SULFONAMIDES FOR BACILLARY DYSENTERY

I. The Antibacterial Activity of Sulfacarboxythiazoles and Sulfathiadiazole

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Since a rational approach to the chemotherapeutic control of intestinal infections was established through the introduction of sulfaguanidine by Marshall in 1940 (1), this drug and others with similar properties have been used with considerable success in the treatment of bacillary dysentery.

The basic idea at present is to use active drugs, highly soluble at intestinal pH's, but absorbed and excreted in such a manner as to maintain high concentrations in the intestinal contents and low concentrations in the blood. If the blood level remains low because of a very rapid rate of excretion, then, in order to avoid kidney complications, a high urine solubility is also necessary.

In a search for new chemotherapeutic drugs which may have more of the desirable properties outlined above, we have turned our attention to some of the more acidic sulfonamides. Increased acidity greatly increases the limiting solubilities of these compounds in the intestinal and urinary tracts. Furthermore, oral dosage with this type of compound does not result in high blood levels. This may possibly be due either to low permeability of the intestinal mucosa to the ionic form of the drugs, or to a rapid rate of excretion in the urine, or both (2).

In this connection we have investigated 2-sulfanilamido-1,3,4-thiadiazole (sulfathiadiazole), 2-sulfanilamido-5-carboxythiazole (sulfacarboxythiazole), 2-sulfanilamido-4-methyl-5-carboxythiazole and 2-sulfanilamido-4-carboxythiazole. Data will be presented to show that sulfacarboxythiazole and 2-sulfanilamido-4-methyl-5-carboxythiazole owe their activities to decarboxylation to sulfathiazole and to 2-sulfanilamido-4-methylthiazole, respectively. Because of the ease with which decarboxylation of the methyl compound occurred and because of the possibility of toxic reactions from the sulfamethylthiazole derived from it (3), this compound was not considered further. The 2-sulfanilamido-4-carboxythiazole did not decarboxylate and had only a slight degree of antibacterial activity. Of the four compounds mentioned above, only sulfathiadiazole (which appeared to be chemically stable) and sulfacarboxythiazole (which appeared to decompose to sulfathiazole) had characteristics warranting further study with respect to possible usefulness in the treatment of intestinal infections.

The synthesis and physical properties of sulfathiadiazole (4, 5) 2-sulfanilamido-4-methyl-5-carboxythiazole (6) and 2-sulfanilamido-4-carboxythiazole (7) have been reported. Sulfacarboxythiazole was synthesized in this laboratory by H.

W. Marson. The material melted with decomposition at 209–210°C. This decomposition point depends somewhat on the rate of heating. The *in vitro* activity of sulfathiadiazole has been reported to be about equal to that of sulfanilamide (4, 5, 8). A recent preliminary clinical study has indicated that sulfacarboxythiazole may be of use in the treatment of enteric infections (9).

The results of a study of the comparative antibacterial activity of sulfathiadiazole and sulfacarboxythiazole, together with sulfathiazole, sulfadiazine, sulfaguanidine and sulfathalidine (N^4-phthalysulfathiazole) are given in the present paper. Experimental studies on the absorption, excretion, conjugation and toxicity of sulfathiadiazole and sulfacarboxythiazole will be reported elsewhere.

EXPERIMENTAL. *Buffer solubilities:* Buffers consisting of 0.05 M phosphate plus 0.025 M citrate and covering the physiological range of urinary and intestinal pH, were saturated with the compound in question by stirring 24 hours at 37°C. The excess solid was filtered off at 37°C.; final pH was measured; and determinations of the dissolved compound were made by the Bratton and Marshall method (10). The results obtained with some of the sulfonamides which have been used in bacillary dysentery, along with sulfacarboxythiazole and sulfathiadiazole, are given in fig. 1. The sulfadiazine values are those of Gilligan and Plummer (11). With respect to fig. 1, it should be remembered that this solubility scale cannot be extrapolated due to the fact that it is logarithmic. The curves as drawn cover the pH range actually measured. The results for sulfacarboxythiazole include any sulfathiazole which might have been formed during the 24 hour stirring period. However, as will be shown in the next section, this would be only a very small amount of sulfathiazole.

