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RECENT CONTRIBUTIONS TO ANTIBACTERIAL THERAPY

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PHARMACOLOGICAL STUDIES WITH SULFADIMETHOXINE

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Earlier investigations have shown sulfadimethoxine (Madribon*) to be a potent antibacterial agent. High chemotherapeutic activity was exhibited by this drug toward experimentally induced infections in animals and clinically in the treatment of various diseases of infectious origin.[1-3] Following oral administration of sulfadimethoxine to rats, high blood and tissue levels were rapidly attained, and the drug was excreted slowly by the kidneys over a period of several days.[4] In man, Brandman *et al.* demonstrated that sulfadimethoxine also remained at high concentrations in the blood and urine for an extended period; measurable amounts of drug were detected in the blood 4 days after administration.[5] Chronic toxicity experiments of 3 months' duration with dogs and rats indicated that sulfadimethoxine exerts a low toxicity.[4]

A low incidence of untoward reactions is particularly important when a drug having a prolonged duration of action is employed clinically. Therefore, tolerance studies in animals and man were extended to obtain additional information about potential side effects following chronic administration of sulfadimethoxine. The results of these investigations are presented in this paper.

Methods

Sulfadimethoxine was incorporated in the diet and administered to 20 male and female Sprague-Dawley rats. Another group of 20 rats served as controls. The animals were maintained on ground Purina Chow pellets ad libitum. After 11 weeks of treatment the rats were paired and bred to obtain a first generation. After weaning, rats comprising the first generation were also given the drug as a dietary admixture. The parent and first generations have received sulfadimethoxine continuously in the diet for 34 and 19 weeks, respectively.

Sulfadimethoxine was determined in tissues and body fluids by the method of Bratton and Marshall.[6] Hematological determinations, including total and differential leukocyte counts, hematocrit, and hemoglobin, were performed on the parent generation at the tenth experimental week. Oxygen consumption of surviving liver and kidney slices was determined by conventional manometric methods; Krebs-Ringer phosphate buffer with the calcium concentration reduced to one half was used in these measurements.[7] The tissue slices were rapidly prepared and placed in the main compartment of Warburg flasks containing cold buffer solution. The flasks were gassed with 100 per cent oxygen and incubated with shaking at 37.5° C. for 30 min. Readings were made at 30-min. intervals over a 2-hour period. Results were expressed as QO_2 = μl. O_2 taken up per milligram wet weight of tissue. Serum glutamic-pyruvic trans-

* Hoffmann-La Roche.

aminase levels were determined spectrophotometrically.[8] Estimations of the cecal bacterial flora were made, using plain and differential culture media.

Clinical observations were made in 35 paraplegic patients who were afebrile and had not shown signs of acute bladder flare-up for at least 2 months prior to receiving sulfadimethoxine as prophylactic therapy.[9] Most of these patients were engaged in a rehabilitation program designed for paraplegics. All received an initial loading dose of 1 gm. of the drug daily for the first 3 days and then were maintained on a daily dose of 0.5 gm. for more than 1 year. When diagnostic procedures such as cystoscopy, intravenous and retrograde pyelograms, or treatment for decubitus ulcers were required, the dosage of sulfadimethoxine was increased twofold for a few days. Complete hemograms were

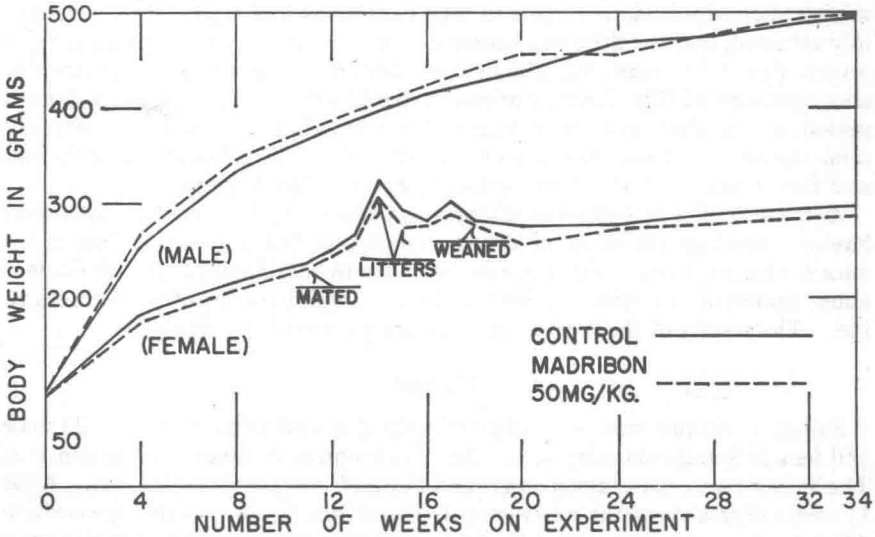

FIGURE 1. Growth curves of parent-generation rats that received sulfadimethoxine (50 mg./kg.) in the diet for 34 weeks.

