Brit. J. Pharmacol. (1947), **2**, 149

2-*p*-AMINOBENZENESULPHONAMIDO-4 : 6-DIMETHOXYPYRIMIDINE : EXPERIMENTAL EVALUATION

BY

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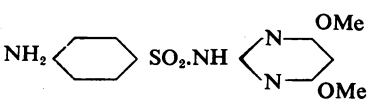
(Received January 16, 1947)

The introduction of an α-pyridyl residue into the molecule of sulphanilamide by Ewins and Phillips (1937) was a major advance in the development of sulphanilamide therapy, since not only was there produced a marked improvement in intrinsic antibacterial activity, but the new substance, sulphapyridine, was the starting point for an extensive research on the preparation of sulphanilamide drugs derived from other heterocyclic systems. The pyrimidine ring system has been particularly fruitful and at least three drugs derived from this nucleus are in common use: sulphadiazine, sulphamerazine, and sulphamezathine. They are characterized by antibacterial activity of a high order against a wide range of organisms, by rapid and efficient absorption from the gastrointestinal tract, and by a degree of persistence in the blood which provides economy in use. The work of Bell and Roblin (1942) suggests that the discovery of sulphanilamide drugs possessing a higher intrinsic antibacterial activity than that exhibited by the sulphapyrimidine group is becoming more remote. Any improvement in sulphanilamide therapy must therefore arise from research devoted to the production of drugs exhibiting reduced toxicity and enhanced persistence in the blood. We have interested ourselves for some time particularly in the latter aspect, and have found this property to be outstanding in sulphanilamides derived from 2-amino-4 : 6-dialkoxypyrimidines. A series of such compounds has been prepared (Rose and Tuey, 1946) and we propose to publish in due course detailed pharmacological and bacteriological findings on these substances as a class. This memoir is concerned with the parent compound 2-*p*-aminobenzenesulphonamido-4 : 6-dimethoxypyrimidine (sulphadimethoxypyrimidine), which is the most effective of the many homologues which have been examined.



While this paper was in preparation our attention was drawn to the researches of van Dyke, Tupikova, Chow, and Walker (1945), who, in the course of an

extensive study of sulphapyrimidines, have examined some of the 4:6-dialkoxy derivatives with which we have been concerned. In the main, these authors confirm our findings.

We describe the pharmacology and *in vitro* antibacterial properties of sulphadimethoxypyrimidine, the combination of these factors in therapeutic activity, the toxicity and the physico-chemical properties. A note on the unusual properties of the acetyl derivative of sulphadimethoxypyrimidine is included.

The clinical activity and pharmacology of the drug are now being investigated at Crumpsall Hospital, Manchester, and will be reported later by the workers concerned.

PHARMACOLOGY

The blood concentrations attained by sulphadimethoxypyrimidine have been examined in the mouse, rat, rabbit, dog, chick, sheep and calf. For data on the last two species we are indebted to Mr. J. Francis, of our Veterinary Research Laboratories.

In the mouse.—Absorption was examined by the standard technique already described (Rose and Spinks, 1946). Three mice received 250 mg./kg. by stomach tube as a 1 g./100 ml. solution of the sodium salt, and the drug was estimated at intervals in pooled tail blood by the method of Rose and Bevan (1944). In a series of 14 analyses of 0.05 ml. samples of blood containing 2 to 20 mg./100 ml., the mean recovery was 98.5 per cent ±standard deviation 6.95 per cent; limit of error for a probability level of 0.05, ±4.00 per cent.

The single experiment was repeated 22 times, so that 66 animals contributed to the mean results (Table I); in Fig. 1 the mean absorption curve is compared with that of sulphamerazine.

TABLE I

BLOOD CONCENTRATIONS OF SULPHADIMETHOXYPYRIMIDINE

250 mg./kg. in groups of 3 mice

Route of administration	Mean blood concentrations in mg./100 ml. after								
	20 min.	40 min.	1 hr.	1.5 hr.	2.5 hr.	3.5 hr.	5 hr.	7 hr.	24 hr.
Oral .. .. (22 expts.)	11.6	12.7	13.2	14.1	13.8	14.4	14.3	14.0	9.4
Intraperitoneal .. (13 expts.)	17.7	21.5	21.1	20.2	20.4	19.3	19.1	17.0	9.4
Subcutaneous .. (6 expts.)	14.1	19.1	18.8	19.9	17.0	20.6	20.3	18.6	7.7

