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Antibiotic Combinations and Resistance to Antibiotics: Penicillin with Other Antibiotics Against Penicillin-Resistant Staphylococci.* (20433)

MAXWELL FINLAND AND CLARE WILCOX.

From the Thorndike Memorial Laboratory, 2nd and 4th Medical Services, Boston City Hospital and the Department of Medicine, Harvard Medical School, Boston, Mass.

In a previous study(1) it was shown that the development of resistance to penicillin, streptomycin or erythromycin *in vitro* may be delayed and depressed by the use of a combination of erythromycin with either penicillin or streptomycin. The organisms used in that study were originally susceptible to small or moderate concentrations of each of these antibiotics, and when they were repeatedly subcultured in the combination of 2 antibiotics, these were present in increasing concentrations, but always in a constant ratio. In the present study an attempt was made to determine whether the presence of a moderately high concentration of an antibiotic to which bacteria are originally resistant would also serve to delay or depress the development of resistance to another antibiotic following repeated exposures to increasing concentrations of the latter.

Materials and methods. The 8 strains of *Staphylococcus aureus* used in the present study had been isolated from infected materials during a recent survey(2); they were each inhibited by 25 μg/ml of streptomycin and 0.2 μg/ml of erythromycin but grew well in the presence of 1000 μg/ml of penicillin. They were all hemolytic, coagulase-positive and produced penicillinase. Each of the strains was subcultured on 3 parallel series of blood agar plates: one contained no antibiotic, the second contained penicillin 200 μg/ml plus graded (2-fold) concentrations of streptomycin [as in the plate-dilution test for sensitivity(3)], while the third contained the same amounts of penicillin and similarly graded concentrations of erythromycin. The inoculum for the antibiotic-containing plates was obtained in each instance by scraping a 3 mm loopful of the surface growth from the plate containing the maximum concentration of antibiotic on which nearly full growth occurred after 48 hours incubation, suspending this in 0.2 ml of broth and streaking a 1 mm loopful of the suspension on a similar series of plates. This method is similar to the one employed in previous studies(1,4-8). At the completion of 25 such subcultures (except strain 31, which was subcultured 29 times) all of the resulting strains were tested simultaneously for sensitivity to penicillin, streptomycin, erythromycin, carbomycin and neomycin by the usual plate-dilution method(3). Sensitivity of any strain to a given antibiotic is defined as the minimum concentration of that antibiotic in which there is no visible growth after 48 hours.

Results. Resistance to streptomycin and erythromycin. The changes in the sensitivity of the strains during successive transfers in the presence of a constant concentration of penicillin and increasing concentrations of either streptomycin or erythromycin are depicted in Fig. 1. The development of resistance to streptomycin in the presence of penicillin was uniformly rapid and was virtually complete after from 5 to 8 transfers. Resistance to erythromycin under similar circumstances developed somewhat more irregularly,

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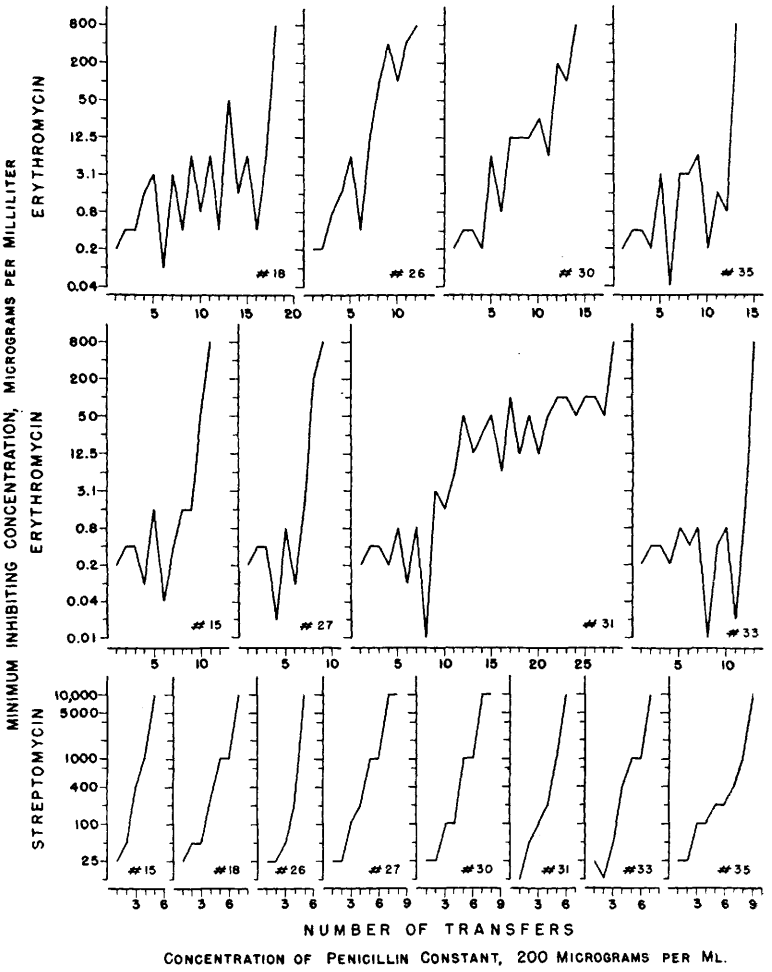