Chemical stability of the carboxythiazoles: Early in the course of investigating the carboxythiazoles, several observations seemed to indicate that sulfacarboxythiazole and 2-sulfanilamido-4-methyl-5-carboxythiazole were undergoing decomposition. When solutions of these compounds were autoclaved, a decrease in solubility occurred. Also, titration with alkali and acid indicated that a change was taking place in solution which increased titratable acid in the carbonate pH range.

In order to ascertain whether sterilization of bacteriostatic test solutions by autoclaving resulted in decomposition of the compounds, the following comparison was made. A portion of a solution sterilized by Seitz filtration was autoclaved and then titrated for bacteriostatic activity along with the original filtered solution. The results given in table 1 clearly indicate that autoclaving increased the activity of solutions of the two above-mentioned compounds.

In another experiment, more concentrated solutions of the three carboxythiazoles, prepared in 1.0 M phosphate buffer at pH 7.0, were autoclaved and incubated at 37°C. for 72 hours. During this period crystals separated from the sulfacarboxythiazole and 2-sulfanilamido-4-methyl-5-carboxythiazole solutions. These solids were identified by melting points, mixed melting points, chemical analysis and ultraviolet spectral curves, as sulfathiazole and sulfamethylthiazole, respectively. There was no evidence of any decarboxylation of 2-sulfanilamido-4-carboxythiazole under these conditions. With respect to the methyl com-

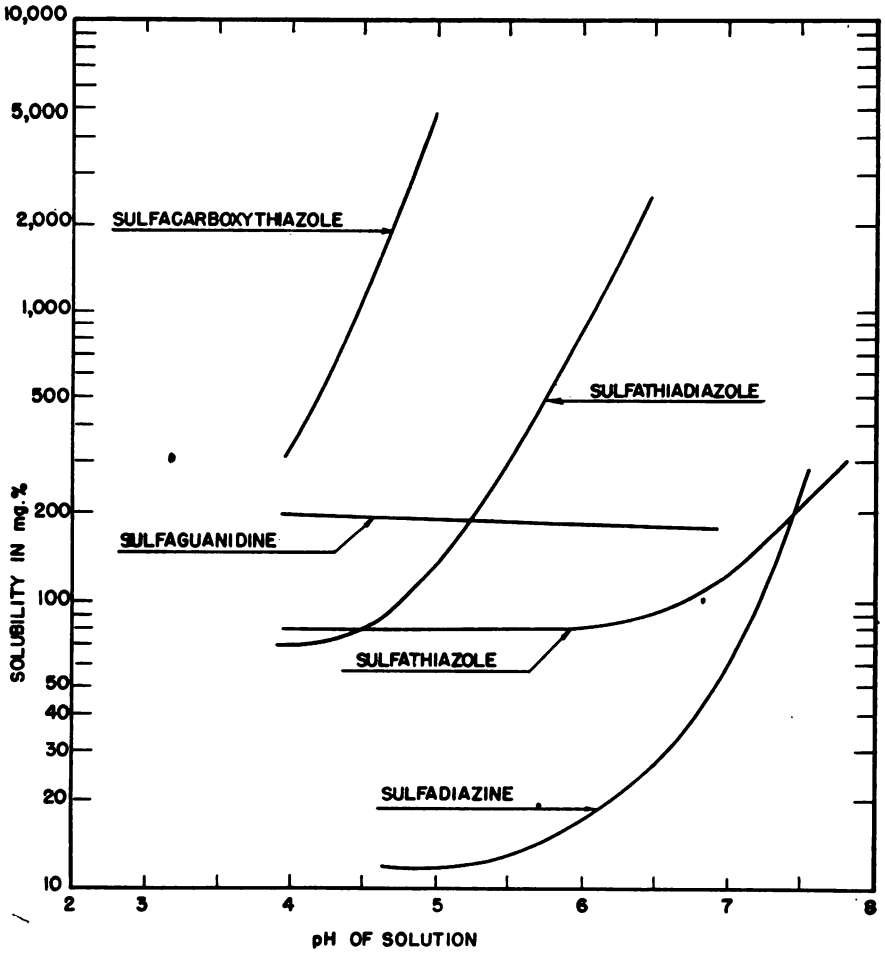

FIG. 1. SOLUBILITIES OF SULFANILAMIDES IN BUFFERS

Phosphate (0.05 M) and citrate (0.025 M) at 37°C. pH values are those of saturated solutions. Data for sulfadiazine (Gilligan and Plummer) in 0.066 M phosphate (11).