In an earlier paper (Rose and Spinks, 1946) the expression C7, which is the time required for the blood concentration to fall from that attained 7 hours after dosing to two-thirds of this figure, was suggested as a convenient means of defining the rate of disappearance of a sulphonamide from the blood. Sulphadimethoxypyrimidine is clearly more persistent than sulphamerazine, the values

of C7 taken from the mean curves being 16.2 and 7.3 hours respectively. By obtaining the characteristic values from individual curves (Rose and Spinks, 1946) and submitting the results to statistical analysis, the difference is readily shown to be decisive ($P<0.01$). Sulphadimethoxypyrimidine is thus 2.2 times as persistent as sulphamerazine in mice, the term persistence being taken to mean the retention of free drug in the blood. The new drug reaches fairly high concentrations; the mean of the maximum concentrations from individual curves is 15.3 ± 1.25 mg./100 ml., which is higher than the corresponding figure for

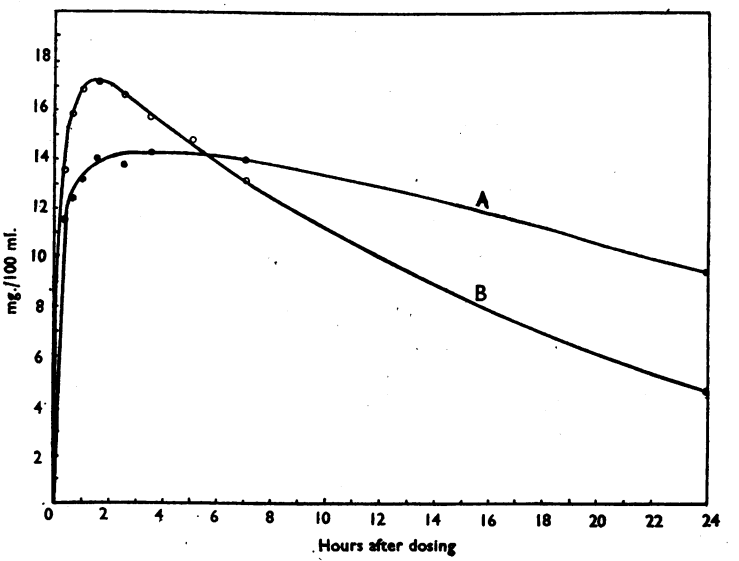


FIG. 1.—Blood concentrations in mice of sulphadimethoxypyrimidine (A) and sulphamerazine (B), following the administration of 250 mg./kg. orally.

sulphanilamide, sulphapyridine, or sulphathiazole, although lower than that for sulphamezathine, sulphamerazine, or sulphadiazine. Sulphadimethoxypyrimidine is rather slowly absorbed, the time at which the maximum blood concentration is attained, taken from the mean curve, being 220 minutes. Using the method of statistical analysis already described (Rose and Spinks, 1946), the drug can be shown to be significantly more slowly absorbed than any of the other three sulphapyrimidines.

The blood concentrations attained by the drug following intraperitoneal and subcutaneous administration of 250 mg./kg. to mice are shown in Table I. From these it must be concluded that the drug is more rapidly and more completely absorbed by these routes than when given orally. Persistence in the blood is again marked, indeed there is no significant difference in this respect between the intraperitoneal and oral routes. The mean maximum blood concentration

of individual intraperitoneal curves (20.7 mg./100 ml.) is decisively higher than that of individual oral curves (15.3 mg./100 ml.), and the difference between the mean times of attaining these maximum values is also decisive. Owing to the difficulty of drawing some of the individual subcutaneous curves, no statistical comparison has been attempted in this case.

Sulphadimethoxypyrimidine can be detected in the blood of mice for several days after the administration of a single oral dose of 250 mg./kg. Blood concentrations recorded in a typical experiment with three mice were:

Time (hr.)	2	22	27	42	46	66	71	73	89	139
mg./100 ml.	13.2	13.85	13.4	5.0	5.3	4.0	4.2	1.2	0.25	0.14

The maintenance of high concentrations for so extended a time suggested that repeated dosing might have a cumulative effect, giving dangerously high concentrations. This possibility was examined by administering two oral doses of 250 mg./kg. at an interval of