FIG. 1.

but each of the strains had become resistant to 800 μg/ml after from 9 to 18 subcultures, except in the case of Strain 31 which required 28 transfers before this degree of resistance was acquired.

Cross-resistance. It was previously shown that high degrees of cross-resistance to carbomycin quite regularly accompanied the development of resistance to erythromycin following exposures to the latter *in vitro*(8). Cross-resistance between streptomycin and neomycin have also been demonstrated although less regularly(6). In the present study, the sensitivity of all of the strains after completion of the 3 series of transfers was tested simultaneously to the 3 antibiotics used in the study and also to carbomycin and neomycin. The results are shown in Table I. No cross-resistance developed to erythromycin following the subcultures in streptomycin, and *vice versa*. Resistance to carbomycin developed to a high degree in each of the strains as a result of the exposures to erythromycin. A significant increase in resistance to neomycin occurred in only 1 strain and a slight increase to another at the end of the series of subcultures on streptomycin. One of the strains (No. 32) became streptomycin-dependent during the course of the exposures to streptomycin; none of the other 4 antibiotics (including neomycin) in any of a wide range of concentrations could support the growth of this streptomycin-dependent strain in the absence of streptomycin. All strains of the streptomy-

TABLE I. Effect of Repeated Subcultures of Penicillin-Resistant Staphylococci on Agar Containing Constant Concentration of Penicillin (200 μg/ml) plus Graded Concentrations of Streptomycin or Erythromycin on Their Resistance to Each of These Antibiotics and also to Carbomycin and Neomycin.

Strain	Subcultures*	Minimum inhibiting concentration, μg/ml				
		P	S†	E†	C	N
15	0 × 25	>6400	25	.4	12.5	1.6
	P + S × 25	>6400	>10000 (5)	.2	3.1	1.6
	P + E × 25	>6400	25	>800 (11)	>800	.8
18	0 × 25	6400	25	.4	12.5	1.6
	P + S × 25	800	>10000 (7)	.2	3.1	1.6
	P + E × 25	>6400	25	>800 (18)	400	1.6
26	0 × 25	6400	25	.4	12.5	1.6
	P + S × 25	800	>10000 (5)	.2	.8	3.1
	P + E × 25	6400	25	>800 (12)	400	.4
27	0 × 25	6400	25	.4	12.5	.8
	P + S × 25	800	>10000 (8)	.2	3.1	3.1
	P + E × 25	6400	12.5	>800 (9)	400	.8
30	0 × 25	>6400	25	.4	12.5	1.6
	P + S × 25	>6400	>10000 (8)	.2	3.1	3.1
	P + E × 25	>6400	25	>800 (14)	800	.8
31	0 × 29	1600	25	.4	12.5	1.6
	P + S × 29	1600	>10000 (6)	.2	3.1	6.3
	P + E × 29	3200	12.5	>800 (28)	200	3.1
32	0 × 25	>6400	25	.4	12.5	1.6
	P + S × 25	S-Dep.‡	>10000 (7)	S-Dep.	S-Dep.	S-Dep.
	P + E × 25	6400	12.5	>800 (13)	>800	1.6
35	0 × 25	>6400	25	.4	12.5	1.6
	P + S × 25	3200	>10000 (9)	.2	6.3	25
	P + E × 25	6400	12.5	>800 (14)	>800	1.6

P = penicillin; S = streptomycin; E = erythromycin; C = carbomycin; N = neomycin.
* 0 = No antibiotic; concentration of penicillin in mixtures was constant (200 μg/ml).
† Number of transfers required to achieve maximum concentration is shown in parentheses. The organisms at start of experiment were each inhibited by streptomycin, 25 μg/ml and by erythromycin, 0.2 μg/ml.
‡ Streptomycin-dependent.

cin series became somewhat more sensitive to carbomycin, but their neomycin sensitivity was not affected by the exposures to erythromycin.

