3. Penicillin injected into an area of rash of patients with scarlet fever did not cause blanching.

4. Dick positive patients receiving penicillin for an unrelated disease did not become Dick negative after a week of penicillin therapy.

5. It is concluded that penicillin has no neutralizing effect upon Dick or Schick toxin.

We wish to thank Dr. John H. Hanks for his interest and advice.

15537

Hypertonic Sodium Chloride Solution as Serum Diluent in Agglutination Tests with *Rickettsia burneti.**

NATHAN GALLENSON. (Introduced by J. H. Dingle.)

From the Respiratory Diseases Commission Laboratory, Regional Hospital, Fort Bragg, N.C.

In the course of agglutination tests employing as antigen a killed suspension of the Balkan Grippe strain of *Rickettsia burneti*,[1] it was found that a prozone effect occurred with considerable frequency. In addition, the fine granular quality of the aggregates some-

TABLE I.
Effect of Varying Concentrations of Sodium Chloride, as a Diluent for Sera, on Agglutination of a Suspension of *Rickettsia burneti.*

Convalescent serum	Conc. of diluent % NaCl	Dilution of serum 1:4	1:8	1:16	1:32	1:64	1:128	1:256	Diluent control
1	0.85	4	4	4	2	1	0	0	0
	2.5	4	4	4	4	2	±	0	0
	5.0	4	4	4	4	2	1	0	0
	7.5	4	4	4	2	1	0	0	0
	10.0	4	4	3	2	0	0	0	0
	12.5	4	4	3	2	0	0	0	0
	15.0	4	4	3	1	0	0	0	0
2	0.85	4	4	4	2	2	±	0	0
	2.5	4	4	4	4	2	1	0	0
	5.0	4	4	4	4	2	1	0	0
	7.5	4	4	4	2	1	0	0	0
	10.0	4	4	3	2	0	0	0	0
	12.5	4	4	3	2	0	0	0	0
	15.0	4	4	2	1	0	0	0	0
3	0.85	4	4	4	4	3	±	0	0
	2.5	4	4	4	4	4	2	0	0
	5.0	4	4	4	4	3	2	0	0

0—No agglutination.
±, 1, 2, 3—Increasing degrees of agglutination.
4—Complete agglutination.

* This investigation was supported through the Commission on Acute Respiratory Diseases, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army, and by grants from the Commonwealth Fund, the W. K. Kellogg Foundation, the John and Mary R. Markle Foundation, and the International Health Division of the Rockefeller Foundation to the Board for the Investigation and Control of Influenza and Other Epidemic Diseases for the Commission on Acute Respiratory Diseases.

[1] Commission on Acute Respiratory Diseases, *Am. J. Hyg.*, 1946, **44**, 110-122.

TABLE II. Comparison of 0.85 and 5.0% NaCl Solutions, as Serum Diluents, on Agglutination of 2 Dilutions of Suspension of *Rickettsia burneti*.

Convalescent serum	Antigen dilution	Serum dilutions in 0.85% NaCl 1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Serum dilutions in 5.0% NaCl 1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
1	1/2	2	3	4	4	4	2	±	0	0	4	4	4	4	3	2	1	0	0
	1/4	0	1	4	4	2	1	0	0	0	4	4	4	3	2	1	±	0	0
2	1/2	3	4	4	4	4	4	3	1	0	4	4	4	4	3	2	1	1	0
	1/4	2	4	4	4	4	4	2	1	0	4	4	4	4	3	2	1	±	0
3	1/2	2	3	4	4	4	3	1	0	0	4	4	4	4	4	2	1	0	0
	1/4	0	1	4	4	3	2	1	0	0	4	4	4	3	2	1	0	0	0
4	1/2	4	4	4	3	1	0	0	0	0	4	4	4	3	2	0	0	0	0
	1/4	1	2	3	3	±	0	0	0	0	4	3	3	2	1	0	0	0	0
5	1/2	3	4	4	4	3	2	1	0	0	4	4	4	3	2	1	0	0	0
	1/4	1	2	4	4	3	1	±	0	0	4	4	3	2	1	1	0	0	0
6	1/2	3	4	4	4	3	1	0	0	0	4	4	4	4	3	1	0	0	0
	1/4	2	4	4	4	4	1	0	0	0	4	4	3	2	1	1	0	0	0

0—No agglutination. ±, 1, 2, 3—Increasing degrees of agglutination. 4—Complete agglutination.

times caused uncertainty in determining end points. These difficulties were most often encountered with sera of high titer and with light antigen suspensions.

As is well known, the electrolyte concentration influences the rate of antigen-antibody reactions and the ratio in which antibody combines with antigen in flocculation and agglutination tests.[2] Accordingly, the effect of varying salt concentrations on the agglutination test with Balkan Grippe antigen was investigated.