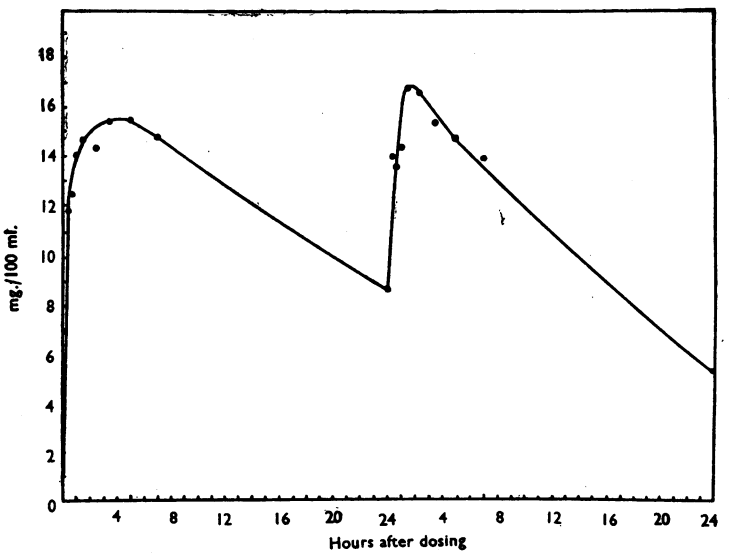


FIG. 2.—Blood concentrations of sulphadimethoxypyrimidine following the administration to mice of two oral doses of 250 mg./kg. at an interval of 24 hours.

24 hours to a group of three mice, and estimating the concentration of free drug in the blood at suitable intervals after each dose. The experiment was repeated ten times, with the results shown in Fig. 2. Clearly there is no marked rise in maximum blood level on such repeated dosing. This has been confirmed by statistical treatment, which shows that there is no significant difference between the mean maximum concentrations, but there are decisive differences between the mean times at which these maxima were attained and between the respective rates of disappearance of the drug from the blood stream. We have no explanation to offer for the greater rapidity with which the second dose is absorbed, but it may be that this phenomenon is associated in some way with the lower persistence.

Tissue concentrations in mice have been estimated following the usual dose of 250 mg./kg., three mice being used for each point recorded in Table II. In Table III the corresponding values for sulphamezathine are given for comparison; these have not been previously recorded. With both drugs, the concentrations in tissues approximated to those in blood in magnitude and persistence. Figures showing tissue distribution in the nephrectomized cat are given in the following section.

TABLE II

TISSUE CONCENTRATIONS OF SULPHADIMETHOXYPYRIMIDINE

250 mg./kg. orally in groups of 3 mice

Tissue	Concentrations in mg./100 g. of wet tissue after								
	30 min.	2 hrs.	4 hrs.	6½ hrs.	19 hrs.	31 hrs.	48 hrs.	72 hrs.	96 hrs.
Lung	14.5	16.9	19.6	17.6	16.3	10.1	2.6	3.8	2.1
Liver	15.1	13.6	12.9	15.0	14.6	4.0	2.7	2.5	1.1
Kidney	14.1	12.9	12.2	7.9	8.9	6.7	2.7	2.6	0.5
Spleen	7.0	6.8	9.5	9.5	10.8	5.5	1.6	0.5	0.6
Fat..		9.8							

The urinary excretion of the drug in the mouse has been examined in two groups of three animals. Following the administration of 250 mg./kg. orally, 46 and 32 per cent of the administered drug was excreted over three days in the two groups, and of these amounts 39 and 44 per cent respectively were acetylated. These results confirm the conclusion drawn from estimations of blood concentration after intraperitoneal injection, namely, that the drug is poorly absorbed in the mouse. The observation that, although much more persistent than sulphamezathine or sulphamerazine, sulphadimethoxypyrimidine gives lower blood concentrations than either of these compounds, can be explained on the same basis.

Experiments to determine the extent of conjugation of the new drug in the blood stream of the mouse are described below in the section dealing with acetylsulphadimethoxypyrimidine.

TABLE III

TISSUE CONCENTRATIONS OF SULPHAMEZATHINE

250 mg./kg. orally in groups of 3 mice

Tissue	Concentrations in mg./100 g. of wet tissue after				
	30 min.	2 hrs.	4 hrs.	7 hrs.	24 hrs.
Lung	21.8	17.1	12.3	6.5	2.3
Liver	18.0	18.8	10.7	3.1	3.0
Kidney	19.9	20.8	17.3	13.2	1.6
Spleen	14.8	11.1	6.3	4.2	1.4
Fat	10.7	6.6	7.1	5.3	1.9

In species other than the mouse.—The blood concentration-time curves presented have in each case been chosen as typical from at least three available in the particular species. The drug was given orally as a solution of the sodium salt in the following amounts: dog, calf, sheep, 100 mg./kg.; rabbit, 150 mg./kg.; rat, chick, 200 mg./kg. The

