of thiamin, riboflavin, pantothenic acid, factor W, or inositol in the diet.

The authors are grateful to Prof. M. B. Visscher for his helpful advice and criticisms.

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Effects of Sulfanilamide, Sulfapyridine, Sulfathiazole and Sulfanilylguanidine upon Colon-Typhoid-Dysentery Group.

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Recent studies of Marshall, *et al.*,[1] indicate that sulfanilylguanidine compares favorably with the *in vitro* effects of sulfanilamide and sulfapyridine upon microörganisms, particularly those of the colon-typhoid-dysentery group. Following incorporation of sulfanilylguanidine in the diet of normal mice the *in vitro* effects were largely reproduced under *in vivo* conditions in that such medication caused a distinct reduction in the usual number of coliform organisms in the stools.

Since earlier observations[2, 3, 4] indicated that sulfathiazole was equal to, or slightly superior to, sulfanilamide and to sulfapyridine in its bacteriostatic action upon organisms of the colon-typhoid-dysentery group, it appeared worth while to further study the effects of sulfanilamide, sulfapyridine and sulfanilylguanidine upon additional organisms of the group mentioned and to compare them with sulfathiazole and sodium sulfathiazole under corresponding experimental conditions.

The *in vitro* test procedures used in evaluating the several compounds were, with slight modifications, those described by Marshall.[1] Small inocula (0.2 cc) containing 50 to 750 organisms of highly diluted 18-24-hour cultures in tryptone water, were exposed to 5 cc quantities of varying drug concentrations ranging from 100 to 200

[1] Marshall, E. K., Jr., Bratton, A. C., White, H. J., and Litchfield, J. T., Jr., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 163.

[2] Long, P. H., and Bliss, E. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 324.

[3] Lawrence, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 162.

[4] Libby, R. L., and Joyner, A. L., *J. Inf. Dis.*, 1940, **67**, 67.

mg %. The organisms tested included: 5 strains of *E. typhosa,* 6 strains of *S. dysenteriae,* 2 strains each of *E. coli, A. aerogenes* and *Ps. aeruginosa,* and 1 strain each of *S. paratyphi, S. schottmuelleri, S. suipestifer, S. psittacosis, S. enteritidis* and *B. proteus.*

Our observations confirm and extend the observations made by Marshall, *et al.,* in that sulfanilylguanidine corresponds in activity to sulfanilamide and is equal to or slightly inferior to sulfapyridine when tested against the organisms of the colon-typhoid-dysentery group.

In general, these investigations indicate that sulfathiazole and sodium sulfathiazole are somewhat more effective *in vitro* both as to bacteriostatic and bactericidal actions than sulfapyridine and distinctly superior to sulfanilamide and to sulfanilylguanidine against the strains tested.

A study was also made on the influence of oral medication (through incorporation in the diet) of sulfanilamide and the several derivatives on the bacterial flora of the intestinal tract of normal mice. The method and procedures followed, duplicated as closely as possible those described by Marshall, *et al.*[1] Healthy albino mice properly marked and of approximately equal weight and age were placed in coarse wire screen cages in groups of 5 animals each and fed a normal laboratory diet (Rockland mouse diet). Stool specimens were obtained directly from each mouse and emulsified in sterile broth (1.0 cc broth to each 10 mg quantity of stool). Pour plates were made in desoxycholate agar with 1 cc amounts of a 1:100 broth dilution of the heavy stool suspension. After 24 hours' and 48 hours' incubation at 37°C., the coliform colonies were counted or estimated. At least 3 control fecal specimens were taken at the same time of day on different days in order to observe the range of a normal mouse colon-bacillus count. Considerable variation was noted in the coliform population in the stools from day to day in individual mice as well as in different mice of a group on the normal diet as indicated in the tables. The magnitude of the variation noted was such that the method can be considered as of qualitative value only, if carefully controlled over a period of days.