TABLE 1

The effect of autoclaving on the bacteriostatic activities of solutions of the sulfathiazoles

COMPOUND	BACTERIOSTATIC CONC. IN MGM. %			
	Autoclaved		Filtered	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
2-S-Thiazole (Sulfathiazole)	1/64	1/32	1/64	1/32
2-S-5-Carboxythiazole (Sulfacarboxythiazole)	1/8	1/4	1	2
2-S-4-Methylthiazole	1/32	1/16		
2-S-4-Methyl-5-carboxythiazole	1/16	1/8	1	1
2-S-4-Carboxythiazole	4	8		

S = sulfanilamido group.

Test medium: McLeod's synthetic (12) buffered at pH 7.2.

Inoculum: *E. coli*, about 200 bacteria per ml.

Incubation at 37°C.

pound, our results confirm those of Jensen and Thorsteinsson (6). These qualitative results indicated that possibly all of the activity of the carboxythiazoles tested in table 1 might be due to the corresponding sulfathiazole being present as an impurity, or being released during the experiment.

Quantitative estimates of the amount of this breakdown were based upon ultraviolet absorption curves obtained with a Beckman spectrophotometer using 3.74×10^{-5} M solutions of the compounds in 0.05 M phosphate buffer at pH 7.0. The results obtained before and after autoclaving and incubating are given in

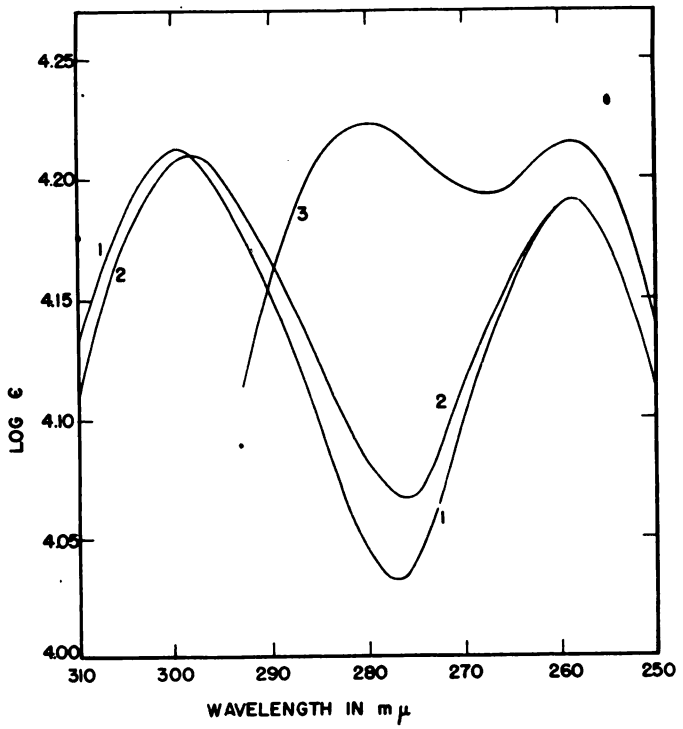

FIG. 2. ULTRAVIOLET ABSORPTION CURVES OF SULFACARBOXYTHIAZOLE AND SULFATHIAZOLE

3.74×10^{-4} M solutions in 0.05 M phosphate buffer at pH = 7.0. Curve 1, sulfacarboxythiazole before autoclaving; Curve 2, solution of Curve 1 after autoclaving and 72-hour incubation at 37°C.; Curve 3, sulfathiazole before and after similar autoclaving and incubation treatment.

figs. 2 and 3. From a study of mixtures of the proper compounds it was possible to show that the position of the peak, associated with the thiazole portion of the molecule, (wavelength 300-280 mμ), could be related quantitatively with the percentages of the compounds in the solution being measured. On the basis of such calibration curves, it was estimated that 12.0 ± 2.5% of the sulfacarboxythiazole decomposed to sulfathiazole and that 100% decarboxylation of the 2-sulfanilamido-4-methyl-5-carboxythiazole occurred. By use of the same method of analysis, the rates of breakdown of the two compounds, at pH 7.0 and 37°C.,

were determined. Both appeared to decompose according to a first order rate law:

$$k = \frac{2.303}{t} \log \frac{C_0}{C}, \quad \text{where} \quad \begin{aligned} k &= \text{reaction rate constant} \\ C_0 &= \text{initial concentration} \\ C &= \text{concentration at time, } t. \end{aligned}$$

