

benadryl. The possibility of correlation between anesthetic action and antihistamine effect deserves further study.

Summary. A study was made of the local anesthetic effect of β -dimethylaminoethyl benzhydryl ether hydrochloride (benadryl) when injected into the skin of human beings. The results were as follows: 1. By means of electric algometric determinations, benadryl in dilutions of 1:500, 1:1,000, 1:5,000,

1:10,000 and 1:20,000 was found to possess anesthetic potencies similar to those of procaine in dilutions of 1:200, 1:400, 1:800, 1:1,600 and 1:3,200 respectively. 2. Benadryl in concentrations greater than 1:500 (2 mg per cubic centimeter) proved to be exceedingly irritating when injected into human skin, causing tissue necrosis and ulceration in 4 of 10 subjects tested.

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Comparative Inhibitory Effect of Penicillin and Streptomycin Upon the Action of Staphylocoagulase.*

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One of the properties of pathogenic staphylococci is the capacity to induce the formation of a fibrin clot when the organisms are added to citrated human plasma. Though the significance of this biologic phenomenon is not clear, Hale and Smith¹ have pointed out that the *in vitro* phagocytosis of coagulase-positive staphylococci is inhibited in the presence of coagulable plasma and suggested that the invasiveness of these strains is associated with the *in vivo* production of a coagulum around the cocci which prevents efficient phagocytosis and destruction of the bacteria. Spink and Vivino² showed that the sulfonamides had a variable effect on the action of staphylocoagulase.

The present study revealed that streptomycin had a marked inhibitory effect on the action of staphylocoagulase in contrast to the results obtained with penicillin.

Material and Methods. Eighteen strains of coagulase-positive staphylococci were utilized; 5 strains being stock cultures, and 13

of them recently isolated from the lesions of patients. The strains were grown for 24 hours in tryptose phosphate broth and then 1 ml of each culture was added to a series of 11 sterile test tubes. Serial dilutions of penicillin and streptomycin were freshly prepared in physiologic saline solution and 1 ml amounts of the desired dilutions were added to each of the tubes containing organisms. The initial concentrations per ml of each antibiotic were 50,000 units, or 15 mg of crystalline penicillin G sodium, and 250 mg of streptomycin (base equivalent). The coagulase tests were performed by adding 0.2 ml of each culture-antibiotic mixture to 0.5 ml of citrated human plasma contained in small test tubes. The tubes were placed in a water bath at 37°C and examined for the presence of a coagulum after 2, 12 and 24 hours. Coagulation was graded from 1+ to 4+, 1+ indicating a coagulum that was just visible, while 4+ meant the formation of a solid clot, and 2+ and 3+ were designated as intermediate coagulation. The contents of each tube of antibiotic-culture mixture were tested for the presence of viable cells by streaking standard loopful amounts on agar plates, and incubating at 37°C for 24 hours.

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¹ Hale, J. H., and Smith, W., *Brit. J. Exp. Path.*, 1945, **26**, 209.

² Spink, W. W., and Vivino, J. J., *Proc. Soc. Exp. Biol. and Med.*, 1942, **50**, 37.

TABLE I.

Results with Strain 19-11 in Which Tests for Activity of Staphylocoagulase and Growth Inhibition Were Made Immediately After Mixing Culture with Penicillin or with Streptomycin.

Penicillin						Streptomycin				
Concentration per ml		Action of staphylocoagulase				Action of staphylocoagulase				
Mg	Units	2 hr	12 hr	24 hr	Growth 24 hr	Mg per ml	2 hr	12 hr	24 hr	Growth 24 hr
15.	25,000	0	0	±	0	125.	0	0	0	0
7.5	12,500	±	1+	1+	0	63.	0	0	0	0
3.75	6,250	1+	2+	2+	0	31.	0	0	0	0
1.87	3,125	1+	2+	2+	0	16.	0	0	0	0
.93	1,562	1+	2+	3+	+	8.	0	0	0	0
.47	780	1+	2+	3+	+	4.	0	0	0	+
.23	390	1+	3+	3+	+	2.	0	0	0	+
.12	195	1+	3+	2+	+	1.	0	0	0	+
.06	98	2+	3+	2+	+	0.5	0	0	0	+
.03	49	2+	3+	2+	+	0.25	0	0	0	+
Control	0	3+	3+	2+	+	0	0	0	0	+

Two procedures were followed in detecting the effect of the antibiotics on the action of staphylocoagulase. In procedure A, the coagulase and viability tests were set up immediately after the antibiotics and organisms had been mixed. In procedure B, mixtures of antibiotics and organisms were incubated for 24 hours at 37°C before carrying out the coagulase and viability tests. Comparative studies were made with the 2 procedures to determine whether the length of time the bacterial cells were exposed to the antibiotics affected the action of staphylocoagulase. In each instance, control tubes containing only staphylococci and physiologic saline solution were used.

