The antibody content of the serums used in this investigation was determined by titration of the agglutinin titer against *Brucella abortus* suspensions.

The results of the test given in Table II show that the major part of the agglutinins were still intact after ketenization.

Conclusion. The treatment of anti-Brucella horse serum with ketene for 35 minutes or more prevents anaphylactic shock in animals sensitized to the original antiserum. Such a serum still retains the major part of its agglutinating antibody.

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Pathogenesis of Hemorrhagic-Necrotic Skin Lesions in Intradermal Infection of Rabbits with Pneumococci.*

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The hemorrhagic necrotic changes occurring in certain endermal infections of rabbits closely resemble those described by Shwartzman. This induced us to examine various species of pathogenic microörganisms as to their capacity to produce the lesion in question. Previously reported experiments[1-4] revealed 3 different types of reactions represented by the following bacteria:

(1) *H. influenzæ,* when injected into the skin over the abdomen produces a localized swelling and infiltration. There is no trace of hemorrhagic necrosis to be observed. When, 24 hours after the intracutaneous injection of *H. influenzæ,* a suspension of *H. influenzæ* is given intravenously, the site of the dermal infection may be transformed into a bluish hemorrhagic lesion within a few hours. Not only living and heat-killed influenza bacilli but also agar-washing filtrates of *B. typhosus, B. coli,* or meningococcus may induce the same change. On the other hand, those filtrates may be activated by the intravenous injection of *H. influenzæ.* (2) Some

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1 Witebsky, E., and Salm, H., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 351.

2 Witebsky, E., and Salm, H., *J. Exp. Med.*, 1937, **65**, 43.

3 Witebsky, E., Neter, E., and Salm, H., Second International Congress for Microbiology, London, Report of Proceedings, 1936, 414.

4 Witebsky, E., and Neter, E., *Arch. Path.*, 1937, **24**, 271.

strains of Friedländer bacilli spontaneously may induce the formation of hemorrhagic necrotic skin lesions in rabbits. When a heat-killed suspension of such a strain is injected intracutaneously into a rabbit, the site of the infection may be transformed into a hemorrhagic necrotic lesion by means of a subsequent intravenous injection of a suspension of living or heat-killed Friedländer bacilli. (3) Pneumococci, finally, display a very interesting picture: When injected intracutaneously certain rabbit-virulent strains produce a severe dermal lesion followed by hemorrhagic necrosis and accompanied by bacteremia as described by Goodner. The following experiments are concerned with the experimental conditions which would bring about the hemorrhagic change of the lesion in the dermal pneumococcal infection of rabbits.

For this purpose rabbits were inoculated intracutaneously with a highly virulent strain of Type I pneumococcus (obtained through the courtesy of Dr. Paul R. Cannon). Twenty-four hours later, the rabbits showed a severe lesion characterized by swelling and œdema, followed by hemorrhagic necrosis and death of the animals after 2 to 4 days. The influence of an intravenous injection of a potent *B. typhosus* agar-washing filtrate prepared according to Shwartzman is shown by the following experiment:

Six rabbits (Nos. 1-6) weighing about 4-5 pounds, were shaved over the abdomen; then 0.2 cc. of a 1:50 diluted 18-hours meat-infusion broth culture of pneumococcus Type I was injected intracutaneously. Eighteen hours later all the rabbits exhibited severe swelling and œdema at the site of the dermal infection without any trace of hemorrhagic changes. At this stage 3 rabbits (Nos. 1, 2, and 3) were injected intravenously with 2 cc. of a 1:20 diluted *B. typhosus* agar-washing filtrate. Rabbits Nos. 4, 5, and 6 were used as controls.

The results of this experiment (Table I) show that the injection of Shwartzman filtrate may bring about a severe hemorrhagic necrotic lesion at the site of the pneumococcal infection, the intensity and development of which exceeds that of the untreated rabbits. The 3 rabbits injected with *B. typhosus* filtrate died in 12-24 hours previous to the controls. Similar observations were made on several occasions.