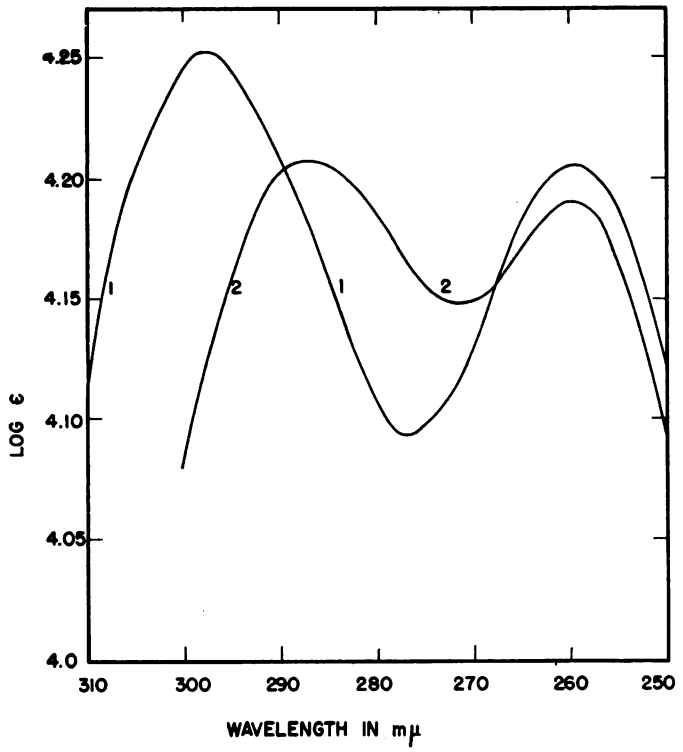

FIG. 3. ULTRAVIOLET ABSORPTION CURVES OF 2-SULFANILAMIDO-4-METHYL-5-CARBOXYTHIAZOLE AND 2-SULFANILAMIDO-4-METHYLTHIAZOLE

3.74×10^{-4} M solutions in 0.05 M phosphate buffer at pH = 7.0. Curve 1, 2-sulfanilamido-4-methyl-5-carboxythiazole before autoclaving; Curve 2, solution of Curve 1 after autoclaving and 72-hour incubation at 37°C. This curve is identical with that obtained for 2-sulfanilamido-4-methylthiazole before and after autoclaving.

The rate constants, with t in hours, and corresponding half-lives were as follows: sulfacarboxythiazole, $k = 6.35 \times 10^{-4}$ hr.$^{-1}$, $t_{1/2} = 1090$ hrs.; 2-sulfanilamido-4-methyl-5-carboxythiazole, $k = 6.65 \times 10^{-3}$ hr.$^{-1}$, $t_{1/2} = 104$ hrs.

In the light of these decomposition rates and the results in table 1, it is at once obvious that it is not necessary to assume any activity due to the carboxy forms of sulfacarboxythiazole and the sulfamethylcarboxythiazole. Also, the presence of sulfathiazole as an impurity to the extent of only 0.4% would account for the observed activity of 2-sulfanilamido-4-carboxythiazole.

On the basis of acid strengths of the amide hydrogen (second acid constant)

the carboxy forms of these compounds should be very active (13). pK_a's obtained by alkali titration and not corrected for dilution were: sulfacarboxythiazole, $pK_a(1) = 3.4$, $pK_a(2) = 7.0$; 2-sulfanilamido-4-methyl-5-carboxythiazole, $pK_a(1) = 3.4$, $pK_a(2) = 7.7$; and 2-sulfanilamido-4-carboxythiazole, $pK_a(1) = 3.3$, $pK_a(2) = 6.9$. However, at thepH of the bacteriostatic tests (7.2), the carboxyls of all of these compounds are better than 99 per cent ionic. If one assumes that such ionic forms do not penetrate bacteria (14, 15) then it is not surprising that the carboxy compounds, as such, were found to be relatively inactive.