performed on all patients every 10 days during the first 6 months of the clinical study and at frequent intervals thereafter. Urine cultures with sensitivity tests were done weekly on all patients during the initial 6 months of therapy.

Results

Ten male and 10 female rats of the Sprague-Dawley strain were administered sulfadimethoxine (50 mg./kg.) continuously for 34 weeks. One male control rat expired at the twenty-second experimental week, and another male in the treatment group succumbed during the twenty-seventh week. These deaths were attributed to pneumonia and are unrelated to the administration of the drug. The health of the remaining animals has been optimal during the entire experimental period. The average weekly body weights of the parent generation are shown in FIGURE 1. The rates of growth, activity, food consumption, and general condition have not been adversely influenced, and these rats have

not exhibited signs of toxicity. The lack of an effect on growth supports the conclusion that higher doses of sulfadimethoxine are required to exert goitrogenic activity in this species.

Complete hematological determinations, including measurements of hematocrit, hemoglobin, and total and differential leukocytes were performed during the tenth experimental week; these results are shown in TABLE 1. Sulfadimethoxine had no deleterious effects on circulating blood cells or hemopoietic organs and tissues.

After 11 weeks of treatment the rats were paired and bred to obtain a first generation. These data provide information concerning the influence of sulfa-

TABLE 1

AVERAGE BLOOD COUNTS OF PARENT GENERATION RATS GIVEN SULFADIMETHOXINE (50 MG./KG.) FOR 10 WEEKS

Group	No. and sex of rats	WBC in thousands	Hematocrit per cent vol. RBC	Hb in gm./100 ml.	Differential							Per cent Nucl. RBC
					NSN	SN	L	M	E	B	Pl. cells	
Sulfadimethoxine	10 Females	10.9	46.2	14.8	0	7.6	89.6	1.7	1.1	0.0	0.0	0
Sulfadimethoxine	10 Males	18.7	46.4	14.7	0	8.0	89.3	1.7	0.9	0.1	0.0	0
Controls	10 Females	13.7	46.6	15.2	0	10.9	86.4	1.4	1.0	0.1	0.2	0
Controls	10 Males	16.2	46.9	15.0	0	11.2	85.5	1.6	1.5	0.2	0.0	0

TABLE 2

INFLUENCE OF SULFADIMETHOXINE ON REPRODUCTION

Sulfadimethoxine (50 mg./kg.) for 11 weeks

No. females bred	Litters obtained		Days to parturition	No. per litter	Survivors at weaning	Wt. (gm.) at weaning (21 days)
10	9	Av. of 8 rats*	26	9	6	45
			Controls			
10	10	Av. of 10 rats	26	10	8	42

* One female devoured her litter.

dimethoxine on fertility, pregnancy, lactation, and growth of the newborn; the results of these experiments are recorded in TABLE 2. The absence of congenital malformations, absorption of fetuses, or other abnormalities indicates that chronic administration of this drug does not exert deleterious effects on the process of reproduction.

After weaning, rats constituting the first generation were given sulfadimethoxine (50 mg./kg.) for 19 weeks, and mortality or signs of toxicity have not occurred. In the corresponding group of controls 3 rats contracted a severe respiratory infection and expired during the ninth to thirteenth experimental weeks. The body weights of the first-generation rats at weekly intervals are shown in FIGURE 2; these animals have exhibited a normal rate of growth.

