JCI The Journal of Clinical Investigation

SULFONAMIDE-RESISTANT STAPHYLOCOCCI: CORRELATION OF *IN VITRO* SULFONAMIDE—RESISTANCE WITH SULFONAMIDE THERAPY

Wesley W. Spink, J. J. Vivino

J Clin Invest. 1944;23(2):267-278. https://doi.org/10.1172/JCI101490.

Research Article

SULFONAMIDE-RESISTANT STAPHYLOCOCCI: CORRELATION OF *IN VITRO* SULFONAMIDE—RESISTANCE WITH SULFONAMIDE THERAPY[1]

By WESLEY W. SPINK AND J. J. VIVINO

(From the Division of Internal Medicine, University of Minnesota Hospitals and Medical School, Minneapolis)

(Received for publication September 20, 1943)

A feature of sulfonamide therapy for bacterial infections is that species of bacteria differ in their resistance to the antibacterial action of the compounds. It is also recognized that strains of microorganisms within the same species display variations in susceptibility. A disconcerting and confusing factor associated with chemotherapy, which appears to be assuming increasing clinical significance, is the ease and frequency with which some species of bacteria may develop *in vitro* and *in vivo* resistance to the bacteriostatic action of the sulfonamides. In the literature, the term "sulfonamide-fast" has been applied to those strains which become resistant to the antibacterial action of the compounds. This is particularly applicable to studies involving species of bacteria whose progenitors were known to be sensitive to the sulfonamides. Because the development of resistance is a relative phenomenon, and because, under proper experimental conditions, the growth of even the most resistant strains of bacteria may be inhibited by the drugs, the term "sulfonamide-resistant" is believed to be a more accurate description.

The purpose of this report is to review briefly the problem of sulfonamide-resistant bacteria in general, and to record the results of investigations with several strains of staphylococcus isolated from patients. An attempt has been made to answer the following questions: If a standard *in vitro* test is used for quantitating the inhibitory effect of the sulfonamides upon the growth of staphylococci, do strains of this species vary in their susceptibility to the antistaphylococcic action of the compounds? Is there any correlation between the isolation of sulfonamide-resistant strains of staphylococci from patients and previous sulfonamide therapy carried out in these patients? Is the development of strain resistance a permanent characteristic of the bacteria?

Several species of bacteria have been rendered resistant to the *in vitro* and *in vivo* action of the sulfonamides. Many of the investigations have been carried out with different strains of the pneumococcus (1 to 5). Sesler and Schmidt (6) observed the development of sulfonamide-resistant pneumococci, and concluded that strains vary in developing this sulfonamide-resistance; that a given strain develops resistance to the several sulfonamides at different rates; that the more susceptible a parent strain is to the action of a sulfonamide, the more difficult it is to develop resistance; and that strains which become resistant to one sulfonamide, are resistant to all the other compounds tested. There is evidence that the development of sulfonamide-resistant pneumococci is more than a temporary phenomenon (7 to 9). On the other hand, sulfonamide-resistant pneumococci are sensitive to the action of specific antipneumococcus serum. Hemolytic streptococci, particularly Lancefield group A strains, are usually quite susceptible to the action of sulfanilamide, and yet strains in this group may acquire *in vitro* and *in vivo* resistance to the drug (10, 11). While staphylococci as a species are more refractory to the bacteriostatic effect of all of the sulfonamides than are pneumococci and hemolytic streptococci, it has been demonstrated that strains of staphylococci may develop an increased resistance to the compounds (12, 13). Gram-negative species of bacteria, whose growth is usually inhibited *in vitro* by the sulfonamides, have been shown to develop sulfonamide-resistance. These include *E. coli* (14, 15), *B. abortus* (16), meningococci (17), and *Shigella paradysentariae* (strains of Flexner and Sonne) (18). Strains of gono-

[1] Aided by grants from the Committee on Scientific Research, American Medical Association, and the Charles P. DeLaittre Research Fund, University of Minnesota Medical School.

cocci, a species which is highly susceptible to the bacteriostatic action of the sulfonamides, have been made resistant to sulfanilamide and sulfapyridine (19, 20). Carpenter and his associates (21) could not develop an increased tolerance of 10 strains of gonococci to sulfathiazole over a period of 3 months. Nevertheless, this has been accomplished by Kirby (22).

If invasive strains of microorganisms develop resistance to the sulfonamides both *in vitro* and in experimental animals, the question immediately arises as to whether such a phenomenon may transpire in human subjects while they are being treated with one of the sulfonamides, and whether the development of sulfonamide-resistant strains will affect the ultimate recovery of the patient. While caution must be exercised in transposing quantitatively *in vitro* data or the results of animal protection tests with the sulfonamides to the problem of chemotherapy in human subjects, these data are often quite helpful in directing the clinical application of the drugs. Several investigators have confirmed the observation that specific types of pneumococci isolated from patients have shown an initial sensitivity to a sulfonamide, but as chemotherapy proceeded, subsequent isolation of the same type of pneumococcus revealed the development of sulfonamide-resistance (8, 23 to 28). In some instances, coincident with the detection of sulfonamide-resistant pneumococci, the patients' condition became worse or they failed to respond as anticipated. Similar observations have been made with other species of bacteria. Francis (29) encountered a group of 13 individuals on a plastic surgery ward whose lesions were infected by a group A, type 12, strain of beta hemolytic streptococcus, and the local application of sulfanilamide was without effect. *In vitro* tests showed the organisms to be resistant to sulfanilamide, although other strains of this type had been shown to be quite sensitive. It is of interest that the local application of gramicidin in one case eradicated the infection. Strains of *Brucella abortus*, sensitive to the *in vitro* action of sulfanilamide, have been made sulfanilamide-resistant by repeated exposure of the organism to the drug. Green (30) reported that 2 individuals in a laboratory became infected with these sulfanilamide-resistant strains. The patients finally recovered, although a strain isolated from the blood of one of the victims was still resistant to sulfanilamide. It was observed by Cohn and his associates (31), in gonorrheal patients who did not respond favorably to therapy with sulfathiazole, that strains of gonococci from these individuals frequently showed *in vitro* evidence of resistance to sulfathiazole. Similar observations have been recorded by Petro (32). Strains of staphylococci have been shown to develop sulfonamide-resistance in patients undergoing therapy with one of the sulfonamides (13).

