

TABLE I.
Failure to Demonstrate Alkaline Phosphatase in
Inclusion Bodies.

Virus	Source inclusion	Histochemical reaction
Vaccinia	rabbit cornea	negative
"	" "	"
"	chorio-allantoic membrane	"
"	chorio-allantoic membrane	"
Fowlpox	chicken epidermis	"
Herpes simplex	chorio-allantoic membrane	"
Tracheo-laryngitis	chorio-allantoic membrane	"

The corneas of 2 rabbits were inoculated with vaccinia virus and the rabbits killed after 24 and 48 hours respectively. Vaccinia virus was inoculated into the chorio-allantoic membrane of 2 chick embryos of 12 days and allowed to incubate for 6 and 7 days. Similar preparations were made with herpes simplex

and tracheo-laryngitis virus. A section of epidermis from a chicken dead of naturally occurring fowlpox was studied. Each of these histochemical preparations revealed the presence of their corresponding large inclusion bodies, but in none of the inclusions was there any apparent alkaline phosphatase activity. Likewise, there was little or no phosphatase activity in the chorio-allantoic membrane in the immediate vicinity of the virus. The lesions in skin of the chicken, however, showed slight and scattered enzyme activity.

Summary. We failed to demonstrate alkaline phosphatase in the inclusion bodies of vaccinia, herpes simplex, fowlpox, and tracheo-laryngitis by the use of a slightly modified Gomori technic.¹⁰ While our evidence is not conclusive, it strongly favors the hypothesis of Hoagland, Ward, Smadel, and Rivers that the phosphatase activity of the inclusion body is due to tissue contamination.

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Comparative *in vitro* Resistance of Staphylococci to Penicillin and to Sodium Sulfathiazole.*†

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Investigations to be reported elsewhere¹ revealed that sulfonamide-resistant strains of staphylococcus were being recovered with increasing frequency at the University Hospitals, and that the majority of the resistant strains were being obtained from patients who had received sulfonamides prior to isolation of the organisms. In view of this, it appeared highly desirable to compare the *in vitro* resistance of a large number of strains of staphylococcus to the antistaphylococcic action of penicillin and to sodium sulfathiazole. Recent studies carried out with a relatively small number of cultures indicate that some strains of *Staphylococcus aureus* varied only slightly in their resistance to penicillin,² while other strains were quite insensitive.^{3,4,5,6}

Materials and Methods. Sixty-eight

assigned by the Committee on Medical Research for investigations recommended by the Committee on Chemotherapeutic and Other Agents of the National Research Council.

† Aided by grants from the Committee on Scientific Research of the American Medical Association and from Sharp and Dohme, Inc.

¹ Spink, W. W., and Vivino, J. J., *J. Clin. Invest.*, in press.

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

³ Fleming, A., *Lancet*, 1942, **1**, 732.

⁴ Hobby, G. L., Meyer, K., and Chaffee, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, **50**, 277.

⁵ Schnitzer, R. J., Camagni, L. J., and Buck, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1943, **53**, 75.

⁶ Abraham, E. P., Chain, E., Fletcher, C. M., Gardener, A. D., Heatley, N. G., Jennings, M. H., and Florey, H. W., *Lancet*, 1941, **2**, 177.

* The penicillin was provided by the Office of Scientific Research and Development from supplies

TABLE I.
Effect of Size of Inoculum Upon Resistance of Staphylococci to Action of Penicillin.

Strain No.	No. of organisms	Penicillin (Oxford units per cc)									
		0.1	0.2	0.5	1.0	5.0	0.05	0.1	0.2	0.4	0.6
35	22,000,000	4+	4+	4+	4+	4+					
	220,000						4+	4+	4+	4+	3+
	22,000						4+	4+	4+	4+	0
	2,200						4+	4+	4+	3+	0
10	20,000,000	4+	4+	4+	4+	4+					
	200,000						4+	4+	4+	0	0
	20,000						4+	4+	4+	0	0
	2,000						4+	4+	4+	0	0
41	10,000,000	2+	+	0	0	0					
	100,000						1+	0	0	0	0
	10,000						2+	0	0	0	0
	1,000						3+	0	0	0	0
39	30,000,000	1+	0	0	0	0					
	300,000						1+	0	0	0	0
	30,000						0	0	0	0	0
	3,000						0	0	0	0	0
42	11,000,000	1+	1+	0	0	0					
	110,000						1+	0	0	0	0
	11,000						0	0	0	0	0
	1,100						1+	0	0	0	0