The suspension of rickettsiae used as antigen was prepared in a density comparable to Barium Sulphate Standard No. 2.[3] The sera were obtained from patients in a laboratory outbreak of Q fever caused by the Balkan Grippe strain of *R. burneti*.[4] The effect of varying salt concentrations was determined by the use of a single antigen and 3 sera serially diluted in solutions of 0.85, 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0% sodium chloride, respectively.

The technic of the agglutination test[1] was as follows: sera were centrifugalized to remove particulate material and diluted serially in multiples of 2 in the respective saline solutions. To 0.2 ml of each serum dilution was added 0.2 ml of antigen dilution in 0.85% solution of sodium chloride. The tubes were incubated at 48°C for 10 minutes in a water bath, shaken for 3 minutes, returned to the 48°C water bath for 3 hours, and then placed at 4°C for 18 hours. The tubes were examined at the end of 1, 2, 3 and 21 hours. Readings were made with the aid of a concave mirror against a dark background.

The initial experiments indicated that agglutination occurred to a slightly higher titer when the sera were diluted with 2.5 and 5.0% solutions of sodium chloride (Table I). In addition, the rate of combination was somewhat more rapid with the 5.0% solution, than with the 2.5% solution. The 5.0% solution of sodium chloride was therefore selected

[2] Boyd, W. C., *Fundamentals of Immunology*, 1943, Interscience Publishers, Inc., New York.

[3] Wadsworth, A. B., *Standard Methods*, 1939, Williams and Wilkins Co., Baltimore.

[4] Commission on Acute Respiratory Diseases, *Am. J. Hyg.*, 1946, **44**, 123-157.

as the one giving a final electrolyte concentration which was most nearly optimal. Additional experiments confirmed this finding. They indicate further that the prozone effect was reduced or eliminated when the 5.0% diluent was employed, and that a more dilute antigen could be used (Table II).

The application of these observations has made possible the use of a screen test consisting of one or 2 low-serum dilutions, for the rapid detection of rickettsial antibodies in large numbers of sera at a considerable saving of antigen.

Summary. The use of a 5.0% solution of sodium chloride as a serum diluent in agglutination tests with *Rickettsia burneti* reduced or eliminated the prozone effect and permitted the employment of a more dilute antigen.

15538

Induced *in vitro* Resistance of Staphylococci to Streptomycin and Penicillin.

OTTO E. GRAESSLE AND BETTINA M. FROST. (Introduced by H. Molitor.)
From the Merck Institute for Therapeutic Research, Rahway, N.J.

It has been recognized that certain strains of staphylococci acquire resistance to penicillin when grown in a medium containing this antibiotic.[1-6] Streptomycin[7] resistant strains can also be produced *in vitro* by exposure to this agent.[8-10]

The following study was undertaken to determine (1) the rate and degree to which various staphylococcal strains acquire resistance to streptomycin and to crystalline penicillin (G); (2) whether strains made resistant to penicillin simultaneously acquire resistance to streptomycin; (3) whether the resistant strains revert to their original sensitivity after repeated transfer in nontreated broth or when stored on dry ice for long periods of time; (4) if differences occur in the metabolic activity of the resistant and sensitive strains with respect to carbohydrate fermentation, and (5) to observe if changes in morphological characteristics occur after the organisms develop resistance to streptomycin.

Materials and Methods. The potency of the penicillin used was constant since crystalline penicillin (G) was used, however the streptomycin varied in potency from 250 to 600 units per mg. Bacto Brain Heart Infusion Broth was used as the liquid broth and standard F.D.A. agar served as the solid medium. Possible changes in the carbohydrate reactions of the cultures rendered resistant to streptomycin were investigated in Bacto Peptone Colloid Medium to which was added 0.002% phenol red and 1% of the carbohydrate. The media were inoculated from a 24-hour culture, incubated at 37°C and read after 24, 30, 48 and 120 hours.

Six strains of staphylococci obtained from different sources were used and designated as

1 Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **2**, 177.

2 Rommelkamp, C. H., and Maxton, T., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 386.

3 McKee, C. M., and Houck, C. L., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 33.

4 Demerec, M., *Proc. Nat. Acad. Sci.*, 1945. **31**, 16.

5 Todd, E. W., Turner, G. S., and Drew, L. G., *Brit. Med. J.*, No. 4386, 1945.

6 Rake, G., McKee, C. M., Hamre, D. M., and Houck, C. L., *J. Immunol.*, 1944, **48**, 271.

7 Schatz, A., Bugie, E., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 66.

8 Waksman, S. A., Reilly, H. G., and Schatz, A., *Proc. Nat. Acad. Sci.*, 1945, **31**, 157.

9 Youmans, G. P., Williston, E. H., Feldman, W. H., and Hinshaw, H. C., *Proc. Staff Meet., Mayo Clinic*, 1946, **21**, 126.

10 Miller, C. P., and Bohnhoff, M., *J. A. M. A.*, 1946, **130**, 485.