Similar plate counts were made on stool specimens from mice 48 and 96 hours after being placed on a diet containing 1% of one of the several sulfanilamide compounds. Additional plates were made several days after the animals had been returned to a normal diet. Several of the compounds were tested alternately after intervening suitable rest periods on the same group of animals. The results of these studies are presented in Tables I, II, III.

TABLE I.

Effects of Sulfanilylguanidine and Sulfathiazole upon Bacterial Flora (*B. coli*) of Intestinal Tracts of Mice.

	Desoxycholate agar plate (*B. coli*) count. Mouse No.																			
Test day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Normal diet.																			
1st	*59	H	85	H	51	10	T	H	37	H	M	71	H	27	H	M	H	H	52	H
3rd	—	T	H	H	H	50	T	T	H	T	H	H	T	28	H	T	M	0	H	H
13th	5	T	H	T	T	H	M	M	H	M	H	T	T	T	T	T	M	0	M	M
	1% sulfanilylguanidine in diet on 13th day.																			
15th	8	H	50	60	H	H	16	22	15	20	M	31	H	19	65	51	M	T	T	H
17th	H	H	H	H	16	0	1	4	1	9	H	21	M	39	H	32	H	M	M	T
	Normal diet begun 17th day.																			
21st	50	52	M	M	18	0	T	H	H	0	M	4	H	1	H	T	M	H	M	M
	1% sulfathiazole in diet on 21st day.																			
23rd	22	18	M	H	2	2	H	4	M	0	0	20	M	4	M	55	M	H	M	T
25th	0	75	69	M	H	1	H	0	M	M	0	0	38	0	17	M	T	55	H	1
	Normal diet begun 25th day.																			
36th	M	H	50	H	M	M	65	T	M	M	H	T	M	—	T	M	M	M	M	M
43rd	M	T	M	H	H	30	M	20	T	M	30	H	M	5	H	H	H	H	T	M

* Figures represent actual number of lactose-fermenting colonies in plate; H, Hundreds; T, Thousands; M, Innumerable; 0, No lactose-fermenting colonies observed; —, No test made.

TABLE II.

Effects of Several Sulfonamide Compounds upon Bacterial Flora (*B. coli*) of Intestinal Tracts of Mice.

	Desoxycholate agar plate (*B. coli*) count. Mouse No.																			
Test day	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
	Normal diet.																			
1	*H	M	H	H	H	M	H	T	M	H	H	H	3	T	H	H	50	36	M	H
3	H	H	50	H	H	M	M	H	M	M	H	18	H	M	44	M	4	M	M	H
7	H	H	M	M	T	M	M	30	M	T	H	M	70	T	M	T	0	50	T	M
	Drug diet begun 7th day.																			
	1% Sulfanilylguanidine										1% Sulfathiazole									
9	T	50	0	6	2	T	H	20	H	3	15	H	0	H	H	2	0	0	2	6
11	T	H	1	H	50	H	H	25	11	50	15	H	35	H	H	60	0	10	3	10
	Normal diet begun 11th day.																			
13	M	50	70	M	M	T	H	M	M	50	32	M	M	H	20	T	H	2	M	H
15	T	H	20	M	T	M	46	30	M	H	50	M	H	H	M	50	M	1	T	H
16	T	M	10	T	M	M	18	35	M	15	H	M	0	H	M	20	M	15	H	H
	Drug diet begun 16th day.																			
	1% Sulfapyridine										1% Na Sulfathiazole									
18	H	T	2	10	M	H	25	30	55	30	4	20	2	0	6	8	0	13	19	5
20	H	M	3	28	65	H	H	23	T	20	40	5	0	0	20	8	1	6	60	4
	Normal diet begun 20th day.																			
23	H	4	H	H	T	H	12	67	H	31	51	46	24	M	M	20	16	21	44	52
27	T	M	T	H	M	H	0	0	T	M	H	6	T	T	T	13	H	0	2	T
28	H	M	65	90	H	T	T	H	T	H	H	50	H	M	M	7	90	0	T	H

*See legend under Table I.

TABLE III.
Effects of Sulfanilamide and Sulfathiazole upon Bacterial Flora (*B. coli*) of Intestinal Tracts of Mice.