As a species, the staphylococcus is generally more resistant to the sulfonamides than are several other species of pyogenic bacteria. However, several experimental and clinical studies, carried out at the University of Minnesota Hospitals, have revealed that the sulfonamides are effective antistaphylococcic agents (13, 33 to 37). As a result of these investigations, sulfathiazole has been accepted at the University Hospitals as the most effective of the available sulfonamides in the therapy of staphylococcic sepsis, but this still leaves much to be desired. While the failure of patients with staphylococcic infections to respond to therapy with sulfathiazole is dependent upon several factors, the apparent increasing incidence of patients having infections due to staphylococci which are highly resistant *in vitro* to sulfathiazole merits further inquiry.

MATERIALS AND METHODS OF STUDY

Investigations have been carried out with a total of 57 strains of staphylococci, obtained from an equal number of patients who had various types of staphylococcic infections. Isolation of the parent strains was made in brain broth or on veal agar-blood plates. The strains were then grown on slants of veal agar, having a pH of 7.8, and kept in the refrigerator until ready for transfer to a synthetic medium. Only those strains were selected for study which gave a positive coagulase test. This test provides the most simple procedure for identifying pathogenic strains of staphylococci (39). Sodium sulfathiazole was selected for testing the resistance of the microorganisms. Comparative observations with sulfanilamide, sodium sulfapyridine, sodium sulfadiazine, and sodium sulfathiazole revealed that staphylococci were inhibited in their growth to a greater degree by sodium sulfathiazole than by the other sulfonamides. Furthermore, strains that were resistant to sodium sulfathiazole were more resistant to the

aforementioned compounds. A water-clear medium of known chemical constituents, buffered to give a pH of 7.4, was employed in testing the staphylococci for their resistance to sodium sulfathiazole (40). The preparation of this medium will be described below. As far as is known, this medium has a neglible amount of sulfonamide inhibitor. A standard *in vitro* test for sulfathiazole-resistance was used throughout. Strains to be tested were grown for several generations in the synthetic medium. As will be pointed out, variable results will be obtained if the initial inoculum of bacteria is not standardized. In performing the test, 10-fold dilutions of a 24-hour bacterial suspension were made in the synthetic medium. Then 0.1 cc. of the 10^{-3} dilution was added to each of several test tubes containing the synthetic medium. The approximate number of cocci seeded to each tube was determined by making duplicate agar pour plates with 0.1 cc. of the 10^{-7} dilution. A freshly prepared, aqueous solution of sodium sulfathiazole was used. Each strain was tested against varying concentrations of the compound. This was done by starting with an initial concentration of 1 mgm. per 100 cc. and then increasing the concentration of the drug in each of a series of tubes until a maximum concentration of 360 mgm. was reached. The final total volume of each tube was 10 cc. The bacterial suspensions, with and without added sodium sulfathiazole, were incubated for 24 hours at 37° C. At the end of this period, the degree of bacterial growth was determined according to the turbidity of the contents in each tube. Sulfathiazole-resistance was quantitated by selecting the tube which showed complete inhibition of bacterial growth with the lowest concentration of sodium sulfathiazole.

In a few instances, cultures of staphylococci were isolated from patients before they had been given a sulfonamide. In the remaining cases, this was not possible because chemotherapy had been instituted before the patients were seen.

INGREDIENTS AND PREPARATION OF SYNTHETIC MEDIUM

For the preparation of 50 liters of synthetic medium, the following weighed ingredients are mixed together in a large mortar. The mixture may then be stored in a clean container in the refrigerator until ready for further use.

KH_2PO_4	225.0 grams
$MgSO_4 \cdot 7H_2O$	2.05 grams
$FeSO_4(NH_2)_2SO_4 \cdot 6H_2O$	1.25 grams
$NaNO_3$	8.5 grams
Glucose	112.5 grams
Cystine	1.2 grams
d1 methionine	1.5 grams
1 tryptophane	0.51 grams
d1 valine	7.5 grams
d1 leucine	7.5 grams
1 aspartic acid	5.0 grams
d1 alanine	5.0 grams
d glutamic acid	5.0 grams
d1 iso-leucine	5.0 grams
d1 B phenylalanine	5.0 grams
d1 lysine·2 HCl	5.0 grams
glycine	2.5 grams
1 proline	2.5 grams
1 hydroxy-proline	2.5 grams
1 tyrosine	2.5 grams
d arginine·HCl	2.5 grams
1 histidine·HCl	2.5 grams

To prepare a liter of medium, 8.2 grams of the above mixture are placed in a volumetric flask of 1 L. capacity. Twenty-six cc. of 1N NaOH are then added and 0.0337 mgm. of thiamin chloride. This quantity of thiamin chloride may be conveniently added by making up a standard solution in distilled water in which there are 10 mgm. of thiamin chloride per cc. A dilution of 1 to 296.7 is made with this standard solution, and then 1 cc. of this dilution added to the volumetric flask. Ten cc. of a M/1,000 solution of nicotinic acid are added, and then enough distilled water to bring the total volume up to 1 L. After thorough mixing in the flask, the pH of the solution is adjusted to 7.4. The solution is sterilized by passing it through a fine Berkefeld candle (size W) and collecting it in a sterile flask having a capacity of 2 L. The medium is then tested for sterility.