At the sixth experimental week, 6 rats were sacrificed and the levels of drug in blood and various tissues determined. In these measurements, material that reacted directly with the Bratton-Marshall reagents was termed free or unbound drug. This material represents all compounds containing a free N^4 aminophenyl group, including the glucuronide conjugate. Metabolites that required acid hydrolysis prior to analysis were taken to be acetylated or bound drug; these results are shown in TABLE 3. Each value represents the average of duplicate determinations performed in 6 rats. Prolonged administration of

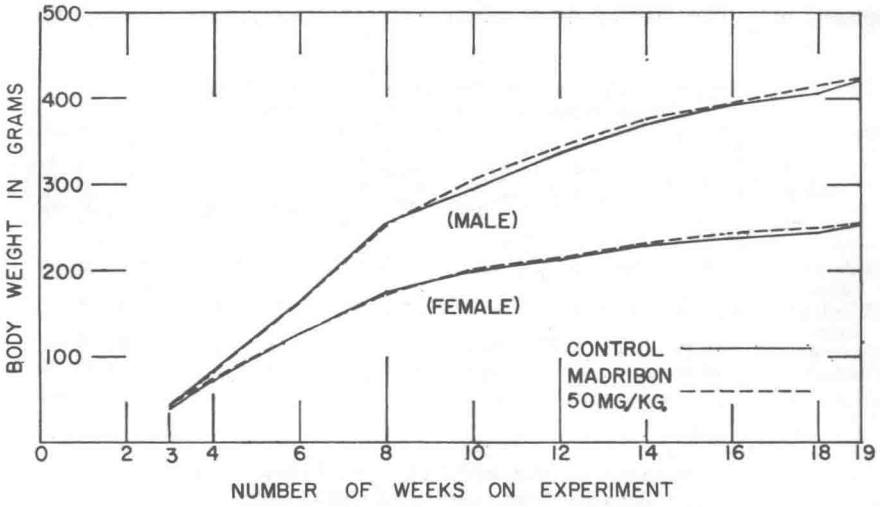

FIGURE 2. Growth curves of first-generation rats that received sulfadimethoxine (50 mg./kg.) in the diet for 19 weeks.

TABLE 3

FIRST GENERATION RATS GIVEN SULFADIMETHOXINE (50 MG./KG.) FOR 6 WEEKS

Levels of drug in blood and tissues (mg. per cent)

	Blood	Kidneys	Liver	Muscle	Brain
Unbound	13.70	3.60	2.70	1.61	0.78
Bound	0.90				

sulfadimethoxine did not result in an excessive accumulation of the drug in blood and tissues. These concentrations of drug are not significantly different from the blood and tissue levels achieved in rats after 4 successive daily doses of sulfadimethoxine.[4] These findings indicate that the rat excretes excessive amounts of the drug, and in this manner abnormal amounts of sulfonamide are prevented from accumulating in the tissues.

At the eighth experimental week groups of 6 treated and 6 control rats were sacrificed, and cultures of the cecal contents were examined to determine if alterations of the normal bacterial flora had occurred.* *Escherichia coli* flora

* These determinations were performed by E. Grunberg.

were found to be diminished by administration of sulfadimethoxine when compared with controls; cultures taken from the cecal contents of 4 of the 6 control rats exhibited a heavy growth of the organism, in contrast to 1 rat in the group of 6 sulfadimethoxine-treated animals. Sulfadimethoxine had no effect against *Streptococcus fecalis* or other bacterial flora normally present in the intestinal tracts of rats.

Despite long-term administration of sulfadimethoxine, evidence of hepatic or renal damage has not been detected. In addition to histological examination of tissue sections,[4] selected biochemical measurements were made to ascertain if changes in cellular metabolism had been produced by the drug.

The serum glutamic-pyruvic transaminase (SGP-T) levels of 4 rats given sulfadimethoxine for 10 weeks remained within normal limits. Elevations of this enzyme have been shown to reflect hepatocellular injury.[10] Another group of 4 rats from the first generation given the drug continuously for 10 weeks was sacrificed together with 4 control animals, and the oxygen consumption of surviving liver and kidney slices was determined by manometric methods. The average QO_2 of liver and kidney slices of control rats was found to be 1.1 and 2.3, respectively; similar values of 0.7 and 2.1 were obtained, using liver and kidney slices of rats receiving the drug over an extended interval. These results indicate that chronic administration of high doses of sulfadimethoxine to rats does not significantly impair endogenous respiration of liver and kidney tissue.