Effect on coagulase and penicillinase. Each of the strains was tested for coagulase activity on several occasions during the course of the study. For the most part, coagulase was demonstrated in all of the strains throughout the study; some irregularities were noted, however, on a few strains at times when it was necessary to resort to the use of plasma from different donors for the tests. All of the parent strains produced penicillinase(9) and this property was retained essentially intact throughout the 3 series of subcultures.

Summary and conclusion. Eight strains of staphylococci which were originally resistant to penicillin but sensitive to streptomycin and erythromycin were repeatedly subcultured in the presence of 200 μg/ml of penicillin along with increasing concentrations of one of the other 2 antibiotics. Resistance to streptomycin or erythromycin resulted from the exposures to the homologous agent in spite of the presence of the high but ineffective concentration of penicillin. Cross-resistance between streptomycin and erythromycin did not occur, but high degrees of resistance to carbomycin were demonstrated in all of the strains which had become resistant to erythromycin and some increase in resistance to neomycin occurred in 2 of the strains which had become resistant to streptomycin. These findings suggest that for the suppression or delay in development of resistance to one of a pair of antibiotics to which bacteria are simultaneously exposed, both of the antibiotics must exert

an inhibitory activity; if the strain is resistant to one of the pair of antibiotics, its presence in a high but ineffective concentration may not influence the development of resistance to the second agent.

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Fatty Acid and Ketone Metabolism during Hemorrhage and Shock in the Rat.* (20434)

FRANK L. ENGEL AND KATHLEEN HEWSON.

From the Departments of Medicine and Physiology, Duke University, Durham, N. C.

Although there is now available considerable information concerning protein and carbohydrate metabolism during hemorrhagic shock in the rat, little or nothing is known about fat metabolism in this syndrome(1-3). Since hepatic metabolism appears to be particularly disturbed during shock, it seemed pertinent to investigate some aspects of ketone body metabolism since the liver is the chief site of synthesis of these substances. It has been shown that the oxygen consumption and the rates of a variety of oxidative reactions by liver slices from shocked rats may be profoundly depressed, but the degree of depression of oxygen consumption cannot be accounted for by the metabolic disturbances so far demonstrated in the liver. Since, during fasting, fat oxidation and ketone production may account for a very substantial part of the oxygen requirements of the liver, this study was initiated in an attempt to determine whether fatty acid and ketone metabolism might be sufficiently disturbed during hemorrhage and shock in the rat to contribute to the observed lowered hepatic oxygen consumption.

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Materials and methods. Male albino rats of the Vanderbilt strain, weighing between 200 and 270 g, were fasted 24 hours prior to bleeding. Shock was induced under light nembutal anesthesia by bleeding from the cut tail a volume of blood equivalent to 2.5% of the body weight in one hour. Blood samples for ketone and amino nitrogen determinations were withdrawn at appropriate intervals, and the animals observed for 3 to 5 hours and then sacrificed for other studies(4,5). In one group of rats, adrenalectomies were performed 4 to 5 days prior to bleeding, the animals being maintained with 0.5 mg of desoxycorticosterone acetate intramuscularly daily and saline in their drinking bottles. Ketone levels were determined on 0.4 ml samples of heparinized whole blood by a modification of the method of Greenberg and Lester(6,7) and amino nitrogen in plasma by the method of Frame *et al.*(8).

Results. The effects of hemorrhage and shock on blood ketone levels are depicted in Fig. 1. The control rats, which had blood samples drawn just for analyses every hour for 7 hours, exhibited a slow progressive rise in ketone levels, as might be anticipated during a continuing fast. The bled rats were divided into 2 groups, designated as "sublethal hemorrhage" and "hemorrhagic shock"