RESULTS

Standard in vitro *test for detecting sulfonamide-resistance*

After many *in vitro* tests for sulfonamide-resistance had been carried out, it became quite obvious that variable results would be obtained if the number of cocci inoculated into the test medium was not controlled. Furthermore, in no instance was a completely resistant strain of staphylococcus encountered. The resistance of staphylococci to the antibacterial action of the sulfonamides is relative, and the degree of resistance is directly related to the size of the inoculum. No matter how resistant a strain was, sodium sulfathiazole inhibited growth when higher concentrations of the compound were employed. The effect of the size of the inoculum upon growth inhibition by sodium sulfathiazole is shown in Table I. The strains of staphylococcus cited in Table I were considered to be sensitive to the action of sodium sulfathiazole. The results with 2 sulfonamide-resistant strains are presented in Table II. On the basis of many similar observations, the standard inoculum selected for use in all the comparative studies was 0.1 cc. of a 10^{-3} dilution of a 24-hour culture. The number of organisms in such an inoculum varied between 40,000 and 180,000 colonies.

TABLE I

Influence of size of inoculum of staphylococci upon inhibition of growth by sodium sulfathiazole
Incubation for 24 hours at 37° C.

Strain number	Size of inoculum	Concentration of sodium sulfathiazole							
		1	5	10	20	40	60	80	100
		mgm. per 100 cc.							
39	0.1 cc. 10^{-4} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-3} dilution	+	+	0	0	0	0	0	0
	0.1 cc. 10^{-2} dilution	++	+	+	+	+	+	+	+
	0.1 cc. 10^{-1} dilution	++++	++++	+++	+++	+++	++	++	++
33	0.1 cc. 10^{-4} dilution	+	+	0	0	0	0	0	0
	0.1 cc. 10^{-3} dilution	++++	++	0	0	0	0	0	0
	0.1 cc. 10^{-2} dilution	++++	++++	+++	++	+	0	0	0
	0.1 cc. 10^{-1} dilution	++++	+++	+++	+++	+++	+++	+++	++
1	0.1 cc. 10^{-4} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-3} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-2} dilution	+	0	0	0	0	0	0	0
	0.1 cc. 10^{-1} dilution	+++	++	++	++	++	+	0	0
2	0.1 cc. 10^{-4} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-3} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-2} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-1} dilution	++++	+++	++	++	++	++	+	+
35	0.1 cc. 10^{-4} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-3} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-2} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-1} dilution	++++	++	++	++	++	++	+	+

0 = No growth.
++++ = Maximum growth.

TABLE II

Influence of size of inoculum of sulfonamide-resistant staphylococci upon inhibition of growth by sodium sulfathiazole
Incubation for 24 hours at 37° C.

Strain number	Size of inoculum	Concentration of sodium sulfathiazole						
		100	140	180	220	260	300	340
		mgm. per 100 cc.						
42	0.1 cc. 10^{-4} dilution	++++	++++	0	0	0	0	0
	0.1 cc. 10^{-3} dilution	++++	++++	++	0	0	0	0
	0.1 cc. 10^{-2} dilution	++++	+++	+++	++	0	0	0
	0.1 cc. 10^{-1} dilution	++++	++++	+++	+++	+++	+++	++
41	0.1 cc. 10^{-4} dilution	++++	++++	++	++	0	0	0
	0.1 cc. 10^{-3} dilution	++++	++++	++++	++	+	0	0
	0.1 cc. 10^{-2} dilution	++++	++++	++++	++++	+++	+++	++

0 = No growth.
++++ = Maximum growth.

Correlation between strains of staphylococci sensitive to sodium sulfathiazole in vitro *and the results of sulfonamide therapy*

Data for 32 strains of staphylococci, whose *in vitro* growth was inhibited by less than 1 mgm. per 100 cc. of sodium sulfathiazole, are given in Table III. These strains were considered to be the most sensitive to the antibacterial action of sodium sulfathiazole. It will be noted that 9 of 32 patients from whom the strains were isolated received one or more of the sulfonamides prior to the time when the culture of staphyloccus

TABLE III

Summary of non-resistant strains of staphylococcus

Growth inhibited by less than 1 mgm. per 100 cc. of sodium sulfathiazole

Patient and strain number	Age and sex	Type of lesion	Sulfonamide therapy prior to isolation of strain	Comments
1	78 M.	Bacteremia, Prostatitis, Thrombophlebitis	13 grams sod. sulfathiazole i.v. in 3 days.	Died. Some improvement following penicillin.
2	43 F.	Osteomyelitis, left femur	42 grams sulfathiazole, 11 days.	No improvement
3	3 M.	Impetigo, Acute hemorrhagic nephritis	None	Died
4	51 M.	Diabetes mellitus, Bacteremia, Osteo. rt. foot	34 grams sulfadiazine in 9 days. 33 grams sulfathiazole in 8 days. Sulfathiazole locally to osteo.	Recovery. Bacteremia persisted with sulfonamide therapy. Blood sterile after penicillin. Amputation rt. leg.
5	37 M.	Tbc. rt. wrist	Sulfathiazole locally for 2 months and 4 grams daily orally for 1 month. No drug for 2 months prior to obtaining culture.	No improvement from chemotherapy. Surgery, rt. wrist.
6	43 M.	Osteo. rt. femur	Large amounts sulfathiazole and sulfadiazine for 3 months.	No improvement
7	25 F.	Subacute bacterial endocarditis	None	Died. Temporary improvement from sulfapyridine.
8	23 F.	Cellulitis	None	Died. Strain produced lethal toxin.
9	16 M.	Bacteremia, Osteo. left femur	None	Recovery. No chemotherapy.
10	20 M.	Bacteremia, Osteo., Pneumonia, Empyema	Sulfanilamide, amount not known.	Died
11	39 F.	Chronic osteo. with exacerbation and bacteremia	None	Recovery. Improvement from sulfanilamide and sulfapyridine.
12	14 M.	Bacteremia, Thrombophlebitis, Meningitis	Sulfapyridine and sulfathiazole, amounts not known.	Died
13	7 M.	Chronic osteo.	None	Improvement following surgery.
14	13 F.	Chronic osteo.	None	Improvement following surgery and sulfapyridine.
15	40 M.	Chronic osteo.	None	Improvement following surgery. Questionable value of sulfonamide therapy.
16	36 M.	Chronic osteo., Perinephritic abscess, Empyema	None	Recovery following surgery.
17	84 M.	Ca bladder	None	No change
18	4 M.	Chronic osteo. left femur and humerus	Large amounts sulfapyridine and sulfathiazole, none for 6 months prior to isolating culture.	Improvement
19	7 F.	Bacteremia, Osteo., Pericarditis, Empyema	None	Recovery following penicillin therapy.
20	9 F.	Osteo., Pericarditis, Empyema	Sulfadiazine for 9 days, amount not known.	Recovery following penicillin therapy.
21	13 F.	Bacteremia, Osteo., Pneumonia	None	Recovery following penicillin therapy.
22	74 M.	Tbc. adenitis	None	Improvement
23	23 M.	Reticulo-endotheliosis, Abscess of neck	None	Died
24	26 M.	Bacteremia, Pneumonia, Osteo. of mandible, Abscess of neck	None	Recovery following surgical drainage. Also received sulfanilamide and staphylococcus antitoxin.
25	19 M.	Abscess rt. thigh	None	Recovery
26	16 mo. M.	Hydrocephalus, Meningitis	None	Recovery. Received sulfanilamide
27	14 F.	Bacteremia, Chronic osteo., Pulmonary abscesses, Meningitis	None	Died
28	32 F.	Left pyelonephritis and hydronephrosis	None	Recovery following surgery.
29	68 M.	Diabetes mellitus, Bacteremia	None	Died
30	22 M.	Actinomycosis liver and peritoneum	None	Died
31	12 M.	Bacteremia, Osteo.	None	Recovery. Received sulfanilamide and sulfapyridine.
32	26 M.	Bacteremia, Osteo.	None	Died