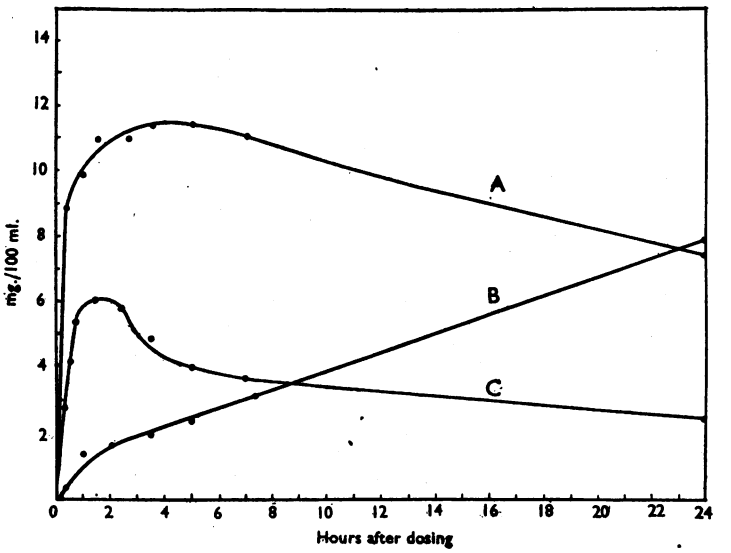


FIG. 3.—Blood concentrations of sulphadimethoxypyrimidine following the oral administration of 200 mg./kg. to the rat (A) and chick (B) and of 150 mg./kg. to the rabbit (C).

resultant blood concentrations are given in Figs. 3 and 4. In the rabbit, the urinary excretion of the drug has been measured, and the amounts appearing as free amine and in conjugated form estimated (Table IV). Rabbit A, which received 150 mg./kg. of the drug, excreted approximately 80 per cent in the urine, of which some 47 per cent was conjugated.

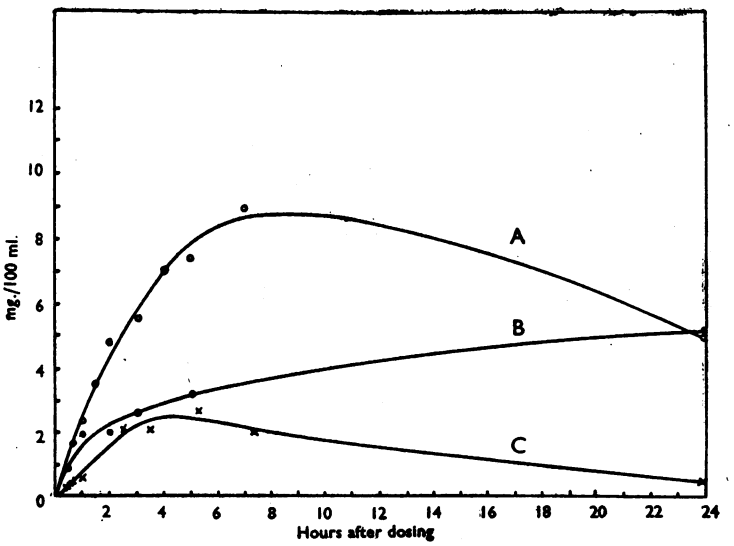


FIG. 4.—Blood concentrations of sulphadimethoxypyrimidine following the oral administration of 100 mg./kg. to sheep (A), calf (B) and dog (C).

TABLE IV

EXCRETION OF FREE AND CONJUGATED SULPHADIMETHOXYPYRIMIDINE IN THE URINE OF TWO RABBITS

Rabbit A (1.2 kg.; dose 180 mg.)					Rabbit B (2.17 kg.; dose 217 mg.)				
Day	Free drug		Total drug		Day	Free drug		Total drug	
	mg.	% of dose	mg.	% of dose		mg.	% of dose	mg.	% of dose
1	62.7	34.8	120.3	66.8	1	35.5	16.3	134.9	62.1
2	5.7	3.2	13.8	7.7	2	5.4	2.5	14.8	6.8
3	5.8	3.2	6.4	3.6	3	7.6	3.5	13.7	6.3
4 + 5	3.1	1.7	5.9	3.3	4 + 5	2.2	1.0	4.3	2.0
Total	77.3	42.9	146.4	81.4	Total	50.7	23.3	167.7	77.2

The proportion of total drug excreted by rabbit B, which was given a smaller dose of 100 mg./kg., was similar, but the percentage conjugated was much higher (70 per cent). Despite the high recovery, indicative of efficient absorption from the gastro-intestinal tract, the maximum blood concentrations attained in the rabbit were appreciably lower than those reached in the rat and mouse although a similarly high degree of persistence was observed. The expression C7, obtained from Fig. 3, had a value of 14 hours for the rabbit, as against 17 hours for the rat and 16.5 hours for the mouse. In the sheep, calf, and chick the drug was slowly absorbed but persisted well, particularly in the last two. Absorption was poor in the dog and removal from the blood stream rapid (C7=2 hours). Conjugation in rabbit and sheep is described in the section dealing with acetylsulphadimethoxypyrimidine.