4+ = Maximum growth.

strains of coagulase-positive staphylococcus were investigated, all of which had been isolated from the lesions or body fluids of patients. A standard method was used for detecting the resistance of the strains to penicillin or to sodium sulfathiazole with the aid of a chemically-defined liquid medium, buffered at pH 7, in which the presence of a sulfonamide inhibitor was a negligible factor.¹ Just prior to carrying out a test, each strain was grown for several generations in the synthetic medium. A standard inoculum of staphylococci was used since it was observed that sulfonamide-resistance was a relative phenomenon, and the degree of resistance was directly related to the number of organisms employed in a test. On the contrary, it has been reported that the size of the inoculum was of minor importance in carrying out *in vitro* tests with penicillin.⁶

The *in vitro* tests were carried out by preparing 10-fold dilutions of a 24-hour culture in synthetic medium. Then 0.1 cc of the 10⁻³ dilution was added to each of a series of test tubes containing the medium. The approximate number of organisms in such an inocu-

lum was determined by making pour plates with the 10⁻⁷ dilution. Under these circumstances, there were between 100,000 and 300,000 organisms. Freshly prepared, aqueous solutions of sodium sulfathiazole were used for each test. Increasing concentrations of the sulfonamide, ranging from 0.5 mg per 100 cc up to 360 mg, were employed in the series of tubes containing the bacterial suspensions. Aqueous solutions of the sodium salt of penicillin from the same lot were freshly prepared and added to the bacterial suspensions with the final concentrations varying between 0.05 and 5 Oxford units per cubic centimeter. The final volume in all of the tubes was 10 cc. The bacterial suspensions with and without penicillin or sodium sulfathiazole were incubated for 48 hours at 37°C. Bacterial growth was quantitated on the basis of turbidity, and the inhibitory effect of penicillin or sodium sulfathiazole was determined by selecting that tube which revealed complete inhibition of growth in the presence of the lowest concentration of the antibacterial agent.

Results. The first series of observations were concerned with the effect of the size of

TABLE II.
Comparative *in vitro* Action of Penicillin and Sodium Sulfathiazole Against 68 Strains of Staphylococcus.

Strain No.	Penicillin inhibiting growth completely (Oxford units) per cc	Sodium sulfathiazole inhibiting growth completely (mg per 100 cc)	Strain No.	Penicillin inhibiting growth completely (Oxford units) per cc	Sodium sulfathiazole inhibiting growth completely (mg per 100 cc)
18	0.8	Less than 1	16	0.1	Less than 1
43	0.8	30	17	0.1	" " "
7	0.6	Less than 1	19	0.1	" " "
34	0.6	10	21	0.1	" " "
35	0.6	Less than 1	23	0.1	" " "
40	0.6	20	24	0.1	" " "
58	0.6	100	27	0.1	" " "
10	0.4	Less than 1	29	0.1	" " "
2	0.2	" " "	32	0.1	" " "
5	0.2	" " "	33	0.1	5
6	0.2	" " "	38	0.1	80
8	0.2	" " "	42	0.1	200
12	0.2	" " "	44	0.1	100
13	0.2	" " "	50	0.1	200
14	0.2	" " "	52	0.1	160
15	0.2	" " "	59	0.1	140
20	0.2	" " "	60	0.1	Less than 1
22	0.2	" " "	61	0.1	" " "
25	0.2	" " "	62	0.1	" " "
26	0.2	" " "	64	0.1	" " "
28	0.2	" " "	66	0.1	" " "
30	0.2	" " "	45	0.05	200
31	0.2	" " "	46	0.05	180
36	0.2	20	47	0.05	200
37	0.2	5	48	0.05	180
39	0.2	10	49	0.05	200
41	0.2	250	51	0.05	200
63	0.2	Less than 1	53	0.05	200
65	0.2	" " "	54	0.05	160
67	0.2	" " "	55	0.05	180
3	0.1	" " "	56	0.05	200
4	0.1	" " "	57	0.05	200
9	0.1	" " "	68	0.05	Less than 1
11	0.1	" " "			

the bacterial inoculum upon the inhibitory action of penicillin. For this purpose, 2 strains of staphylococcus relatively resistant to penicillin were selected, and compared with 3 strains considered to be sensitive to penicillin. The results are presented in Table I. The inhibitory effect of penicillin upon the growth of staphylococci sensitive to the action of the drug is but little affected by the number of cocci used in the test. This does not apply to the more resistant strains, and in such instances the interpretation of *in vitro* resistance studies might be confusing because of the number of bacteria utilized in a test.