In contrast, heat-killed pneumococci of the same strain when injected endermally, apparently did not prepare the skin for a subsequent transformation into a hemorrhagic lesion when living pneumococci or Shwartzman filtrate respectively were injected intravenously 24 hours later. In this respect pneumococci differ

TABLE I.
Production of Hemorrhagic Necrosis in Skin Areas of Rabbits Infected with Pneumococcus Type I.

Hours after reinjected with Shwartzman filtrate	A			B Controls		
Rabbit No.	1	2	3	4	5	6
1 hr.	+	++++	+	—	—	—
4 hr.	++++	++++	++++	+	—	—

— = no hemorrhagic necrosis.
+ to ++++ = various degrees of hemorrhagic necrosis.

markedly from *H. influenzæ*. It was impossible thus far to use successfully living or heat-killed pneumococci for the second—intravenous—injection in order to produce hemorrhagic necrotic lesions in the skin previously prepared by the intracutaneous injection of living rabbit-virulent pneumococci. On the other hand, rabbits previously injected intradermally with pneumococci developed hemorrhagic reactions following the intravenous injection of bacterial filtrates, 0.2% agar and occasionally of heavy suspensions of killed pneumococci as reported by Wadsworth and Sickles.[5] Goodner and Horsfall[6] recently produced a purpuric reaction in a skin-area infected with Type I pneumococcus by means of pneumococcal autolysate prepared according to the technic described by Julianelle and Reimann.[7]

There are some strains of pneumococci that produce a certain degree of swelling but fail to cause a hemorrhagic necrotic lesion in the skin of the rabbit and do not kill rabbits. The influence of bacterial agar-washing filtrates on such a lesion is shown in the following experiment:

Seven rabbits weighing about 4-5 pounds were shaved over the abdomen and injected with 0.2 cc. of a Type 3 pneumococcal suspension. Eighteen hours later the rabbits exhibited definite redness and swelling at the site of the infection without any hemorrhages or necrosis. Four of the rabbits (Nos. 1-4) were then injected intravenously with 2 cc. of a 1:20 diluted Shwartzman agar-washing filtrate. Rabbits Nos. 5, 6, and 7 were used as controls.

The results of this experiment show that it may be possible to induce a hemorrhagic change at the site of a pneumococcal skin-lesion that fails to develop spontaneously into a hemorrhagic necrotic lesion.

[5] Wadsworth, A., and Sickles, G. M., *J. Bact.*, 1933, **25**, 80.

[6] Goodner, K., and Horsfall, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 178.

[7] Julianelle, L. A., and Reimann, H. A., *J. Exp. Med.*, 1926, **43**, 87, 97; 1927, **45**, 609.

TABLE II.
Production of Hemorrhagic Necrosis in Skin Areas of Rabbits Infected with Pneumococcus Type III.

Hours after reinjected with Shwartzman filtrate	A				B Controls		
Rabbit No.	1	2	3	4	5	6	7
4 hr.	++++	—	++	—	—	—	—
18 hr.	++++	+	++++	—	—	—	—

— = no hemorrhagic necrosis.
+ to ++++ = various degrees of hemorrhagic necrosis.

One would be tempted to draw the conclusion from these experiments that the severe bacteremia thus far usually considered to be nothing more than a symptom in the pneumococcal infections of rabbits—and human beings—may be a contributing etiological factor in the development of the hemorrhagic necrotic change of the primary lesion. Indeed, substances present in bacterial agar-washing filtrates, prepared according to Shwartzman, are able to induce the changes in question. The above-mentioned investigations of Wadsworth and Sickles and of Goodner and Horsfall point in the same direction. Living as well as dead pneumococci, however, when injected intravenously proved to be ineffective in our experiments so far. Further experiments are necessary in order to elucidate this problem.