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 Keefer[9] 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.

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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 investigations 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

by penicillin. Prior observations had revealed that normal human blood, with but few exceptions, possessed little or no killing effect for small numbers of pathogenic staphylococci.[2] In general, coagulase-positive strains of staphylococcus resisted the bactericidal action of human whole blood, whereas coagulase-negative strains were killed in large numbers.[3]

It has been reported that cultures of pneumococci, streptococci, and staphylococci may develop an increased resistance to penicillin following *in vitro* and *in vivo* exposure of the organisms to the drug.[4,5,6,7]

Materials and Methods. Five strains of coagulase-positive staphylococci were utilized. The *in vitro* sensitivity of these strains for both penicillin and sodium sulfathiazole was first ascertained by the dilution method with a standardized inoculum of organisms in a synthetic medium.[1,8] The Eden strain was isolated from the blood stream of a child, while Eden I was obtained from the exudate of an osteomyelitic sinus after this child had received large doses of penicillin and sulfathiazole. The Rutgers strain was cultured from the exudate present in an extensive subcutaneous lesion of a child. The Ked and Nelson strains were obtained from draining osteomyelitic sinuses, present in each of 2 children. Of the five strains, Rutgers and Nelson were highly resistant to the *in vitro* action of sodium sulfathiazole.

The sensitivity of each strain for penicillin was first determined, and then resistance to the drug was developed in the following manner: each of several tubes contained 9 cc of the synthetic medium to which was added 1 cc of sodium penicillin dissolved in isotonic saline solution. A standard loopful of a 24- to 48-hour culture of organisms in synthetic medium was seeded to medium containing the added penicillin in a concentration which permitted demonstrable growth within 24 hours. Loopful transfers of the organisms were made every 24 to 48 hours in a given concentration of penicillin until maximum growth could be attained within 24 hours. Then the concentration of penicillin was increased and the serial transfers repeated. Simultaneously, serial transfers were made with the parent strain in 5 cc of synthetic medium. Comparative observations were also made to ascertain whether there was any difference in the rate of development of resistance in veal infusion broth and in synthetic medium. When the maximum degree of resistance to penicillin had been attained in the synthetic medium, the resistance of the strains to sodium sulfathiazole was compared with the parent strains.

The bactericidal action of human blood for the resistant strains and their susceptible parent strains was performed in the same manner as described elsewhere.[3] Briefly, the method was as follows: fresh defibrinated blood from normal individuals was used. Tenfold dilutions were prepared in broth from a 24-hour culture grown in synthetic medium, and 0.1 cc of each dilution (up to 10^{-7}) was added to 0.5 cc of the defibrinated blood in small pyrex test tubes. The approximate number of organisms seeded to each tube was determined by making pour plates with 0.1 cc of the 10^{-7} dilutions. The tubes were sealed in a gas-oxygen flame, and then rotated for 48 hours at 37°C in a box contained in an incubator. At the end of this time, the contents of each tube were cultured for viable

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organisms. In a second series of observations, the bactericidal action of whole blood with and without added sodium sulfathiazole was quantitated.

Results. Development of in vitro *resistance to penicillin.* Strains Eden and Ked were initially inhibited respectively by 0.04 and 0.08 Oxford units per cc of penicillin, but maximum growth took place in the presence of 0.01 units. The growth of both strains was inhibited by less than 1 mg per 100 cc of sodium sulfathiazole. After 109 serial transfers for each of the strains in increasing concentrations of penicillin maximum growth was obtained in 1 unit per cc. Both strains were then transferred to veal-agar slants and placed in a refrigerator for 3 months. At the end of this period, the cultures were seeded to synthetic medium for 4 generations, and then exposed to penicillin. Both strains had retained their resistance to penicillin. After 101 additional transfers in increasing concentrations of penicillin, both strains were capable of growing in the presence of 50 units per cc of penicillin. Comparative studies with these 2 strains revealed that both could be more readily adapted to grow in the presence of increasing concentrations of penicillin when veal infusion broth was used as the medium instead of synthetic medium. Although both cultures developed a high degree of resistance to the antistaphylococcic action of penicillin, both remained sensitive to the inhibitory effect of sodium sulfathiazole. While the resistance of both of these strains to penicillin had been induced by growing the cultures in synthetic medium containing penicillin, it was observed that the cultures retained this resistance when veal infusion broth was used as the medium.

Studies were made with the Ked culture to determine if there was any difference in the rate at which this strain developed resistance to penicillin as compared to sodium sulfathiazole. Organisms were exposed simultaneously to increasing concentrations of each of the drugs in synthetic medium. Under these circumstances, Ked became more readily adapted to grow in the presence of the sulfonamides than in the penicillin.

The Rutgers and Nelson strains were both highly resistant to sodium sulfathiazole, the former requiring a concentration of 160 mg per 100 cc before growth was completely inhibited, and the latter 180 mg. On the other hand, both cultures were quite sensitive to penicillin, the growth of Rutgers being inhibited by 0.06 Oxford units per cc, and Nelson by 0.05 units. After 94 serial transfers in the presence of increasing concentrations of penicillin, both cultures grew readily in 4 cc units per cc, with complete inhibition of growth taking place in 6 units per cc.