The comparative results of the *in vitro* action of penicillin and sodium sulfathiazole upon 68 strains of staphylococcus are summarized in Table II. There appeared to be no relationship between the resistance of the cultures to penicillin and their resistance to sodium sulfathiazole, an observation which has been made by others with the pneumococcus.^{7,8} Strain 58 was highly resistant to

⁷ Powell, H. M., and Jameson, W. A., PROC. SOC. EXP. BIOL. AND MED., 1942, 49, 387.

⁸ McKee, C. M., and Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1942, 51, 275.

the antibacterial action of both penicillin and sodium sulfathiazole. Strain 41 is the most resistant culture for the sulfonamides that has been encountered over a period of 3 years, and yet its growth was inhibited by 0.2 unit of penicillin.

Since several strains were found to be relatively resistant to penicillin but sensitive to sodium sulfathiazole, it appeared that simultaneous exposure of the organisms to both agents would require lower concentrations of each to inhibit growth completely than that found to be necessary when either penicillin or sodium sulfathiazole was used alone. Rammelkamp and Keefer⁹ observed that small amounts of penicillin without demonstrable inhibitory effect for staphylococci enhanced the antistaphylococcic action of sulfathiazole in defibrinated blood. Observations were made with strains 18, 34, 40, and 43, and in each instance it was observed that smaller concentrations of penicillin and sodium sulfathiazole were necessary to inhibit growth than was required when either agent was used alone.

Discussion and Conclusions. When 68 strains of pathogenic staphylococcus were subjected to the *in vitro* action of sodium penicillin, 8 of the strains, or approximately 12%, were found to be quite resistant, requiring from 0.4 units per cc to 0.8 units of penicillin before growth was completely inhibited. The

growth of the remaining 60 strains necessitated the following amounts of penicillin before growth was inhibited: 22 strains, or about 32%, required 0.2 units; 25 or approximately 37%, required 0.1 unit; while 12 or about 18%, failed to grow in the presence of 0.05 unit. About 28% of the strains were found to be highly resistant to the antistaphylococcic action of sodium sulfathiazole. There was no relationship between resistance to penicillin and to the sulfonamide. The evidence at hand would indicate that the mechanism whereby staphylococci become resistant to the sulfonamides is different and unrelated to the development of resistance to penicillin.¹⁰⁻¹⁴

Since some strains of staphylococcus isolated from patients appear to be naturally resistant to penicillin, and yet, sensitive to sodium sulfathiazole, it would not be unreasonable to assume that a combination of therapy with penicillin and sulfathiazole might be advantageous in the management of some types of staphylococcic sepsis. Further clinical observations are necessary to substantiate this, but preliminary investigations appear to bear out this assumption.

¹⁰ Landy, M., Larkum, N. W., Oswald, E. J., and Streightoff, F., *Science*, 1943, **97**, 265.

¹¹ Spink, W. W., Wright, L. D., Vivino, J. J., and Skeggs, H. R., *J. Exp. Med.*, in press.

¹² Gardner, A. D., *Nature*, 1940, **146**, 837.

¹³ Smith, L. D., and Hay, T., *J. Franklin Inst.*, 1942, **233**, 598.

¹⁴ Hobby, G. L., Meyer, K., and Chaffee, E., *Proc. Soc. Exp. Biol. and Med.*, 1942, **50**, 281.

⁹ Rammelkamp, C. H., and Keefer, C. S., *J. Clin. Invest.*, 1943, **22**, 649.

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Antibacterial Effect of Whole Blood upon Strains of Staphylococci Sensitive and Resistant to Penicillin.*†

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In a previous report,¹ it was shown that coagulase-positive strains of staphylococcus isolated from human subjects varied in their susceptibility to the *in vitro* action of penicillin. The purpose of the present investiga-

tions was to compare the antibacterial action of defibrinated human blood upon strains of staphylococcus adapted to grow in increasing concentrations of penicillin with the parent strains, whose growth was readily inhibited