Blood concentrations in mice[1]*:* Comparative blood concentration-time values following a single oral dose of 0.5 grams per kgm. in mice are given in table 2. Sulfacarboxythiazole blood levels were very low as was the case with sulfathalidine. Sulfathiadiazole blood levels were somewhat higher than those

TABLE 2

Drug concentrations in blood of mice after a single oral dose of 0.5 gm./kgm. in 10% acacia solution

COMPOUND	DRUG CONCENTRATION IN BLOOD AT HOUR				
	1	2	4	8	24
	mgm. %	*mgm. %*	*mgm. %*	*mgm. %*	*mgm. %*
Sulfathiazole	12.0	7.4	4.6	4.6	0.4
Sulfadiazine	18.9	20.0	18.9	10.3	0.4
Sulfathiadiazole	6.1	2.8	2.0	1.2	0.7
Sulfaguanidine	2.6	1.7	0.5	0	0
Carboxythiazole	1.0	0.5	0.3	0.3	0
Sulfathalidine	0.7	0.6	0.6	0.3	0.3

Each determination (Bratton and Marshall method) was made as free drug on pooled tail blood samples from ten mice. Mice were without food for 18 hours prior to administration of drug.

for sulfaguanidine, but appreciably lower than those obtained with sulfathiazole and sulfadiazine. The fact that sulfathiadiazole gave blood levels higher than those of sulfaguanidine was not considered to be necessarily disadvantageous with respect to usefulness in treating bacillary dysentery, as will be discussed later in this paper.

Blood levels which were more or less constantly maintained in mice as a result of continual feeding on drug-diets for seven days are given in table 4. Under these conditions, the sulfacarboxythiazole and sulfathalidine levels were again found to be very low, while those of sulfathiadiazole and sulfaguanidine were higher, but not as high as the sulfathiazole and sulfadiazine values.

Bacteriostatic activity: The dysentery strains used in this study were obtained

[1] We wish to thank Miss Dorothea Babbitt for assistance in determinations on blood concentration values.

through the courtesy of Dr. A. J. Weil of the Lederle Laboratories. The test procedure has previously been described in detail (16). The activity of each drug was titrated by making serial two-fold dilutions in 5 ml. volumes of Trypticase-Soy Phosphate Broth (Baltimore Biological Laboratory) buffered at pH 7.2. After autoclaving, each tube in each drug series and control tubes without drug were inoculated with 0.2 ml. of a 10^{-6} broth dilution of a 22-hour broth test culture. The relative values in table 3 were confirmed in repeated comparisons.

On the basis of bacteriostatic endpoints for all four drugs, the dysentery strains may be divided into a resistant group and a relatively susceptible group, with six strains in each group. In view of a clinical study (17) which indicated that Flexner varieties of *Shigella paradysenteriae* were more sensitive than Sonne

TABLE 3

Comparative activity of sulfonamides against dysentery strains in 0.4% peptone broth

DYSENTERY STRAINS		MINIMAL CONCENTRATION OF DRUG IN MGM. % REQUIRED TO PREVENT VISIBLE GROWTH			
		Sulfathiazole	Sulfadiazine	Sulfaguanidine	Sulfathiadiazole
Sonne.....	B-151	256	>256	>256	256
Flexner.....	5733	128	256	>256	256
Shiga.....	70-151	128	256	128	512
Sonne.....	B-152	64	256	>256	256
Schmitz.....	B-161	64	256	>256	256
Shiga.....	B-111	16	>256	>256	64
Flexner.....	63-143-Z	4	8	128	16
Flexner.....	63-143-V	1/4	1/2	8	1
Schmitz.....	B-162	1/8	1	16	2
Flexner.....	63-143-X	1/8	1	16	2
Flexner.....	63-143-Y	1/8	1/2	8	1
Flexner.....	63-143-W	1/64	1/64	2	1/2

Test medium: 0.4% Trypticase-Soy-Phosphate Broth buffered at pH 7.2.
Inoculum: 50 ± 20 bacteria per ml.
Incubation: 48 hours at 37°C.

varieties to sulfonamide treatment, it is of interest that, in our tests five of the six Flexner strains were relatively susceptible to one or more drugs, whereas each of the two Sonne strains were relatively resistant to all of the drugs.