It had been previously reported that a strain of *Staphylococcus aureus* having acquired resistance to penicillin showed no fundamental change in biological or metabolic activities as compared to the parent strain, except that the rates at which these activities were carried out were slower for the resistant strain.[4] These observations had been confirmed in the present study. The resistant strains retained their property of coagulating plasma. Acquired resistance to penicillin was associated with morphological changes of the bacterial cells, which appeared to be permanently acquired characteristics. The cells showed marked pleomorphism with large spherical cell bodies quite apparent. The parent cultures of Rutgers and Nelson had previously been shown to produce a brown-orange pigment in the presence of high concentrations of sodium sulfathiazole.[9] While the cultures of Rutgers and Nelson adapted to resist penicillin still retained the same degree of resistance for sodium sulfathiazole as the parent strains, the pigment produced by the adapted strains was much less than that produced by the parent strains.

Development of in vivo *resistance to penicillin and sodium sulfathiazole.* The growth of Eden, originally isolated from the blood stream of a child, was inhibited by 0.04 Oxford unit per cc of penicillin and by less than 1 mg per 100 cc of sodium sulfathiazole. She was given penicillin parenterally and dramatic improvement followed. However, a complication of her illness was the development of osteomyelitis of the left femur which resulted in a progressive decalcification of the bone.

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TABLE I.
Antibacterial Action of Human Defibrinated Blood upon Strains of Staphylococcus Sensitive and Resistant to the Action of Penicillin.

Strain	Penicillin inhibiting growth completely (Oxford units per cc)	Sod. sulfathiazole inhibiting growth completely (mg per 100 cc)	Dilution of organisms 10^1	10^2	10^3	10^4	10^5	10^6	10^7	No. organisms 10^7 dilution
Eden (parent)	0.04	Less than 1	4+	4+	4+	4+	4+	4+	0	24
Eden (resistant)	80	" " "	3+	2+	2+	0	0	0	0	11
Ked (parent)	0.06	" " "	4+	4+	4+	4+	4+	4+	4+	19
Ked (resistant)	120	" " "	4+	4+	4+	4+	2+	0	0	13
Rutgers (parent)	0.06	160	4+	4+	4+	4+	4+	4+	2+	22
Rutgers (resistant)	6	160	4+	4+	+	+	0	0	0	20
Nelson (parent)	0.05	180	4+	4+	4+	4+	4+	4+	4+	12
Nelson (resistant)	6	180	4+	4+	4+	+	0	0	0	25
Eden (parent)	0.04	Less than 1	4+	4+	4+	3+	3+	0	0	25
Eden I (resistant)	0.8	200	4+	4+	3+	2+	0	0	0	6

0 = Growth.
4+ = Maximum growth.

Over a period of weeks she was given 225 g of sulfathiazole. Fourteen months after the onset of her illness, there was still active involvement of the bone, and she was given over two million Oxford units of penicillin. This resulted in improvement. A site over the infected bone became reddened, and finally spontaneous rupture and evacuation of purulent material occurred from which Eden I was cultured. The growth of Eden I was inhibited by 0.8 units of penicillin and 200 mg of sodium sulfathiazole. It was concluded that the resistance of Eden I was induced by the administration of large amounts of penicillin and sodium sulfathiazole.

Bactericidal Tests. The killing effect of whole defibrinated blood upon the 5 parent strains and their subcultures, which had developed a resistance to penicillin, is summarized in Table I. In every instance except in the case of Eden I, the resistant cultures were killed in larger numbers than were the sensitive strains. The degree of resistance to penicillin manifested by Eden I was much less than that shown for the other resistant cultures. The ability of the organisms to grow in the presence of sodium sulfathiazole was unrelated to the bactericidal action of the blood. The important difference in the antistaphylococcic action of the blood appeared to be related to the resistance of the cultures to penicillin. Strains resistant to the sulfonamide were as capable of growing in defibrinated blood as their nonresistant progenitors.

When the same experiments were repeated with and without added sodium sulfathiazole, it was observed that the sulfonamide exerted an additional bactericidal effect provided the strain being tested was sensitive to the action of sodium sulfathiazole.

Discussion and Conclusions. Strains of staphylococcus adapted to grow in the presence of the sulfonamides are just as invasive as other strains which are inhibited in growth by the sulfonamides. Whole, human blood has no greater bactericidal action on the sulfonamide-resistant strains than on the sulfonamide-sensitive strains. Strains of staphylococcus adapt themselves to grow in the presence of penicillin at a slower rate and to a lesser degree than is apparent for the sulfonamides. And probably of considerable clinical importance is that an increased resistance to penicillin is accompanied by the development of strains which are more susceptible to the bactericidal action of whole blood, and possibly to the other defense mechanisms of the host.