Sulfadimethoxine has been given continuously to a group of 35 paraplegics with spinal cord bladder for as long as one year to investigate its effectiveness as a prophylactic and therapeutic agent in the management of chronic cystitis. Urinary tract infections are relatively common complications of paraplegia as a consequence of urine stasis caused by a hypotonic bladder. The patients were given an initial loading dose of 1 gm. of sulfadimethoxine and then were maintained on a single daily dose of 0.5 gm. Urine specimens were obtained weekly, and cultures with sensitivity tests were done during the initial 6-month period. The weekly urine cultures revealed the same spectrum of organisms (*E. coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*) as was found prior to treatment with sulfadimethoxine, and these organisms showed a complete resistance to this sulfonamide *in vitro*. However, none of the patients given sulfadimethoxine as a prophylactic measure exhibited the clinical signs of bladder infection. Another patient who was receiving tetracycline had a recurrence of bladder infection with fever of 102 to 103° F., chills, and general malaise. He was given 1 gm. of sulfadimethoxine twice a day for 2 days and 0.5 gm. daily thereafter. The clinical manifestations subsided in 24 hours and he has continued to be asymptomatic. These results indicate that *in vitro* sensitivity tests should not be relied upon as an indication of clinical effectiveness.

Every patient in this investigation tolerated the drug without the occurrence of blood dyscrasias or neurological, renal, gastrointestinal, or allergic reactions. The complete absence of side effects in these patients despite the extended period of treatment was a particularly impressive finding of this clinical study.

Discussion. Pharmacological and toxicological investigations of sulfadimethoxine indicate that this drug is a superior chemotherapeutic agent. Fol-

lowing oral administration, high blood and tissue levels of the drug are attained, and the drug is slowly excreted by the urinary route.

Chronic administration of sulfadimethoxine to animals and man has not produced the serious toxic manifestations often encountered with the use of sulfadiazine and other sulfonamides.[11, 12] In the present study, blood dyscrasias, renal complications, hepatitis, dermatological manifestations and other hypersensitivity reactions, or involvement of the central and peripheral nervous system were not detected despite the long duration of treatment. The absence of renal obstruction and other urinary tract reactions was not unexpected, since Koechlin *et al.* have demonstrated that sulfadimethoxine is excreted by humans largely as a highly soluble glucuronide.[13] The lack of deleterious actions on tissues and blood cells of the host is a distinct advantage exhibited by sulfadimethoxine over other antibacterial sulfonamides.

Summary

A parent generation of 20 rats was administered sulfadimethoxine (50 mg./kg.) for 34 weeks without the occurrence of toxic manifestations. After 11 weeks of treatment the rats were bred, and the litters obtained have also been maintained on the drug continuously for 19 weeks. Chronic administration of sulfadimethoxine to rats did not adversely affect growth, activity, general condition, food consumption, or the process of reproduction. Evidence of renal or hepatic injury was not observed, and complete hematological determinations did not reveal any abnormalities of the circulating blood cells or hemopoietic tissues. Examination of the cecal bacterial flora of rats of the first generation after 8 weeks of treatment revealed the facts that the *E. coli* were markedly reduced as compared to untreated controls, but that other intestinal flora were unaffected.

Sulfadimethoxine has been given continuously to a group of 35 paraplegics for over 1 year in a daily maintenance dose of 0.5 gm. to investigate the effectiveness as a prophylactic and therapeutic agent in the management of chronic cystitis. The complete absence of side effects in these patients was particularly impressive. The low toxicity exhibited by sulfadimethoxine in these animal and clinical investigations indicates that this drug is a superior antibacterial sulfonamide.

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COMPARATIVE CHEMOTHERAPEUTIC STUDIES WITH THE NEWER SULFONAMIDES

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In a recent study Fust and Boehni have described similarities and differences of the activity of nine sulfonamides.[1] Owing to the chemical relationships of the sulfonamides, their chemotherapeutic efficacy is bound to be similar in experimental tests in which their activity against pathogenic organisms is evaluated. Nevertheless, the history of the sulfonamides and the development of new compounds of this class during recent years has brought forth evidence that different sulfonamides possess different biological properties that can influence their antibacterial qualities. It has long been known that the activity of sulfonamides in infections with various organisms is quantitatively different and that these differences are, to a certain degree, determined by the fate of the drugs in the host organisms; that is, their absorption, distribution, and elimination. The most important quality is, however, the antibacterial activity as such, and it is this property that has been studied in the following experiments in which a comparison of four of the more recently developed sulfonamides has been attempted. Only *in vivo* experiments are presented and discussed.

The following 4 sulfonamides tested (FIGURE 1) were selected on the basis of different pharmacological characteristics: Madribon* (SDM), Gantrisin* (ST), Kynex† (SMP), and Orisul‡ (SP).