The tissue distribution of the drug in nephrectomized cats has been estimated using the technique of Fisher, Troast, Waterhouse, and Shannon (1943). The results recorded in Table V are the means of three experiments and show the tissue/plasma ratio; they are compared with the figures obtained by Fisher *et al.* for sulphamerazine and sulphadiazine.

TABLE V

DISTRIBUTION OF SULPHADIMETHOXYPYRIMIDINE IN THE BILATERALLY NEPHRECTOMIZED CAT

Drug	Tissue/plasma concentration ratios							Vol. of distribution (as % of body weight)
	C.S.F.	Brain	R.B.C.	Lung	'Liver	Pancreas	Muscle	
Sulphadimethoxypyrimidine	0.23	0.23	0.12	0.40	0.68	0.35	0.24	39
Sulphamerazine*	0.38	0.35	0.45	0.56	0.76	0.47	0.39	45.8
Sulphamezathine*	0.31	0.21	0.53	0.60	0.63	0.44	0.45	82.5

*Data of Fisher *et al.* (1943)

It will be seen that sulphadimethoxypyrimidine diffuses rather less into most tissues than the other sulphapyrimidines, and markedly less into the red blood cells; this may possibly be connected with the high protein binding of the drug (*vide infra*).

Antibacterial action in vitro

Comparisons of the antibacterial activities of sulphadimethoxypyrimidine, sulphamezathine and sulphadiazine were made by the method of Harper and Cawston

(1945). The medium used was Wright's broth containing 10 per cent (v/v) of lysed horse blood. Serial two-fold dilutions of the various sulphonamides in this medium were placed in 3 in. × ½ in. tubes in 0.5 ml. amounts, and 0.5 ml. of a 0.2×10^{-6} dilution in plain broth of a 24-hour culture of *Streptococcus pyogenes*, Kruger strain, was added to each tube. (This inoculum gave a count of approximately 5×10^{8} colonies per ml. on blood-agar.) The final

TABLE VI

COMPARATIVE ANTIBACTERIAL ACTIVITIES. RESULTS OF 48-HOUR PLATING ON BLOOD-AGAR

Organism: *Streptococcus pyogenes*

Sulphonamide	Concentration—one part in				Control
	40,000	80,000	160,000	320,000	
Sulphadimethoxypyrimidine	−	−	+	+	+
Sulphamezathine	−	±	+	+	
Sulphadiazine	−	−	±	+	

medium thus contained 5 per cent of lysed horse blood and the concentration of the sulphonamides ranged from 1 : 40,000 to 1 : 320,000. All tubes were incubated at 37° C. for 48 hours. One loopful from each tube was removed and plated upon blood-agar. The end-points were quite sharp and were recorded as − (no growth), ± (partial growth), and + (growth equal to control). The results are shown in Table VI. It will be seen that sulphadimethoxypyrimidine was intermediate in activity between the other two compounds.

Acute toxicity

When a suspension of sulphadimethoxypyrimidine was given orally to a group of 6 mice at the rate of 10 g./kg. none died. The intravenous injection of solutions of the sodium salts of sulphamezathine and of sulphadimethoxypyrimidine gave the results shown in Table VII, which is a summary of two experiments. All doses were contained in a volume of 0.2 ml., and each injection took 1 min. to complete.

TABLE VII

THE TOXICITY OF SULPHAMEZATHINE AND SULPHADIMETHOXYPYRIMIDINE BY INTRAVENOUS INJECTION OF THEIR SODIUM SALTS

Sixteen mice in each group. All mice observed for 5 days

Dose	Sulphamezathine	Sulphadimethoxy-pyrimidine
1.0 g./kg.	16 died	16 died
0.9 „ „	16 „	13 „
0.8 „ „	13 „	12 „
0.7 „ „	2 „	10 „
0.6 „ „	2 „	4 „
0.5 „ „	0 „	0 „

Chronic toxicity

Two separate experiments were carried out in which sulphadimethoxypyrimidine was administered as an aqueous dispersion once a day to rats for 28 days, at the rate of 1 g. per kg. body weight per day. The total number of rats in the two experiments was 54.

