. 6. Other side of rabbit of Fig. 5. Four areas of skin were infiltrated with 0.5 cc. of immune serum. 24 hours later these areas were inoculated with portions of the same dilutions of the streptococcus suspension used on the side shown in Fig. 5. No lesions developed following the inoculations of streptococci into the skin prepared with immune serum. Photographed 2 days after the inoculations.

FIG. 7. Same as Fig. 5, 5 days later.

FIG. 8. Same as Fig. 6, 5 days later.



(Rivers and Tillett: Local passive immunity.)



(Rivers and Tillett: Local passive immunity.)