Under our test conditions, five strains were resistant to sulfaguanidine concentrations approximating its highest solubility. Two of these strains were also resistant to the highest concentration of sulfadiazine which could be obtained at pH 7.2. Inhibition of the sulfaguanidine-resistant strains with high concentrations of the other drugs was readily accomplished by dissolving their sodium salts in the test medium and adjusting the pH to 7.2.

Against the sulfonamide-susceptible strains, sulfathiadiazole was from four to eight times as active as sulfaguanidine.

Anticoliform activity in mice: Full details of the method used for this compari-

son have been given in a previous paper (18). In brief, mice (Carworth CFCW strain) were arranged in groups which were equivalent with respect to fecal coliform counts on the basis of preliminary determinations. Each group was then treated with a different drug-diet. Each mouse in each group was kept in an individual cage and allowed to feed on drug-diet for seven days at which time final coliform counts were made. Coliform counts per unit volume of a standard stool suspension were determined by serial ten-fold dilutions in lactose broth incubated at 44°C. Drug activity was expressed as the difference between coliform counts at the beginning and at the end of drug-diet treatment.

In table 4 the results for six drugs, together with the data on control animals, are summarized and listed in order of decreasing anticoliform activity. With each drug, it is evident that, on the average, the pre- and post-treatment coliform

TABLE 4

Sulfonamide activity against coliform bacteria in mice

DRUG	PER CENT IN DIET	NUMBER OF MICE	DAILY INTAKE		DRUG CONCENTRATION		LOGARITHMIC COLIFORM COUNT PER 9 CC. OF STOOL SUSPENSION		
			Food	Drug	Blood	Stool	Before treatment	After treatment	Reduction
			gm.	*gm./ kgm.*	*mgm./ 100 cc.*	*mgm./ 100 gm.*			
Sulfathiazole	1.0	63	4.4	2.2	9.2	140	6.5	1.5	5.1 ± 0.3
Sulfadiazine	0.5	17	4.4	1.1	26.6	910	6.3	1.8	4.5 ± 0.5
Sulfathiadiazole	1.0	66	4.7	2.4	4.1	580	5.8	1.5	4.3 ± 0.3
Sulfaguanidine	1.0	63	4.7	2.4	2.6	1100	5.7	2.6	3.1 ± 0.4
Sulfacarboxythiazole	1.0	43	4.6	2.3	1.1	2300	5.7	2.7	3.0 ± 0.4
Sulfathalidine	1.0	25	5.0	2.5	0.5	1250	5.4	3.4	2.0 ± 0.4
Control Diet		117	4.6				5.8	5.7	0.1 ± 0.2

Figures above are mean values. Reductions in logarithmic counts are given with their standard errors. In each case, the mice were fed on drug-diet for seven days. Blood concentrations were determined as free drug on the third day; stool concentrations of each drug were calculated on the basis of an average value of 0.275 grams of wet stool per 100 cc. of standard stool suspension.

counts differed significantly. It is also evident that each of the drugs produced, on the average, a reduction in coliform count which was significantly greater than the reduction which occurred in untreated mice. Thus, the anticoliform effect ranged from about a hundred-fold reduction with sulfathalidine to about a hundred thousand-fold reduction with sulfathiazole. On the basis of average reduction in count, sulfathiazole, sulfadiazine and sulfathiadiazole were all more active than sulfaguanidine, sulfacarboxythiazole and sulfathalidine.

DISCUSSION. One aspect of the bacillary dysentery problem is concerned with the treatment of mild cases and the prevention of epidemic spread by eliminating pathogenic organisms from convalescents and carriers. In these individuals, the bacteria presumably reside chiefly in the lumen of the intestinal tract and the situation calls for an active drug which is highly soluble, but slowly absorbed

and rapidly excreted in the urine, in order to minimize toxic effects which might occur in the absence of strict medical supervision.

Another part of the dysentery problem consists of treating cases, of varying severity, in which the pathogenic bacteria are probably not only in the lumen of the intestinal tract, but also in the lining tissue, often covered by a muco-purulent exudate. Under these conditions, optimal results should be achieved by treatment with a drug which is maintained in an effective concentration in the blood as well as in the intestinal contents. Thus, although a low concentration of drug in the blood has been emphasized as a required characteristic, it is quite possible that this has been over-emphasized and that, in many cases, better results would be obtained with a drug which is absorbed to a moderate extent (17).