At the end of the period of administration of the drug the average final body weight of the treated animals was 130 per cent of their initial weight (from 110 g. to 143 g.), whilst the corresponding figure for equal-sized groups of matched control rats was 150 per cent.

Thirteen of the treated animals died during the period of treatment, or were killed because they were losing weight and were obviously ill, usually about 14 days from the start of the experiment. In several of these rats death was undoubtedly hastened by septic broncho-pneumonia brought on by maladministration of the doses of drug, but there were other deaths among rats whose lungs appeared normal. In the majority of the latter death could be ascribed to severe central necrosis of the liver lobules, sometimes affecting the whole lobule. It should be noted that the livers of 36 of the rats showed no abnormalities. Anaemia was very marked in many of the survivors, 5 of them giving readings of 29–46 per cent haemoglobin by the Sahli method. The spleens of many of the animals showed evidence of increased destruction of red blood corpuscles.

Although damage to the kidneys had been expected on account of the low solubility of this compound, such damage was seen in only 7 of the 54 animals examined. In only one of these was it severe, consisting of massive destruction of many convoluted tubules, amounting often to complete disintegration of the cell, with loss of nuclear staining and desquamation of the cells into the lumen of the tubule.

Therapeutic activity

Therapeutic experiments were carried out in mice infected with either *Streptococcus pyogenes*, Kruger strain (Group A), *Streptococcus pneumoniae* Type I, or *Staphylococcus aureus*. The first two organisms were given intraperitoneally and the third intravenously. All drugs were administered by mouth as aqueous solutions or dispersions, the desired dose

TABLE VIII

THERAPEUTIC RESULTS IN GROUPS OF 12 MICE INFECTED WITH *Streptococcus pyogenes*, *Streptococcus pneumoniae* OR *Staphylococcus aureus*

Oral doses of 100 mg./kg. twice daily for 3 days

Drug	*Streptococcus pyogenes*		*Streptococcus pneumoniae*		*Staphylococcus aureus*	
	No. of deaths in 7 days	Mean survival time in days (max. 7)	No. of deaths in 7 days	Mean survival time in days (max. 7)	No. of deaths in 14 days	Mean survival time in days (max. 14)
None (controls)	12	0.8	12	0.8	12	1.6
Sulphanilamide	12	1.0	12	1.2	10	5.0
Sulphapyridine	11	1.7	12	2.8	9	6.1
Sulphathiazole	12	1.0	12	2.1	8	7.2
Sulphamezathine	12	2.3	10	3.6	5	9.4
Sulphadiazine	11	3.8	12	4.3	4	11.2
Sulphamerazine	12	4.0	—	—	—	—
Sulphadimethoxypyrimidine	11	4.3	8	5.6	5	10.7

Streptococcus pyogenes. Infecting dose 0.2 ml. of a 1 : 10^8 dilution of an 18-hour culture (freshly isolated from a mouse) in 5 per cent (v/v) blood-broth; given intraperitoneally.

Streptococcus pneumoniae. Infecting dose 0.2 ml. of a 1 : 10^8 dilution of an 18-hour culture (freshly isolated from a mouse) in 5 per cent (v/v) blood-broth; given intraperitoneally.

Staphylococcus aureus. Infecting dose 0.2 ml. of a 1 : 2 dilution of a plain broth culture, 18 hours old; given intravenously.

being contained in a volume of 0.5 ml. The drugs were administered shortly before the infection and further doses were given 7, 24, 31, 48, 55, 72, and 79 hours after the infection. The dose chosen for all the drugs in the first experiment (100 mg./kg.) was selected because experience had shown that, with streptococcal and pneumococcal infections, it permitted the various compounds to be ranged in order of activity on the basis of mean survival times. Table VIII summarizes the results of these experiments.

Taking all the experiments together, it will be seen that the increasing order of effectiveness of these compounds is approximately that in which they are arranged in the table.

In order to simulate more closely the conditions under which these drugs are used in clinical practice, another experiment was carried out in which infection of the mice preceded drug treatment by seven hours; in this experiment the mice were infected intraperitoneally with streptococci. The results are shown in Table IX.

The results of this experiment range the drugs in substantially the same order as before. In both experiments sulphadimethoxypyrimidine compares very favourably with any of the other drugs.