A quantitative evaluation in infections with representative Gram-positive and Gram-negative organisms is shown in TABLE 1.

The data in TABLE 1 are based on experiments in mice carried out by the methods described earlier,[3, 5] which consist in the single or repeated oral treatment of intra-abdominal infections with approximately 1000 MLD of the different organisms. In all instances where the natural pathogenicity of the bacteria is low, such as staphylococci, salmonellae, shigellae, *Escherichia coli*, or pseudomonas, the conditioning of mice with gastric mucin was employed. The CD_{50} as given in the table was calculated according to Reed and Muench[9] on the basis of survival at the end of a 3-week observation period.

The data in TABLE 1 indicate that these four sulfonamides may differ considerably in their activity toward the various test organisms. Apparently independent of their pharmacological properties, their activity seems to be determined rather by the sensitivity of the different organisms or the character of the infection produced. Although long-acting sulfonamides such as SDM and SMP may exert very marked activity in all infections tested, SMP was, in 2 infections (streptococci and *Salmonella schottmülleri*), 5 or 6 times more active. On the other hand, the differences of activity between SDM and SI were not too great in the majority of instances, with the exception of pneumococci and pseudomonas. SP occupies a particular position in this group of

* Hoffmann-La Roche.
† Lederle.
‡ Ciba (Basel, Switzerland).

compounds on account of a certain specificity in the infections with Gram-positive organisms.[7] Its CD_{50} in streptococci and staphylococci was within the range of those obtained with SDM and SI; in pneumococci it was superior to the latter. In all infections with Gram-negative bacteria higher doses were required than of the three other compounds.

Nevertheless, a certain degree of similarity also governs this accumulation of different values. In all instances streptococci and staphylococci were the

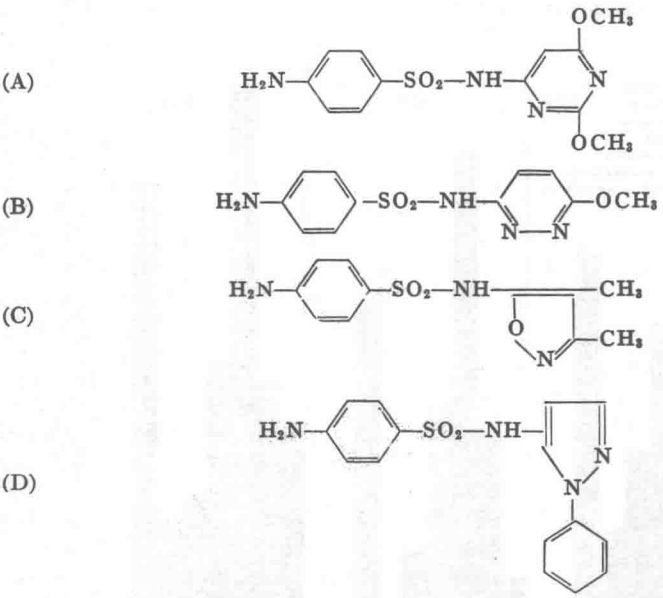

FIGURE 1. Structures of the four sulfonamides investigated: (A) 2,4-dimethoxy-6-sulfanilamido-1,3-diazine (sulfadimethoxine, SDM, Madribon[1-4]); (B) 3-sulfanilamido-6-methoxypyridazine (sulfamethoxypyridazine, SMP, Kynex[1-6]); (C) 5-sulfanilamido-3,4-dimethylisoxazole (sulfisoxazole, SI, Gantrisin[1, 5]); (D) 3-sulfanilamido-2-phenylpyrazole (sulfaphenazole, SP, Orisul[1, 7, 8]).

TABLE 1

IN VIVO ACTIVITY OF SULFONAMIDES AGAINST VARIOUS BACTERIAL INFECTIONS

Organism	No. of doses	CD_{50}: mg./kg.			
		SDM	SI	SMP	SP
Streptococcus hemolyticus No. 4	6	78.5	106.0	15.2	62.0
Diplococcus pneumoniae No. 6301	6	211.0	1000.0	366.0	595.0
Staphylococcus aureus Smith	4	53.2	68.3	—	90.1
Salmonella typhosa P58a	1	10.8	24.1	6.1	54.0
Salmonella schottmülleri	1	91.4	125.0	16.5	369.5
Shigella flexneri	4	99.5	112.0	56.3	235.0
Escherichia coli J	4	88.9	67.8	—	159.0
Klebsiella pneumoniae	6	71.7	119.0	—	—
Pseudomonas aeruginosa B	4	325.0	582.0	162.0	>500.0

more sensitive bacteria of the Gram-positive organisms, *Salmonella typhosa* was always more sensitive than other members of the *coli-Shigella-Salmonella* group, whereas *Pseudomonas aeruginosa* responded only to comparatively high doses of all sulfonamides.