Test day	Desoxycholate agar plate (*B. coli*) count Mouse No. 1	2	3	4	5	6	7	8	9	10
	Normal diet.									
1	*H	H	T	M	M	M	H	M	H	M
3	M	M	H	M	T	M	T	M	M	M
7	50	M	T	T	T	H	T	H	H	T
	1% Sulfanilamide in diet on 7th day.									
9	M	M	H	T	H	H	M	T	H	H
11	M	M	M	T	M	H	M	H	24	H
	Normal diet begun 11th day.									
13	T	M	T	T	M	H	M	H	M	51
15	M	M	M	M	M	T	M	M	M	H
16	M	M	M	M	M	H	M	M	H	H
	1% Sulfathiazole in diet on 16th day.									
18	90	T	70	T	75	30	0	1	10	T
20	50	0	22	T	21	18	19	14	20	dead
	Normal diet begun 20th day.									
23	T	65	T	T	M	35	H	H	34	
27	T	50	M	T	13	H	40	60	H	
28	T	T	H	M	H	60	21	3	5	

*See legend under Table I.

In summarizing the data in the tables, the findings of Marshall were confirmed in that the normal fecal counts of coliform organisms of many of the animals were reduced following dietary medication with sulfanilylguanidine.

Upon extending the feeding experiments to include sulfapyridine, sulfathiazole and sodium sulfathiazole, a similar reduction in the *B. coli* population in the fecal excreta was obtained with all compounds. The animals medicated with sulfanilamide, on the other hand, failed to show a decrease in the *B. coli* count of their stool specimens (Table III). However, substitution of a sulfathiazole, sodium sulfathiazole, sulfapyridine or sulfanilylguanidine for the "sulfanilamide diet" invariably caused a significant reduction in the number of coliform organisms.

The wide variations noted in the *B. coli* counts of fecal specimens of mice on normal diets and the corresponding erratic, although depressed, counts noted after medication with all compounds with the exception of sulfanilamide suggest that this test method for chemotherapeutic compounds is of qualitative value only. Thus, results are influenced primarily by the relative absorbability of the medicaments and secondarily by the dietary drug concentration or dosage fed. Drug action *per se* might, therefore, play only a minor rôle.

Conclusions. *In vitro* studies on several sulfonamide derivatives indicate sulfathiazole and sodium sulfathiazole to be somewhat more

effective than sulfapyridine and distinctly superior to sulfanilamide and to sulfanilylguanidine against the colon-typhoid-dysentery group.

Feeding the several compounds in the diet to mice, caused, with a single exception, a significant reduction of the number of coliform organisms normally present in the intestinal excreta. Sulfanilamide, under the conditions of this test, proved to be ineffective in lowering the fecal *B. coli* count of the animals.

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Mechanism of the Effect of Epinephrine on Bioluminescence of the Firefly.

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There is general agreement[1-4] that flashing of fireflies is regulated by innervated tracheal end-cells which normally occlude tracheoles leading to the light organ. The effect of epinephrine may be interpreted[1] as dilatation of the occluding musculature to permit constant access of O_2 to photogenic areas with consequent prolonged luminescence. Use was made of this action to demonstrate[5] that tensions of ether vapor above the minimal lethal tension will extinguish the constant bright glow of fireflies treated with epinephrine although ether vapor itself causes a constant glow in untreated fireflies exposed to sublethal tensions. Enough specimens were captured from a swarm of the lampyrids, *Photuris pennsylvanica*, during the summer of 1941, at Suncrest, W. Va., to permit the present limited further study.

A recent note by Chakravorty and Ballentine[6] mentions the effect of hydrosulfide on luciferase-oxidized luciferin. Accordingly, 0.01-0.02 cc of 1% freshly prepared NaHS was injected intraäbdominally in 10 fireflies. A moderately bright glow developed immediately and persisted for several minutes, gradually diminishing. Since most available solutions of epinephrine contain 0.1-0.2% $NaHSO_3$, a

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