was isolated from the patient. In 2 of 10 patients, a sulfonamide had not been taken for several months before isolating the strain of staphylococcus (Patients 6 and 19). In most instances, treatment with the sulfonamides was instituted by physicians before the patients entered the University Hospitals. This and other circumstances did not permit us to determine the precise amount of sulfonamide that had been administered.

Since the strains of staphylococcus isolated from the patients in this series were sensitive to the *in vitro* action of sodium sulfathiazole, the next step was to analyze the effect of sulfonamide therapy upon the clinical course of these patients. Sulfonamides were administered to 10 patients after the test cultures were isolated. Patient 7 had subacute bacterial endocarditis. She was given sulfapyridine over a prolonged period of time which was associated with temporary improvement, but her clinical course ended in death. Patient 11 had a chronic osteomyelitis with an acute exacerbation and staphyloccic bacteremia. Following the administration of sulfapyridine, the blood stream became sterile and the patient improved. Chemotherapy had little effect upon the local lesion. Surgical drainage of an osteomyelitic lesion was combined with sulfapyridine in Patient 14, which was followed by improvement. This also applies to Patient 15. In both cases, it was difficult to assay the benefit of treatment with sulfapyridine. Treatment with sulfanilamide was without effect in Patient 24. Patient 26 was a small infant with staphylococcic meningitis and coincident with the use of sulfanilamide, the child recovered. Patient 31 received sulfapyridine for the treatment of acute osteomyelitis and bacteremia. Chemotherapy appeared to be definitely effective in this patient. Penicillin was given to 3 patients (Patients 1, 4, and 20), after either sulfathiazole or sulfadiazine had failed to control the infections.[2] Of the 9 patients (Patients 1, 2, 4, 5, 6, 10, 12, 18, and 20) who received one or more of the sulfonamides prior to isolation of the test culture, only one (Patient 18) appeared to benefit from such therapy.

In summary then, although a group of patients had infections due to a strain of staphylococcus which was sensitive *in vitro* to sulfathiazole, and to a less extent to some of the other sulfonamides, no consistent and outstanding therapeutic results were obtained in 13 of 32 patients who were given one of the sulfonamides.

Attention is called to the fact that several of the strains listed in Table III were isolated from patients before it had been established at the University Hospitals that sulfonamide therapy might be of definite value in selected cases of staphylococcic sepsis. Cultures of many of these strains had been maintained on veal-agar slants under oil for several months before their *in vitro* susceptibility to sodium sulfathiazole was tested. It might be postulated that some of the parent cultures of these strains may have been sulfonamide-resistant, but during the course of many subcultures, this resistance became lost. Evidence will be presented to show that the acquisition of sulfonamide-resistance by staphylococci is more or less a permanent characteristic, and that this resistance does not disappear or diminish following many subcultures.

Correlation between strains of staphylococci moderately resistant to sodium sulfathiazole in vitro *and the results of sulfonamide therapy*

A second series of 8 strains of staphylococcus were tested *in vitro* and all were found to be more resistant to sodium sulfathiazole. The results with this group are presented in Table IV. These strains required more than 1 mgm. per 100 cc. of sodium sulfathiazole and a concentration of less than 100 mgm. before growth was inhibited. In all but 3 cases (Patients 33, 37, and 40), a sulfonamide had been administered prior to isolation of the test strain. There is the possibility that Patients 37 and 40 received a sulfonamide before they were seen at the University Hospitals, but definite evidence is lacking.

In 2 of the patients (Patients 33 and 36), a culture of staphylococcus was isolated before chemotherapy, and then after a sulfonamide had been given. Patient 33 had a severe staphylococcic bacteremia associated with a thrombo-

[2] The penicillin used for experimental and clinical purposes was obtained through the Committee of Chemotherapeutic and Other Agents of the National Research Council.