It is not without interest that a very similar pattern has been observed in the experiments of Fust and Boehni,[1] which were carried out with different strains and sometimes even with an entirely different technique (that is, with staphylococci) and is shown in FIGURE 2.

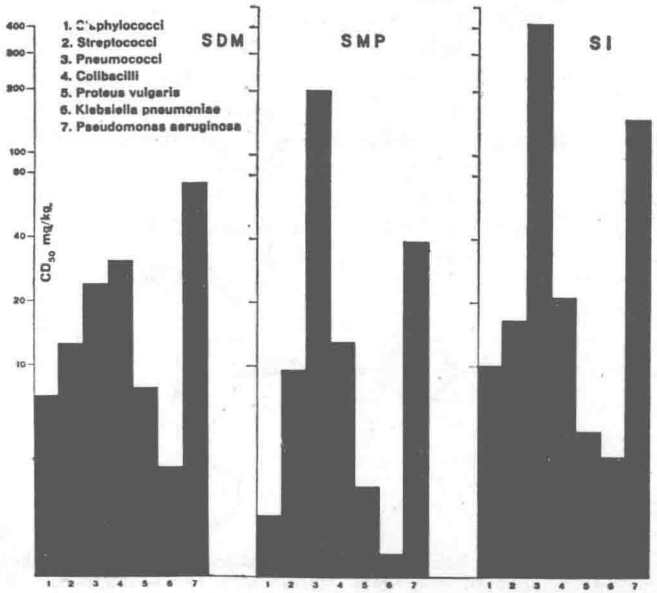

FIGURE 2. Equieffective doses of 3 sulfonamides against infections with 3 Gram-positive and 4 Gram-negative bacteria. Reproduced by permission from *Antibiotics and Chemotherapy*.[1]

One can readily recognize the generally higher sensitivity of the members of the enteric group of organisms and the lower response of pneumococci (most marked in SMP and SI) and pseudomonas.

In the previous publication on antibacterial sulfonamides by The New York Academy of Sciences, Domagk[10] pointed out that the evaluation of a chemotherapeutic agent should be based on the ratio of the tolerated to the effective dose. In other words, Ehrlich's principle of the chemotherapeutic index should be applied also to the sulfonamides, thus correlating the toxicological and the antibacterial aspects. Such ratios, namely, LD_{50}/CD_{50}, both based on oral drug administration in mice, are shown in TABLE 2.

The values of these ratios are influenced by the degree of toxicity, which appears in increasing order as SDM < SI < SMP = SP.

In agreement with the CD_{50} of the sulfonamides, the maxima of the ratios were in all instances obtained in experiments with *S. typhosa*; the minima were

found in the pneumococcal infection, with the exception of SDM, where it was derived from the pseudomonas experiment, although both SI and SP had a higher CD_{50} in this infection. Owing to its particularly low toxicity and high activity, SDM showed the highest ratios.

Notwithstanding the general similarity of the biological properties of the four sulfonamides compared here, it is possible to show that they belong in two distinct groups, as shown in TABLE 3.

The sulfonamides of longest half life have also identical average values of activity, and the same applies to the drugs with shorter persistence in the blood. The half life, however, seems not to be the only factor responsible for the anti-

TABLE 2

ACUTE ORAL TOXICITY IN MICE AND RATIOS LD_{50}/CD_{50} OF 4 SULFONAMIDES

Compound	LD_{50} mg./kg.	Ratio LD_{50}/CD_{50}	
		maximum	minimum
Sulfadimethoxine (SDM)	>16,000	>1481	>49
Sulfisoxazole (SI)	7,500	311	7
Sulfamethoxypyridazine (SMP)	2,500	410	7
Sulfaphenazole (SP)	2,500	46	4