TABLE IX

THERAPEUTIC RESULTS IN GROUPS OF 12 MICE INFECTED WITH *Streptococcus pyogenes*

Infected at 10 a.m. and treated at 5 p.m. of the same day with 500 mg. drug/kg. No further doses given

Drug	No. of deaths in group	Mean survival time in days (maximum 7)
None (controls)	12	0.8
Sulphanilamide	12	0.8
Sulphapyridine	12	1.5
Sulphathiazole	12	1.0
Sulphamezathine	12	1.5
Sulphadiazine	12	2.3
Sulphamerazine..	12	2.5
Sulphadimethoxypyrimidine ..	12	2.8

The infecting dose was 0.2 ml. of a 1: 10^6 dilution of a culture of *Streptococcus pyogenes* prepared as in Table VIII.

Acetylsulphadimethoxypyrimidine

Estimation and occurrence.—As indicated above, sulphadimethoxypyrimidine appeared in a conjugated form in the urine of experimental animals. Since hydrolysis gave a diazotizable amine, the conjugated product was assumed to be the acetyl derivative and all estimations were made against this compound as standard. The method of Rose and Bevan (1944) proved satisfactory for the estimation of acetylsulphadimethoxypyrimidine in urine, but it yielded low recoveries when known amounts of the compound were added to blood and tissue homogenisates. Variable recoveries of the order of 60 per cent were obtained from human blood and 30 per cent or less from mouse tissues. The recovery from blood was increased to 70 per cent by hydrolysis of the conjugated drug in whole blood before the precipitation of proteins. This could not be regarded as satisfactory, and further work

showed that adequate recoveries were obtained from blood and tissue by diluting to 1 : 150 before precipitating protein. A suitable aliquot was then taken, hydrolysed with dilute hydrochloric acid, diazotized and coupled with N-β-sulphatoethyl-*m*-toluidine. A coupling time of 30 min. was required ; this is greater than that of the parent compound and probably indicates breakdown of the pyrimidine ring (cf. Frisk, 1943).

Estimations of free and total drug in the blood of mice receiving 250 mg. of sulphadimethoxypyrimidine/kg. orally, indicated the presence of traces only of conjugated amine. In the rabbit, on the other hand, high concentrations of conjugated drug were reached (Fig. 5). Similar results were obtained in the sheep, it being clear in both species that the acetyl derivative is similar in persistence to the free drug.

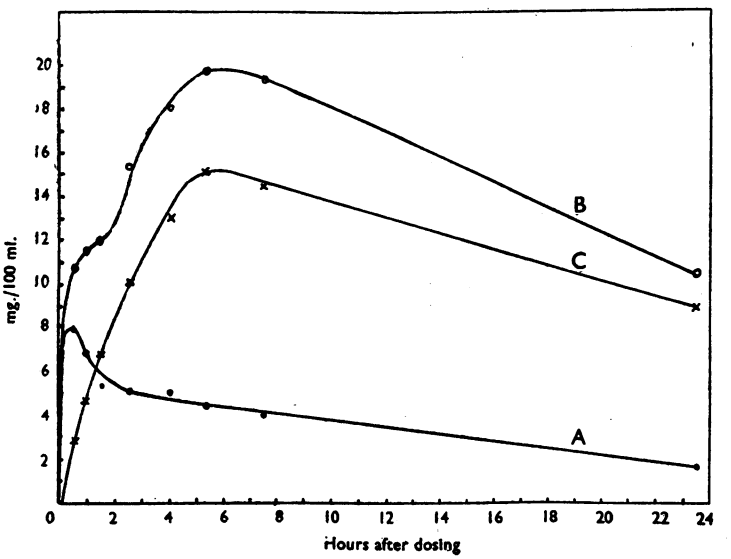


FIG. 5.—Blood concentrations of free (A), total (B), and conjugated (C, by difference) sulphadimethoxypyrimidine in the rabbit following the oral administration of 250 mg./kg. orally.

The administration of acetylsulphadimethoxypyrimidine was examined in the mouse. Four groups of three mice received 250 mg./kg. orally as a 1 per cent solution of the sodium salt. The mean blood concentration-time curves of free and total drug are shown in Fig. 6. That the observed hydrolysis of the acetyl derivative proceeds systemically and not in the lumen of the gut was readily shown by administering it intraperitoneally, when curves very similar to those of Fig. 6 were obtained. This facile hydrolysis of acetylsulphadimethoxypyrimidine undoubtedly accounts for its high activity, observed in the therapeutic experiments described below. In experiments with acetylsulphanilamide we found only traces of free drug in the blood following its administration in doses of 250 mg./kg., a result in agreement with its inactivity in therapeutic tests.