TABLE IV

Summary of resistant strains of staphylococcus
Growth inhibited by less than 100 mgm. per 100 cc. sodium sulfathiazole

Patient and strain number	Age and sex	Type of lesion	Sulfonamide therapy prior to isolation of strain	Mgm. per 100 cc. of sodium sulfathiazole with complete growth inhibition	Comments
33	59 F.	Bacteremia, Thrombophlebitis	None	10	Died. Received 52 grams sulfathiazole in 8 days.
34	12 F.	Bacteremia, Cellulitis, Osteo.	174.5 grams sulfathiazole over 2-year period. Also sulfanilamide.	20	Marked improvement.
35	32 F.	Pneumonia, Empyema, Osteo. of ribs	Sulfonamide in large amounts. Quantity not known.	20	Marked improvement. Osteo. persistent after chemotherapy.
36	46 M.	Bacteremia, Retrobulbar abscess, Osteo.	195.75 grams sulfathiazole orally and parenterally. 78 grams sulfadiazine orally. Sulfathiazole locally.	5	Complete recovery.
37	64 F.	Urethritis and urethral carbuncle	None	5	Improvement after operation.
38	57 M.	Ca prostate with cystitis	21 grams sulfathiazole.	80	Died following operation.
39	32 F.	Bacteremia, Carbuncle, Osteo. of spine, tibia, fibula, and rt. femur	Large amounts of sulfathiazole for several weeks. Quantity not known.	10	Marked improvement following sulfonamide therapy. No evidence of active infection after penicillin.
40	78 M.	Ca prostate and bladder	None	20	Receiving stilbesterol.

phlebitis. Prior to treatment with sulfathiazole, the growth of a strain of staphylococcus isolated from her blood was completely inhibited by less than 1 mgm. of sodium sulfathiazole. There were 100 colonies of staphylococci per cc. in her blood when therapy with sulfathiazole was instituted. After receiving 52 grams of sulfathiazole in 8 days, she appeared moribund. The colony count of a blood culture was 473, and a strain of staphylococcus isolated from her blood required 10 mgm. of sodium sulfathiazole before growth was inhibited. The concentration of free sulfathiazole in her blood at this time was 15 mgm. per 100 cc. While care must be taken in transposing *in vitro* observations of this nature to clinical phenomena, it is not unlikely that the increase in *in vitro* resistance may have been associated in part with a fatal outcome. Similar observations were made with cultures obtained from Patient 36. This individual recovered after a very serious infection, and there is no doubt that the intensive use of sulfathiazole played a rôle in his favorable outcome. At one time, during the course of treatment, a blood culture revealed a colony count of 264 organisms per cc. In spite of the use of large amounts of sulfathiazole, the causative organism apparently developed only a minimal degree of resistance.

The results of therapy with sulfathiazole in the group of cases presented in Table IV were more satisfactory than had been obtained with sulfapyridine. This was to be anticipated, in part, following comparative *in vitro* tests with the 2 compounds when it was shown that sulfathiazole was superior to sulfapyridine in inhibiting growth of the staphylococcus.

Correlation between strains of staphylococci highly resistant to sodium sulfathiazole in vitro *and the results of sulfonamide therapy*

Table V presents a summary of 17 strains of staphylococci which are considered highly resistant to the sulfonamides. A concentration of at least 100 mgm. per 100 cc. of sodium sulfathiazole was necessary before growth was completely inhibited. All of the patients except one (Patient 54) received sulfonamide therapy prior to isolation of the test strain. In this one patient, there remains a possibility that sulfonamide treatment had been carried out before he was admitted to the hospital. An important and interesting aspect of this group of sulfonamide-resistant strains is that 14 of the 17 strains were isolated from the urine of patients having urinary tract infections. In 12 of the 14 patients with infection of the urinary tract, there

TABLE V

Summary of resistant strains of staphylococcus

Growth inhibited by 100 mgm. per 100 cc. or more of sodium sulfathiazole

Patient and strain number	Age and sex	Type of lesion	Sulfonamide therapy prior to isolation of strain	Mgm. per 100 cc. of sodium sulfathiazole with complete growth inhibition	Comments
41	13 F.	Ulcerative colitis, Chronic pyoderma	126 grams sulfathiazole orally, sulfathiazole locally.	250	Died. Only temporary improvement of skin lesions.
42	15 F.	Bacteremia, Osteo.	326 grams sulfathiazole orally over 2 years, 46 grams sulfapyridine, sulfathiazole locally.	200	Marked improvement. Residual active osteo.
43	72 M.	Bacteremia, Cellulitis, Prostatic obstruction	Large amounts sulfonamide, quantity unknown.	100	Died. No improvement.
44	74 M.	Encysted cystitis, Prostatic obstruction with cystitis	15 grams sulfadiazine. Continuous bladder irrigation with 0.8 per cent sulfanilamide solution, 5 days.	100	Improvement following operation. Resistant staph. from urine 5 times.
45	72 M.	Prostatic obstruction with cystitis	Sulfonamides at intervals for several months. 6.5 grams sulfathiazole.	200	Improvement following operation. Resistant staph. persisted in urine after operation.
46	28 F.	Rt. ureter obstructed, Pyelonephritis	Sulfonamides intermittently, amount not known.	180	Improvement following operation.
47	66 M.	Prostatic obstruction with cystitis. Suprapubic cystostomy	Sulfathiazole orally, amount not known. Sulfathiazole locally.	200	Improvement following operation.
48	65 M.	Rt. renal calculi and abscesses	38 grams sulfathiazole. 29 grams sulfadiazine.	180	Improvement following nephrectomy.
49	74 M.	Prostatic obstruction with acute pyelonephritis	51 grams sod. sulfathiazole i.v.	200	Died. Bacteremia due to gamma strept.
50	45 M.	Bilateral renal calculi with left hydronephrosis	Unknown amounts sulfonamide.	200	Improvement following surgery.
51	38 F.	Chronic pyelonephritis left with left tubo-ovarian abscess	29 grams sulfadiazine.	200	Improvement following nephrectomy.
52	4 F.	Purulent bronchiolitis with liver abscesses	21 grams sulfadiazine. 9 grams sulfamerazine.	160	Died
53	80 M.	Prostatic obstruction with cystitis	Continuous bladder irrigation with 0.8 per cent sulfanilamide solution. 5.5 grams sulfathiazole.	200	Died. Acute cardiac failure.
54	75 F.	Ca bladder, Bilateral pyelonephritis	None	160	Died. Cardiac failure after operation.
55	86 M.	Prostatic obstruction with cystitis	39.5 grams sulfathiazole.	180	Improvement following operation.
56	60 M.	Prostatic obstruction with cystitis	12 grams sulfadiazine.	200	Improvement following operation.
57	50 M.	Bilateral polycystic kidneys with uremia	17 grams sulfathiazole.	200	Died. Resistant staph. in urine 3 times.