TABLE 3

COMPARISON OF HALF LIFE, TOXICITY, AND ACTIVITY OF 4 SULFONAMIDES

Compound	Half life* (hours)	LD_{50} mg./kg.	Average CD_{50}† mg./kg.
Sulfadimethoxine	33‡	>16,000	114
Sulfamethoxypyridazine	44§	2,500	104
Sulfaphenazole	9–10‖	2,500	258
Sulfisoxazole	6–7§	7,500	245

* In blood and serum of humans after 2.0 gm. of drug.
† In 6 to 9 infections.
‡ According to E. Gans (personal communication).
§ Calculated (by Struller) from Finland *et al.*[12]
‖ Calculated (by Struller) from Rentchnick.[13]

bacterial activity, because the difference in the half lives of the two groups is fourfold to sevenfold, while the difference in average activity is approximately twofold. Moreover, the toxicity of the compounds introduces still another factor of differentiation, resulting in a different aspect of each of the four sulfonamides compared.

On the basis of half life, one might arrange the compounds in the order SMP > SDM > SP > SI; according to toxicity, SDM < SI < SMP = SP; whereas activity shows the grouping SDM = SMP > SI = SP.

Comparatively small differences of activity can be correlated, therefore, with marked differences of half life and toxicity, thus indicating that the degree of antibacterial effect is, as stated earlier,[8] an intrinsic property of the sulfonamides.

It should be pointed out here that we have discussed laboratory findings. Other considerations might enter into practical medical situations; properties of drugs that do not appear in the experiment in mice might determine their clinical selection, and the desired activity can always be obtained with different drugs by the adjustment of size of dose and frequency of administration.

We have used the expression antibacterial activity so often in the preceding parts of this paper that we believe we are justified in writing at least a few words about the type of activity that has been observed in our experiments. Although most of these experiments have been carried out only with sulfadimethoxine, no claim is made that the findings represent a specific property of this drug. We have some, though not extensive, experience that other sulfonamides act, if not in an identical manner, then at least similarly.

Schnitzer and DeLorenzo[11] have demonstrated that the activity of SDM in the *S. typhosa* infection of mice could not be antagonized by an excess of para-

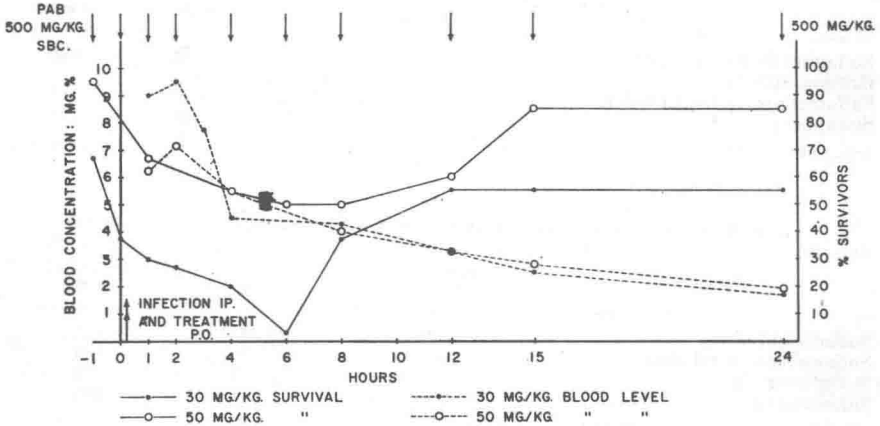

FIGURE 3. The effect of para-aminobenzoic acid (PAB) on the activity of SDM in *S. typhosa* infection of mice.

aminobenzoic acid (PAB) after 8 to 12 hours, although the drug concentration in the blood, and perhaps also the tissues, was declining at that time; this is shown in FIGURE 3.

Ten groups of mice uniformly infected with 1000 MLD of *S. typhosa* P58a and treated once with SDM 30 mg./kg. or 50 mg./kg. orally received a subcutaneous injection of 500 mg. PAB at 2-hour intervals. The survival rate of the different groups of animals after the usual 20-day observation time shows that maximal inhibition occurred at the 6-hour interval when the blood level was still comparatively high, but that after 8 to 15 hours the antagonistic effect decreased to an insignificant level. This finding was interpreted by the assumption that at this time the drug effect on the bacterium had become irreversible.

It was possible to demonstrate that the higher dose of SDM, namely, 50 mg./kg., indeed produced an irreversible damage to the infective organisms.

Such an experiment is presented in FIGURE 4. An intra-abdominal infection with 1000 MLD of *S. typhosa* P58a was treated once with 50 or 200 mg./kg.