Results. Table I presents the data on one experiment with strain 19-11 in which procedure A was followed. It is to be noted that streptomycin markedly inhibited the action of staphylocoagulase even in the presence of viable cells in contrast to the inhibition produced by penicillin. Table II expresses the results of an experiment with the same strain using procedure B. The results are comparable to those given in Table I. It was observed that the incubation of the cells and antibiotics for 24 hours, as carried out in procedure B, delayed the clotting effect of staphylocoagulase longer than that obtained with procedure A in which no preliminary period of incubation took place before testing for coagulase activity. But this retardation of the action of coagulase was

not associated with an increased destruction of organisms.

Similar results were obtained with the remaining 17 strains. It is of interest that low concentrations of streptomycin failed to inhibit the coagulase activity of only one of the 18 strains (strain 6-16). This strain proved to be moderately resistant to the lethal action of streptomycin. Relative resistance to penicillin was also shown with 3 strains, but it is to be pointed out that a large inoculum of organisms was used throughout the experiments. The inhibition of staphylocoagulase activity did not parallel the complete inhibition of growth by streptomycin. In other words, the activity of staphylocoagulase was completely inhibited by much lower concentrations than that required to render the cultures nonviable. The results with streptomycin are in contrast with those obtained with penicillin in which the inhibitory effect was less pronounced. Table III is a summary of the comparative results of coagulase activity in the presence of streptomycin and penicillin, after the mixtures of antibiotics and organisms had been incubated for 24 hours. There is a statistically significant difference between the degree of inhibition of coagulase activity as exhibited both by streptomycin and penicillin in the lower concentrations. Thus, while penicillin exerted some inhibitory effect on the activity of staphylocoagulase in the higher concentrations, its effectiveness was considerably diminished and

TABLE II.

Results with Strain 19-11 in Which Tests for Activity of Staphylocoagulase and Growth Inhibition Were After Mixtures of Culture and Penicillin or Streptomycin Had Been Incubated for 24 Hours at 37°C.

Penicillin						Streptomycin					
Concentration per ml		Action of staphylocoagulase						Action of staphylocoagulase			
Mg	Units	2 hr	12 hr	24 hr	Growth 24 hr	Mg per ml		2 hr	12 hr	24 hr	Growth 24 hr
15.	25,000	0	0	0	0	125.		0	0	0	0
7.5	12,500	0	0	0	0	63.		0	0	0	0
3.75	6,250	0	0	0	0	31.		0	0	0	0
1.87	3,125	0	0	0	0	16.		0	0	0	0
.93	1,562	0+	1+	±	+	8.		0	0	0	0
.47	780	1+	2+	2+	+	4.		0	0	0	0
.23	390	1+	3+	3+	+	2.		0	0	0	+
.12	195	1+	3+	3+	+	1.		0	0	0	+
.06	98	2+	3+	3+	+	0.5		0	0	0	+
.03	49	2+	3+	3+	+	0.25		0	0	0	+
Control	0	1+	2+	1+	+	0					

TABLE III.

Summary of Comparative Inhibition of Staphylocoagulase Activity of 18 Strains by Varying Concentrations of Penicillin and Streptomycin.

Penicillin				Streptomycin			
Concentration, mg/ml	Inhibition	No. of strains	%	Concentration mg/ml	Inhibition	No. of strains	%
15.	None	1	5.55	16. -31	Slight	3	16.65
3.75-14.99	Slight	12	66.25	4. -8	Moderate	1	5.55
.23- 3.74	Moderate	4	22.22	.25- 2	Marked	5	27.77
.03- .22	Marked	1	5.55	<.25	Very marked	9	50.00

lost with lower concentrations while the inhibitory effect of streptomycin was pronounced even in concentrations of 0.25 mg per ml. The comparative results with the 2 antibiotics have been expressed quantitatively with respect to the actual weights of penicillin and streptomycin in each millimeter of mixture. It should be pointed out that streptomycin has a higher molecular weight than crystalline penicillin G sodium, but when comparison is made with the molecular equivalents of each of the antibiotics, streptomycin still has a more marked effect on the action of staphylocoagulase than penicillin.

The mechanism whereby penicillin, and to

a greater extent, streptomycin inhibit the action of staphylocoagulase requires further study. The foregoing results show that inhibition by streptomycin occurred in the presence of viable cells. However, this does not necessarily imply that the antibiotic acted directly upon staphylocoagulase or blocked the reaction between staphylocoagulase and plasma.

Summary. Comparative studies with penicillin and streptomycin on 18 strains of coagulase-positive staphylococci revealed that the action of staphylocoagulase on human plasma is inhibited to a greater extent by streptomycin than by penicillin.