was definite evidence of some degree of obstruction to the free flow of urine. It is well recognized that the efficiency of the sulfonamides is reduced in the treatment of urinary tract disease when a free flow of urine is interrupted. This means that in the latter group of patients, staphylococci were being constantly exposed to relatively high concentrations of one or more of the sulfonamides. It is not unlikely that under these circumstances the organisms become increasingly resistant to the compounds. Another significant feature is that this group of resistant strains, isolated from urine, were obtained over a short period of time. In 2 instances (Patients 44 and 45), sulfonamide-resistant staphylococci persisted in the urine after the obstruction had been corrected by surgical interference. Unfortunately, we were unable to obtain strains of staphylococci from any of the patients given in Table V before the administration of a sulfonamide. Several of these highly resistant strains have been investigated concerning the mechanism whereby they become resistant to the sulfonamides. This will be discussed. A definite

relationship was found to exist between the therapeutic response to a sulfonamide and the presence of sulfonamide-resistant staphylococci. In the cases of urinary tract obstruction, definite clinical improvement occurred only after the obstruction had been eliminated. The most resistant strain of staphylococcus that we have encountered was isolated from Patient 41, who had an extensive infection of the skin. She had received large amounts of sulfathiazole orally, and sulfathiazole ointment had been applied locally. There was only temporary improvement in her skin lesions and she died because of a chronic ulcerative colitis.

Summary of relationship between in vitro *susceptibility of staphylococci to sodium sulfathiazole and sulfonamide therapy prior to isolation of the strains*

A summary of this relationship is given in Table VI. Group I comprises all the strains sensitive to the action of sodium sulfathiazole. Of the 32 patients from whom these strains were obtained, only 9 had received a sulfonamide

TABLE VI

Summary of relationship between the in vitro *susceptibility of staphylococci for sodium sulfathiazole and sulfonamide therapy prior to isolation of the strains*

	Number of strains	Number of patients receiving sulfonamide prior to isolation of staphylococci	Comment
Group I strains—sensitive to sodium sulfathiazole.	32	9	
Group II strains—moderately resistant to sodium sulfathiazole.	8	5	Possibly 2 additional patients received sulfonamide.
Group III strains—highly resistant to sodium sulfathiazole.	17	16	Possibly seventeenth patient received sulfonamide.

prior to isolation of the strains. There were 8 strains in Group II, and these strains were moderately resistant. Five, and possibly 7, of the 8 patients had been given a sulfonamide before the staphylococci were recovered from their lesions. There were 17 strains in Group III, all of which were highly resistant to sodium sulfathiazole. Sixteen of the 17 patients from whom these strains were isolated had had sulfonamide therapy prior to the isolation of the organisms. There is some evidence that the seventeenth patient had been given a sulfonamide. Although it cannot be concluded that the resistance of staphylococci to the inhibitory action of sodium sulfathiazole is due to previous sulfonamide therapy, it is obvious that the majority of the resistant strains were isolated from patients who had been given a sulfonamide.

Acquired sulfonamide-resistance a persistent characteristic

All of the resistant strains of staphylococci included in this report have been subcultured numerous times on veal agar slants, and in synthetic medium, and in no instance did a strain lose its ability to resist the action of the sulfonamides. This relatively permanent feature of sulfonamide-resistance is emphasized by results obtained with strains 41 and 42. As noted in Table V, these 2 strains of staphylococci were quite resistant to sodium sulfathiazole. Each of these strains was subcultured on veal agar and in synthetic medium for 75 generations. Comparative *in vitro* tests revealed that the seventy-fifth generations possessed the same degree of resistance as the parent strains. Both strains remained coagulase-positive. Strains 41 and 42 were each grown on veal agar slants, and then the cultures were covered with oil and stored in a refrigerator for 114 days. At the end of this time, the 2 strains were grown for several generations in synthetic medium and their *in vitro* resistance to sodium sulfathiazole tested. There was no diminution in the resistance of the organisms to the inhibitory effect of the sulfonamide.

DISCUSSION

The foregoing data represent the results of observations that have been made during the past 2 years. It became apparent early in the course of these studies that the mechanism whereby staphylococci developed resistance to the sulfonamides required elucidation. This effort was stimulated by the investigations of MacLeod (41) who showed that a Type I strain of sulfapyridine-resistant pneumococcus produced a substance which inhibited the action of sulfapyridine. Mirick (42) investigated this sulfonamide inhibitor and brought forth considerable evidence that this inhibitor was actually

p-aminobenzoic acid. This information suggested to us that staphylococci became resistant to the sulfonamides by means of a similar mechanism. The most resistant strains included in the present report were subjected to a group of observations with this thesis in mind. As a result, we have concluded that as far as the staphylococcus is concerned, sulfonamide-resistance is dependent at least in part, upon the elaboration of p-aminobenzoic acid by the bacterial cell. These observations will be published in detail elsewhere. This supports the conclusions of Landy and his group (43). It should be emphasized that even the so-called non-resistant strains of staphylococcus produce p-aminobenzoic acid, but to a lesser degree than the resistant strains. There is some evidence at hand which would indicate that the more resistant strains of staphylococci produce relatively large amounts of p-aminobenzoic acid, especially in the presence of the sulfonamides (44). While the mechanism whereby staphylococci resist the inhibitory action of the sulfonamides may be explained in part on the basis of the formation of p-aminobenzoic acid acting as a sulfonamide inhibitor, this mechanism does not necessarily apply to other species of bacteria. Recent evidence would indicate that another mechanism or mechanisms is responsible (9, 43).

