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Potentiating Influence of Urine on Sulfanilamide's Bacteriostatic Effect on *E. coli in vitro.*

RALPH R. MELLON AND LAWRANCE E. SHINN.

From the Western Pennsylvania Hospital, Institute of Pathology, Pittsburgh, Pa.

In a previous publication[1] one of us (Mellon) reported a potentiative effect of sulfanilamide on hemolytic streptococci when the organisms had been exposed to physiological salt solution during the process of dilution preliminary to seeding the test cultures. If this exposure was omitted and dilution in broth substituted, the remarkable bacteriostatic effect of sulfanilamide in low concentrations was not noted. In the presence of normal human serum the effect is enhanced. In other words, a sterilizing effect is obtained from the combined action of these minimal factors which exceeds many times a simple summation effect.

Kenny, Johnston, and von Haebler[2] have recently reported a very favorable series of clinical cases of *E. coli* infection of the urinary tract which were successfully treated with sulfanilamide. They showed that with oral medication of 1.5 gm. a day for 5-7 days sterilization of the urine was obtained during the period of treatment in all of 46 cases of infection with a typical *E. coli.* The concentration of free sulfanilamide obtained in the urine of treated cases ranged from less than 1:100,000 to 1:1,000. It was shown experimentally that the static or bactericidal action of the compound *in vitro* was roughly proportional to its concentration.

It appeared to us that a potentiation similar to that displayed by saline for hemolytic streptococci might be anticipated in this instance with the culture medium itself (the urine) playing the rôle of the potentiating agent. Bacteriostatic tests were accordingly made on a strain of *E. coli* freshly isolated from a case of cystitis which had had no sulfanilamide therapy. Normal pooled urine was adjusted to pH 7.2 and sterilized by Seitz filtration. The broth employed in the following experiments was a 2% proteose-peptone beef infusion of the same pH. Cultures were grown either in urine or broth for 18 hours, and then serially diluted in either urine or broth to the concentration required for seeding the test cultures which were of 2 cc. volume. All seedings and sulfanilamide-additions were in quantities of 0.1 cc. The test cultures were then incu-

[1] Mellon, R. R., and Bambas, L. L., *Med. Record,* 1937, **246**, 247.

[2] Kenny, M., Johnston, F. D., and von Haebler, T., *Lancet,* 1937, **233**, 119.

bated without agitation for 48 hours. Counts were made by plating 0.5 cc. from each at 0, 6, 24, and 48 hours.

TABLE I.

Effect of the Original Culture Medium, Diluent, and Test Medium on the Bacteriostasis of *E. coli in vitro*. Figures averaged from 4 determinations.

Culture medium	Diluent	Test medium	Conc. sulfanil.	Counts per cc. at 0 hr.	6 hr.	24 hr.	48 hr.
Broth	Broth	Broth	0	19	*	*	*
"	"	"	1:10,000	15	*	*	*
"	"	Urine	0	10	*	*	*
"	"	"	1:10,000	14	*	*	*
"	Urine	Broth	0	22	*	*	*
"	"	"	1:10,000	16	*	*	*
"	"	Urine	0	10	*	*	*
"	"	"	1:10,000	15	150	11	4
Urine	Broth	Broth	0	26	*	*	*
"	"	"	1:10,000	19	*	*	*
"	"	Urine	0	10	*	*	*
"	"	"	1:10,000	17	*	*	*
"	Urine	Broth	0	48	*	*	*
"	"	"	1:10,000	53	*	*	*
"	"	Urine	0	42	*	*	*
"	"	"	1:10,000	61	400	1	0

*Uncountable; greater than 20,000.

The comparative effects of broth and urine as initial media, diluents and test media are shown in Table I. Of the 8 possible combinations only 2 show stasis and bactericidal action and these 2 (broth-urine-urine and urine-urine-urine) show it with great clarity.

TABLE II.

Effect of Initial Count and Concentration of Sulfanilamide on the Bacteriostasis of *E. coli* When Grown in Urine, Diluted in Urine, and Tested in Urine. Figures averaged from 4 determinations.

Initial count per cc.	Conc. sulfanil.	Counts per cc. at 6 hr.	24 hr.	48 hr.
1000	0	*	*	*
1000	1:5,000	10,000	5,000	0
1000	1:10,000	10,000	5,000	200
200	0	*	*	*
200	1:5,000	650	11	3
200	1:10,000	900	†	†
200	1:50,000	*	*	*
100	0	*	*	*
100	1:1,000	400	2	0
100	1:10,000	600	0	90
100	1:50,000	*	*	*
50	0	*	*	*
50	1:5,000	450	4	0
50	1:10,000	500	10	0

*Uncountable; greater than 20,000.
†Erratic; some sterilized while others grew out heavily.

The only conclusion which seems possible from this result is that broth exerts either a protective effect on the organism or an inhibitory effect on the drug and that it must be removed by the dilution in urine.

Table II is an attempt to evaluate the minimal concentration of sulfanilamide and the maximal initial count compatible with stasis. It is apparent that initial counts as high at least as 1000 per cc. yield good bacteriostasis with a concentration of 1:10,000. There is furthermore a suggestion that the concentration of 1:10,000 is very near the critical value for a fairly wide range of initial counts, which is particularly interesting in view of the frequent appearance of this particular value *in vivo*.

Initial experiments employing a pH of 6.0 rather than 7.2 indicate that the action of sulfanilamide is considerably enhanced in the more alkaline range. A concentration of 1:1000 at pH 6.0 appears to be effective in somewhat the same degree as a concentration of 1:10,000 at pH 7.2. This agrees with the observations of Helmholz.[3]

Practically, these results effect a correlation between *in vitro* tests and *in vivo* clinical results where, according to Kenny, disparities existed with the technic employed by her.

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Sulfanilamide and the Macrophage Response to Hemolytic Streptococcal Peritonitis in Mice.

RUTH A. MCKINNEY AND RALPH R. MELLON.

From the Western Pennsylvania Hospital, Institute of Pathology, Pittsburgh, Pa.

Because of an apparent disparity between the results of Long and Bliss[1] and Mellon, Cooper, and Gross[2] concerning the rôle of phagocytosis in experimental hemolytic-streptococcal infections in mice under treatment with sulfanilamide, the following inquiry was undertaken. The disparity later came to center about the relative importance of the neutrophils and the clasmatocytes in phagocytosis. In our study we had suggested the importance of strain-differences to explain the original disparity; although it was realized that our experimental set-up was not designed to answer the questions involved. This paper purports to do that.

[3] Helmholz, H. F., *J. A. M. A.*, 1937, **109**, 1039.

[1] Long, P. H., and Bliss, E. A., *J. A. M. A.*, 1937, **108**, 32.

[2] Mellon, R. R., Gross, P., and Cooper, F. B., *J. A. M. A.*, 1937, **108**, 1858.