therefore, that a cell membrane containing substances of this character in the Gram negative bacteria may account for the lack of sensitivity to the anionic agent. We are, therefore, attempting to prepare cell-free enzymes from *S. aureus* and *E. coli* in order to determine the rôle played by the cell membranes in the results shown above.

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Sulfonamide-Resistant Strains of Staphylococci: Clinical Significance.*

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Strains of pneumococci, gonococci, *E. coli* and *S. aureus* have been rendered resistant *in vitro* to the bacteriostatic action of sulfonamide drugs.[1-6] Rammelkamp[7] has also observed that *S. aureus* may become "fast" to tyrothricin, both *in vitro* and *in vivo*. The present report is concerned with the *in vitro* and *in vivo* production of sulfonamide resistant strains of staphylococci.

The *in vitro* observations were made with 2 strains of *S. aureus* isolated from patients with severe staphylococcal infections. A chemically-defined medium as described by Gladstone was used throughout.[8] These strains were made resistant by exposure to increasing concentrations of sodium sulfathiazole in this medium. Initial growth was established with a minute inoculum from an agar slant. Subsequent transfers of the cultures in the synthetic medium were made with a 5 mm wire loop. The two strains showed an initial difference in susceptibility to the action of sulfathiazole. Strain 14

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1 MacLean, I. H., Rogers, K. B., and Fleming, A., *Lancet*, 1939, **1**, 562.

2 MacLeod, C. M., and Doddi, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 69.

3 Lowell, F. C., Strauss, E., and Finland, M., *Ann. Int. Med.*, 1940, **14**, 1001.

4 Westphal, L., Charles, R. L., and Carpenter, C. M., *Ven. Dis. Inform.*, 1940, **21**, 183.

5 Strauss, E., Dingle, J. H., and Finland, M., *J. Immunol.*, 1941, **42**, 313.

6 *Ibid.*, 1941, **42**, 331.

7 Rammelkamp, C. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 346.

8 Gladstone, G. P., *Brit. J. Exp. Path.*, 1939, **20**, 189.

grew readily for several generations in the presence of 0.25 mg per 100 ml of sulfathiazole, but strain 7 was unable to institute growth in this concentration after the second transfer. It was necessary to start strain 7 in 0.1 mg per 100 ml. Control observations were carried out with the parent strains transferred an equal number of times in a drug-free medium.

The degree of growth was estimated regularly by measuring the density of the culture in the Evelyn photoelectric colorimeter, as previously described.[9] The development of drug resistance was determined by seeding approximately 1,000 organisms per ml to tubes of medium containing varying concentrations of sulfanilamide, sulfapyridine, sulfadiazine, and sulfathiazole. Drug resistance was determined by a comparison of growth at the end of 24 and 70 hours of the parent strain and the "fast" strain in the presence of the foregoing drugs.

After 130 transfers in increasing concentrations of sodium sulfathiazole, that is, up to 10 mg per 100 ml, strain 14 grew readily in the presence of 200 mg per 100 ml of sodium sulfapyridine, 100 mg per ml of sodium sulfadiazine, and 50 mg per 100 ml of sodium sulfathiazole. Strain 7 was transferred an equal number of times in increasing concentrations of sodium sulfathiazole up to 5 mg per 100 ml. After this, maximum growth took place in the presence of 200 mg per 100 ml of sodium sulfapyridine, 75 mg per 100 ml of sodium sulfadiazine, and 40 mg per 100 ml of sodium sulfathiazole. Studies are now in progress to determine whether the biologic activity of the "fast" strains differs from the parent strains.

To investigate the *in vivo* development of resistance to sulfonamide action, cultures were made from two patients with staphylococcal bacteremia before and after they had received sulfathiazole. Drug resistance was determined as described for the *in vitro* "fast" strains.

Two strains were secured from a patient having a maximum of 260 colonies per ml of blood, and complicated by the appearance of a retrobulbar abscess.† The patient recovered completely. Strain 116a was isolated from the blood stream at the onset of the illness. The patient was then treated with sulfathiazole, parenterally and orally. Subsequent blood cultures remained sterile, but the localized lesion was treated by daily irrigation with sulfathiazole solution. Strain 116b was cultured from this lesion 11 weeks after isolation of 116a. Strains 117a and 117b were obtained from the blood of a

[9] Spink, W. W., and Jermsta, J., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 395.

† This patient was observed through the courtesy of Dr. Jay C. Davis of Minneapolis.

TABLE I.
In Vivo Development of Sulfonamide-Resistant *S. aureus*.

Strain	Incubation hr	Concentration of drug in mg per 100 ml											
		Sulfathiazole				Sulfadiazine				Sulfapyridine			
		10	5	2	1	10	5	2	1	10	5	2	1
116a	24	0	0	0	0	0	0	0	0	0	0	0	0
	70	0	0	0	0	0	0	0	++	0	0	+	++
116b	24	0	0	+	++	+	++	++	++	+	+	++	++
	70	0	+	++	++	++	++	++	++	++	++	++	++
117a	24	0	0	0	0	0	0	0	0	0	0	0	+
	70	0	0	0	0	0	0	++	++	0	0	++	++
117b	24	0	0	+	++	+	++	++	++	+	++	++	++
	70	++	++	++	++	++	++	++	++	++	++	++	++

a = Isolated before sulfathiazole therapy.
b = Isolated after sulfathiazole therapy.
\+ = Initial growth.
++ = Maximum growth.

second patient having a maximum of 500 colonies per ml of blood. This patient expired. Strain 117b was isolated after 8 days of sulfathiazole therapy, and 3 days prior to death. While under treatment, the sulfathiazole concentration in the blood was 15 mg per 100 ml.

The results of the tests for drug resistance are recorded in Table I. Both strains became markedly resistant to the action of the sulfonamide compounds.

To our knowledge, these observations constitute the first recorded instance of staphylococci becoming resistant to the bacteriostatic action of the sulfonamides in the human body. It is not unlikely that further studies under way may reveal similar cases, and this phenomenon may explain, in part, the failure of sulfathiazole to clear the blood stream of organisms. It is significant that in the case of the patient who expired, coincident with the development of a "fast" strain of staphylococcus, there was a marked increase in the number of colonies in the blood. This "fastness" was detected after only 8 days of therapy.