The use of the sulfonamides in the treatment of staphylococcic infections presents many problems. Even though *in vitro* tests may show that a particular strain of staphylococcus is susceptible to the action of a sulfonamide, attempts at therapy with this sulfonamide may be unsuccessful or not too satisfactory. This is related in large part to the nature of staphylococcic sepsis. Localized lesions, serving as foci for blood stream invasion, are made up of exudate, tissue necrosis, cellular debris, and dead organisms, all of which may inhibit the action of the sulfonamide. If, in addition to these factors, the organism becomes resistant to the sulfonamide, the likelihood of controlling an infection is further reduced. Another disturbing feature in our experience with staphylococcic infections is that sulfonamide-resistant strains of staphylococci are being encountered much more frequently at the present time than 2 or 3 years ago. This may be due to several factors. One is that the sulfonamides are being administered more freely to patients with staphylococcic sepsis before they are brought to the University Hospitals for further treatment. Another possibility is that sulfonamide-resistant strains are being disseminated because of the widespread use of the sulfonamides.

Particular attention should be given to the frequency with which sulfonamide-resistant strains of staphylococci were isolated from the urine of patients with varying types of urinary tract infections. In many cases, a low-grade infection was associated with obstruction to the flow of urine. The development of sulfonamide-resistant organisms is not to be taken too lightly, particularly if operative interference is contemplated. In one patient (Patient 43), a highly resistant strain of staphylococcus was obtained from the urine. This individual had benign prostatic hypertrophy with obstruction. Sulfonamides were administered prior to surgery, and following a transurethral prostatic resection, he developed a fatal staphylococcic bacteremia. The strain isolated from his blood was also resistant to the *in vitro* action of sulfathiazole, and therapy with this compound was of no value in controlling the infection. It is possible that the same sequence of events may take place in patients with other species of bacteria in the urinary tract as brought out in the following observation. Patient 49 developed a fatal bacteremia due to an anhemolytic strain of streptococcus, following a transurethral prostatic resection. This organism was cultured from the urine and the blood, and *in vitro* tests showed it to be highly resistant to sulfathiazole. It is of interest that, in 1926, Feirer and his associates (45) called attention to the development of "drug-fast" organisms in the urine against urinary antiseptics, which were derivatives of the heavy metals. On the basis of this observation, they suggested a rotation of drugs in the treatment of chronic urinary infections.

It is becoming more and more apparent that specific agents, other than the commonly used sulfonamides, are desirable in the treatment of patients with severe staphylococcic infections. There is increasing evidence that the antibiotic agents, such as penicillin, will yield more satisfactory clinical results. We have compared the

in vitro action of sulfathiazole and penicillin against the 57 strains given in this report, the results of which work will be presented elsewhere. It is significant that p-aminobenzoic acid does not inhibit the action of penicillin against the staphylococcus. On the other hand, staphylococci may develop *in vitro* resistance to penicillin, apparently by means of a different mechanism. This feature is undergoing investigation at the present time.

SUMMARY

1. The problem of sulfonamide-resistant bacteria in general is briefly reviewed.

2. Fifty-seven strains of coagulase-positive staphylococci, isolated from an equal number of patients, were tested *in vitro* with a standard procedure against sodium sulfathiazole in a synthetic medium, containing negligible amounts of sulfonamide inhibitor. Thirty-two of the strains were considered non-resistant; 8 were moderately resistant; while 17 strains required a concentration of 100 mgm. per 100 cc. or more of sodium sulfathiazole before growth was completely inhibited.

3. The acquisition of sulfonamide-resistance by staphylococci is a persistent characteristic of the organisms.

4. Although it is apparent that sulfonamide-resistant staphylococci do not necessarily develop as a result of the administration of the sulfonamides, the evidence presented in this paper indicates that resistant strains are almost always isolated from patients who have had previous sulfonamide therapy.

5. The development of sulfonamide-resistance by staphylococci is dependent, at least in part, upon the elaboration of p-aminobenzoic acid by the bacterial cells.

BIBLIOGRAPHY

1. MacLean, I. H., Rogers, K. B., and Fleming, A., M. and B. 693 and pneumococci. Lancet, 1939, **1**, 562.
2. MacLeod, C. M., and Daddi, G., A "sulfapyridine-fast" strain of pneumococcus Type I. Proc. Soc. Exper. Biol. and Med., 1939, **41**, 69.
3. Schmidt, L. H., and Dettwiler, H. A., Development of "sulfapyridine-fastness" in vivo. J. Biol. Chem., 1940, **133**, lxxxv.
4. Dettwiler, H. A., and Schmidt, L. H., Observations on the development of resistance to sulfapyridine by diplococcus pneumoniae. J. Bact., 1940, **40**, 160.
5. Schmidt, L. H., Sesler, C. L., and Dettwiler, H. A., Studies on sulfonamide-resistant organisms. I. Development of sulfapyridine resistance by pneumococci. J. Pharmacol. and Exper. Therap., 1942, **74**, 175.
6. Sesler, C. L., and Schmidt, L. H., Studies on sulfonamide-resistant organisms. II. Comparative development of resistance to different sulfonamides by pneumococci. J. Pharmacol. and Exper. Therap., 1942, **75**, 356.
7. MacLeod, C. M., The antibacterial action of the sulfonamide drugs. Advances in Internal Medicine, 1942, **1**, 83.
8. Frisch, A. W., Price, A. E., and Myers, G. B., Type VIII pneumococcus: Development of sulfadiazine resistance, transmission by cross infection, and persistence in carriers. Ann. Int. Med., 1943, **18**, 271.
9. Tillett, W. S., Cambier, M. J., and Harris, W. H., Jr., Sulfonamide-fast pneumococci. A clinical report of two cases of pneumonia together with experimental studies on the effectiveness of penicillin and tyrothricin against sulfonamide-resistant strains. J. Clin. Invest., 1943, **22**, 249.
10. Hendry, J. L., A study of hemolytic streptococci from a horse treated with sulfanilamide after streptococcal bacteremia developed during immunization. J. Infect. Dis., 1942, **70**, 112.
11. Cutts, M., and Troppoli, A. V., Acquired drug resistance in the hemolytic streptococcus. J. Lab. and Clin. Med., 1942, **28**, 14.
12. Strauss, E., Dingel, J. H., and Finland, M., Studies on the mechanism of sulfonamide bacteriostasis, inhibition and resistance: Experiments with staphylococcus aureus. J. Immunol., 1941, **42**, 331.
13. Vivino, J. J., and Spink, W. W., Sulfonamide-resistant strains of staphylococci: Clinical significance. Proc. Soc. Exper. Biol. and Med., 1942, **50**, 336.
14. Strauss, E., Dingle, J. H., and Finland, M., Studies on the mechanism of sulfonamide bacteriostasis, inhibition and resistance: Experiments with *E. coli* in a synthetic medium. J. Immunol., 1941, **42**, 313.
15. Kirby, W. M. M., and Rantz, L. A., Quantitative studies of sulfonamide resistance. J. Exper. Med., 1943, **77**, 29.
16. Green, H. N., The mode of action of sulphanilamide: With special reference to a bacterial growth-stimulating factor ("P" factor) obtained from Br. abortus and other bacteria. Brit. J. Exper. Path., 1940, **21**, 38.
17. Marshall, E. K., Jr., Bacterial chemotherapy. Ann. Rev. Physiol., 1941, **3**, 643.
18. Cooper, M. L., and Keller, H. M., Sodium sulfathiazole resistant Shigella paradysenteriae Flexner and Sonne. Proc. Soc. Exper. Biol. and Med., 1942, **51**, 238.
19. Boak, R. A., and Carpenter, C. M., Resistance of the gonococcus in vitro to gradually increased concentrations of sulfanilamide. J. Bact., 1939, **37**, 226.

