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Sulfadiazine: Effect on *E. coli* Infections in Mice.

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In a recent report,[1] it was shown that sulfathiazole is more effective than sulfanilamide or sulfapyridine in the treatment of *E. coli communis* infections in mice. Recently, the chemotherapeutic effect of sulfadiazine (2-sulfanilylpyrimidine) has been tested in experimental *E. coli communis* infections in mice, and the results are the subject of this report. The effects of sulfathiazole and sulfadiazine are compared.

Methods. The same strain of *E. coli communis* was used in this experiment as in the previous investigation.[1] The same media and methods of identifying the organism were employed. The pathogenicity of the bacterium for white mice (strain C F 1) remained the same; mucin enhanced its virulence so that less than 5 organisms injected intraperitoneally produced a fatal bacteremia within 24 hours.

Mice were infected intraperitoneally with 0.5 cc of a 1:500,000 dilution in mucin of a 12-hour broth culture of the test organism. The inocula, determined in the same way as in the previous report,[1] varied from 20 to 150 organisms, *i. e.*, from 20 to 100 M.L.D.'s.

The drugs were administered as part of the diet, constituting 1.0% of the diet with sulfathiazole and 0.1% with sulfadiazine. The animals were kept in separate cages and the amount of drug consumed each day was determined.

Results. A. *In vitro.* The bacteriostatic effect of sulfadiazine is compared with that of sulfathiazole in Table I. The method used

TABLE I.
Bacteriostatic Effect of Sulfadiazine and Sulfathiazole on *E. coli communis.*

Inoculum organisms per cc	Growth—Colonies per cc after 18-24 hr		
	Sulfadiazine 10 mg%	Sulfathiazole 10 mg%	Control
15	9,800,000	14,000,000	560,000,000
15	11,700,000	13,100,000	520,000,000
	50 mg%	50 mg%	
15	6,700,000	950,000	560,000,000
15	2,700,000	<100,000	520,000,000

[1] Klinefelter, H. F., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 365.

was similar to that described by McKee, *et al.*,[2] and Long and Bliss.[3]

In 10 mg % concentration, sulfadiazine is slightly more effective than sulfathiazole. In the higher concentration, both drugs exert more bacteriostasis than in the lower, but sulfathiazole is more effective than sulfadiazine.

B. *In vivo.* Mice were given diets containing 1.0% sulfathiazole or 0.1% sulfadiazine for 2 days before infection. On the morning of the third day, the mice that had eaten well of their diets were infected by the intraperitoneal route. The animals were then kept on their diets for 3 days following infection and observed for 7 more days before being discarded. The results are shown in Table II.

It is clear that sulfadiazine is more effective than sulfathiazole in treating *E. coli communis* infections in mice.

Feinstone, *et al.*,[4] have shown that sulfadiazine is more readily absorbed from the gastrointestinal tract of mice than is sulfathiazole. The blood levels in Table II were obtained from mice that had been kept on the drug diets for 5 or 6 days and confirm this observation. With an average daily intake of only one-tenth as much drug, the blood levels of the sulfadiazine mice are slightly higher than the levels of the sulfathiazole group.

Summary. 1. *In vitro* tests show that sulfadiazine has slightly more bacteriostatic action on *E. coli communis* than sulfathiazole in 10 mg % concentrations. 2. *In vivo* experiments show that, with comparable blood levels, sulfadiazine is more effective than sulfathiazole in treating *E. coli communis* infections in mice.

Conclusion. Sulfadiazine is the most effective of the sulfonamide

TABLE II.
Comparative Effect of Sulfadiazine and Sulfathiazole on *E. coli communis* Infections in Mice.

No. of mice	Inoculum colonies	Drug—% in diet	Avg daily intake, mg	Blood level, mg%		Deaths*—Hrs after infection			Survivals	
				Free	Total	24	48	72	No.	%
34	20-150	Sulfadiazine 0.1%	2.8	6.1	8.0				34	100.0
34	20-150	Sulfathiazole 1.0%	23.8	5.4	6.5	9	2	1	22	64.6
19	20-150	Controls				19			0	0.0

**E. coli communis* cultured from heart's blood in 100%.

[2] McKee, C. M., Rake, G., Greep, R. O., and Van Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 417.

[3] Long, P. H., and Bliss, E. A., *Ibid.*, 1940, **43**, 324.

[4] Feinstone, W. H., Williams, R. D., Wolfe, R. T., Huntingdon, E., and Crossley, M. L., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 427.

compounds in treating experimental colon bacillus infections in mice, and would seem to be the drug of choice in treating *E. coli* tissue infections in human beings.

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Studies on Soft Curd Milk Prepared by the Enzyme Treatment Method.

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This is a preliminary report on the investigation of the characteristics of enzyme-treated milk.[1]

The enzyme treatment[1] is carried out by adding one part of pancreatic concentrate, which has a high tryptic value, to 10 to 15 thousand parts of cold raw whole cow milk and then pasteurizing the enzyme-milk mixture immediately in the usual manner. The milk proteins are altered by the pancreatic enzymes as the temperature of the milk rises during pasteurization.

Methods. Amino nitrogen was determined by the Van Slyke gasometric method. All other nitrogen determinations were made with the Kjeldahl method. The precipitin reaction was carried out according to the technic of Hektoen and Welker.[2] All control milk samples were prepared identically the same as the enzyme-treated milk samples and from the same milk without enzyme being added. The technic of the digestibility test which was found to be satisfactory is as follows:

Eight hundred ml of milk at 37°C is mixed with 80 ml of .5 N HCl containing .056% U.S.P. pepsin. The resulting pH is 4.8. Fifty ml portions are removed and filtered after 5 and 30 minutes' digestion at 37°C with mild agitation. The filtrates are set aside for total N analysis. The digest is then neutralized to pH 7.5 with NaOH and .112 g of U.S.P. trypsin is added. After 15 minutes of mild agitation at 37°C a third 50 ml portion is removed and adjusted to isoelectric pH 4.8 with HCl and then filtered to get the non-coagulable isoelectric protein filtrate. This is repeated every 20 minutes thereafter until a total digestion time of about 2½ hours

1 Conquest, V., Turner, A. W., and Reynolds, H. J., *J. Dairy Sci.*, 1938, **21**, 361.
2 Hektoen, Ludwig, and Welker, Wm. H., *J. Infect. Dis.*, 1924, **35**, 294.