Sulfadiazine and sulfathiazole are examples of well absorbed drugs which have been used with a certain amount of success for the treatment of bacillary dysentery (17, 19, 20, 21, 22). These drugs were highly active under our test conditions (tables 3 and 4). But they are absorbed and excreted in a manner which results in rather high concentrations in the blood (tables 2 and 4) and, furthermore, they are soluble to a relatively low degree at intestinal and urinary pH's (fig. 1).

N^4-Succinylsulfathiazole and N^4-phthalylsulfathiazole (sulfathalidine) are examples of very poorly absorbed drugs which have been used for treating enteric infections (17, 22, 23, 24, 25). Apparently one of the drawbacks encountered with the succinyl compound is that it tends to be ineffective in the presence of a watery diarrhea (25). Sulfathalidine, as the sodium salt, is highly soluble at a pH of 7.6 (26). However, according to Poth (25), there is no tangible evidence to show that either succinyl- or phthalylsulfathiazole possesses antibacterial properties which are not entirely dependent upon the presence of sulfathiazole itself, presumably resulting from hydrolysis. This is in agreement with our results (18). Thus, in their inactive form these compounds are highly soluble and very poorly absorbed; and they appear to be effective by means of a slow breakdown to sulfathiazole.

Sulfacarboxythiazole appears to be another type of compound which owes its activity to the release of an active component. It is highly soluble at pH's which may be expected in the intestinal contents (fig. 1); it is poorly absorbed (9); and it presumably releases sulfathiazole at a rate and to an extent sufficient to produce an antibacterial effect about equal to that of sulfaguanidine (table 4).

Sulfaguanidine has been used with considerable success both therapeutically and prophylactically (17, 19, 23, 27, 28, 29, 30). It appears to have been a relatively safe drug for prophylactic use, due to the fairly low blood levels which result from large doses. But it is soluble to only a moderate degree (fig. 1) and it is not as active as sulfathiazole or sulfadiazine (tables 3 and 4).

Sulfathiadiazole, on the basis of the comparative data obtained in the present investigation, appears to be a drug with interesting possibilities for use in bacillary dysentery. It is more active than sulfaguanidine both *in vitro* and *in vivo* (tables 3 and 4); it does not appear to owe its activity to a decomposition product, as do sulfathalidine and sulfacarboxythiazole; and, finally, it is highly soluble at

intestinal and urinary pH's (fig. 1). Preliminary pharmacological studies (31) indicate that, following oral dosage, the absorption and excretion of the compound occurs in such a manner as to result in high concentrations in the intestinal contents, moderately low concentrations in the blood and relatively high but, due to its solubility, probably safe concentrations in the urine.

The final answer to the question as to which sulfanilamide derivative may be most useful in the treatment of bacillary dysentery can only be determined on the basis of carefully controlled clinical and field trials. In addition to this question, there remains the important problem of finding new drugs which will be effective against sulfonamide-resistant strains of Shigella.

CONCLUSIONS

1. Sulfacarboxythiazole (2-sulfanilamido-5-carboxythiazole) was about as active as sulfaguanidine against twelve dysentery strains *in vitro* and against coliform bacteria in mice. With respect to solubility, absorption and mode of action, sulfacarboxythiazole appeared to be similar to sulfathalidine (N^4-phthalylsulfathiazole).

2. Sulfamethylcarboxythiazole (2-sulfanilamido-4-methyl-5-carboxythiazole) decarboxylates quite readily to sulfamethylthiazole. Consequently, further investigation of this compound, as a possible chemotherapeutic agent, does not appear to be warranted.

3. 2-Sulfanilamido-4-carboxythiazole does not decarboxylate readily but is relatively inactive.

4. Sulfathiadiazole (2-sulfanilamido-1,3,4-thiadiazole), when compared with sulfaguanidine, was found to be about ten times as soluble at pH 6.5; from four to eight times as active against dysentery strains *in vitro;* and more active against coliform bacteria in mice. These properties, together with preliminary data on its absorption in mice, indicate that sulfathiadiazole is, at present, one of the most promising compounds available for trial in the treatment and prophylaxis of bacillary dysentery.

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