20. Westphal, L., Charles, R. L., and Carpenter, C. M., The development of sulfapyridine-fast strains of the gonococcus. Ven. Dis. Inform., 1940, **21**, 183.
21. Carpenter, C. M., Charles, R., and Allison, S. D., Effect of gradually increased concentrations of sulfathiazole on the gonococcus in vitro. Proc. Soc. Exper. Biol. and Med., 1941, **48**, 476.
22. Kirby, W. M. M., Development of sulfathiazole-resistant gonococci in vitro. Proc. Soc. Exper. Biol. and Med., 1943, **52**, 175.
23. Ross, R. W., Acquired tolerance of pneumococcus to M. and B. 693. Lancet, 1939, **1**, 1207.
24. Lowell, F. C., Strauss, E., and Finland, M., Observations on the susceptibility of pneumococci to sulfapyridine, sulfathiazole and sulfamethylthiazole. Ann. Int. Med., 1940, **14**, 1001.
25. Moore, F. J., Thomas, R. E., and Hoyt, A., Sensitivity of pneumococci to sulfapyridine. J. A. M. A., 1941, **117**, 437.
26. Frisch, A. W., Sputum studies in pneumonia: In vivo and in vitro susceptibility of pneumococci to sulfapyridine and sulfathiazole. Am. J. Clin. Path., 1941, **11**, 797.
27. Auger, W. J., Sulfapyridine resistance of pneumococci following sulfapyridine therapy in infants and children and comparative potency of three chemotherapeutic agents for pneumococci as shown by laboratory tests. J. Pediat., 1941, **18**, 162.
28. Hamburger, M., *et al.*, Sulfonamide resistance developing during treatment of pneumococcic endocarditis. J. A. M. A., 1942, **119**, 409.
29. Francis, A. E., Sulphonamide resistant streptococci in a plastic surgery ward. Lancet, 1942, **1**, 408.
30. Green, H. N., Laboratory infection with Brucella abortus. Brit. M. J., 1941, **1**, 478.
31. Cohn, A., Steer, A., and Seijo, I., Correlation between clinical and in vitro reactions of gonococcus strains to sulfathiazole: A preliminary report. Am. J. M. Sc. 1942, **203**, 276.
32. Petro, J., Sulphonamide resistance in gonorrhoea. Lancet, 1943, **1**, 35.
33. Spink, W. W., The bactericidal effect of sulfanilamide upon pathogenic and non-pathogenic staphylococci. J. Immunol., 1939, **37**, 345.
34. Spink, W. W., and Jermsta, J., Effect of sulfonamide compounds upon growth of staphylococci in presence and absence of p-aminobenzoic acid. Proc. Soc. Exper. Biol. and Med., 1941, **47**, 395.
35. Spink, W. W., The pathogenesis and treatment of staphylococcal infections. Internat. Clin., 1941, **4**, 236.
36. Spink, W. W., Hansen, A. E., and Paine, J. R., Staphylococcic bacteremia: Treatment with sulfapyridine and sulfathiazole. Arch. Int. Med., 1941, **67**, 25.
37. Spink, W. W., and Haserick, J. R., Treatment of staphylococcal infections. Staff Meeting Bulletin, Hospitals of the University of Minnesota, 1942, **13**, 196.
38. Spink, W. W., Sulfanilamide and Related Compounds in General Practice. Year Book Publishing Co., Chicago, 1942, 2nd ed., p. 104.
39. Spink, W. W., and Vivino, J. J., The coagulase test for staphylococci and its correlation with the resistance of the organisms to the bactericidal action of human blood. J. Clin. Invest., 1942, **21**, 353.
40. Gladstone, G. P., Inter-relationships between amino acids in the nutrition of B. anthracis. Brit. J. Exper. Path., 1939, **20**, 189.
41. MacLeod, C. M., The inhibition of the bacteriostatic action of sulfonamide drugs by substances of animal and bacterial origin. J. Exper. Med., 1940, **72**, 217.
42. Mirick, G. S., Enzymatic identification of p-aminobenzoic acid (PAB) in cultures of pneumococcus and its relation to sulfonamide-fastness. J. Clin. Invest., 1942, **21**, 628.
43. Landy, M., Larkum, N. W., Oswald, E. J., and Streightoff, F., Increased synthesis of p-aminobenzoic acid associated with the development of sulfonamide resistance in staphylococcus aureus. Science, 1943, **97**, 265.
44. Spink, W. W., and Vivino, J. J., Pigment production by sulfonamide-resistant staphylococci in the presence of the sulfonamides. Science, 1943, **98**, 44.
45. Feirer, W. A., Meader, P. D., and Leonard, V., Development of the drug fast character in vitro and its bearing upon drug rotation in the management of chronic urinary infections. J. Urol., 1926, **16**, 97.