Therapeutic activity.—Only activity against *Streptococcus pyogenes* in mice has been examined in this instance, the infecting inoculum being similar to that used in the experiments recorded in Table VIII. The dosage regime was slightly altered in that amounts of 250 mg./kg. mouse (as against 100 mg./kg. mouse of the free amine) were given twice daily for three days, beginning shortly before infection. Acetyl derivatives of other sulphonamide drugs were included for comparison. Two series of experiments were made: in one

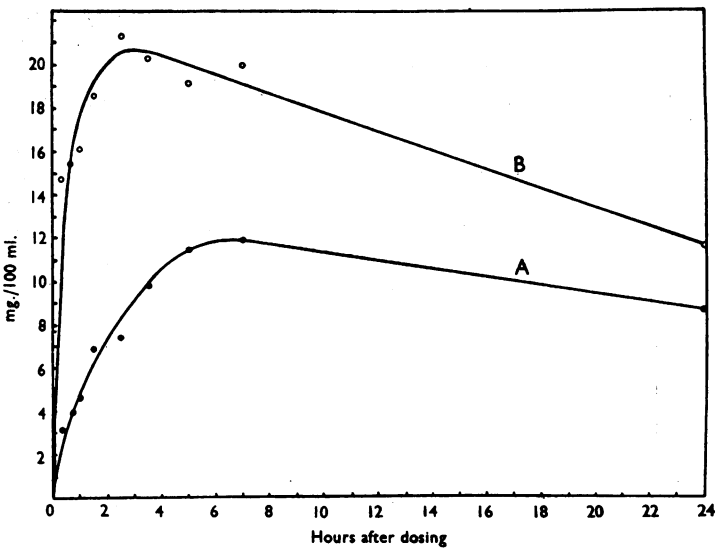


FIG. 6.—Blood concentrations of free (A) and total (B) sulphadimethoxypyrimidine following the oral administration in mice of 250 mg. of the acetyl derivative/kg.

the drugs were administered by mouth, and in the other intraperitoneally. The average survival times are indicated in Table X. Groups of 6 mice only were employed in each experiment.

Since the order of relative effectiveness of the acetyl derivatives is the same by both routes of administration, it appears that in all cases they are largely absorbed as such, and

TABLE X

THERAPEUTIC RESULTS OBTAINED IN GROUPS OF 6 MICE INFECTED WITH *Streptococcus pyogenes* 250 mg./kg. twice daily for 3 days

Drug	Oral		Intraperitoneal	
	No. of deaths in 7 days	Mean survival time (max. 7)	No. of deaths in 7 days	Mean survival time (max. 7)
None (controls)	6	0.8	6	0.8
Acetylsulphanilamide ..	6	0.9	6	0.8
Acetylsulphapyridine ..	6	0.9	6	0.8
Acetylsulphadiazine	6	1.1	6	1.0
Acetylsulphathiazole ..	4	3.2	6	2.3
Acetylsulphamezathine ..	6	1.5	6	1.0
Acetylsulphamerazine ..	6	0.8	—	—
Acetylsulphadimethoxypyrimidine	6	5.0	4	5.0

The infecting dose was 0.2 ml. of a 1 : 10^4 dilution of a broth culture of *Streptococcus pyogenes* prepared as in Table VIII.

absorption is not preceded by extensive hydrolysis to the free amines in the gastro-intestinal tract. This accords with the view expressed above in the case of acetylsulphadimethoxypyrimidine following direct estimation of blood concentrations of the free amine.

Physico-chemical properties

Solubility.—The solubility determinations were made in water at 37° C. by the method of Rose, Martin, and Bevan (1943), increasing *p*H by the addition of sodium hydroxide solution. The concentration of drug in a sample withdrawn through a filter plug into a pipette was determined colorimetrically. With the acetyl derivative, de-acetylation was necessary before the colorimetric estimation, which depended upon a diazo reaction, could be made. The solubility curves so obtained are shown in Fig. 7. In the *p*H range 6.0–7.0, the solubility of the free amine closely resembles that of sulphadiazine (Rose, Martin, and Bevan, 1943); thereafter the curve rises more steeply. Acetylsulphadimethoxypyrimidine differs from acetylsulphadiazine, however, in that it is less soluble than the parent amine in the *p*H range 6.0–7.3, but above the latter limit the acetyl derivative exhibits the greater solubility.

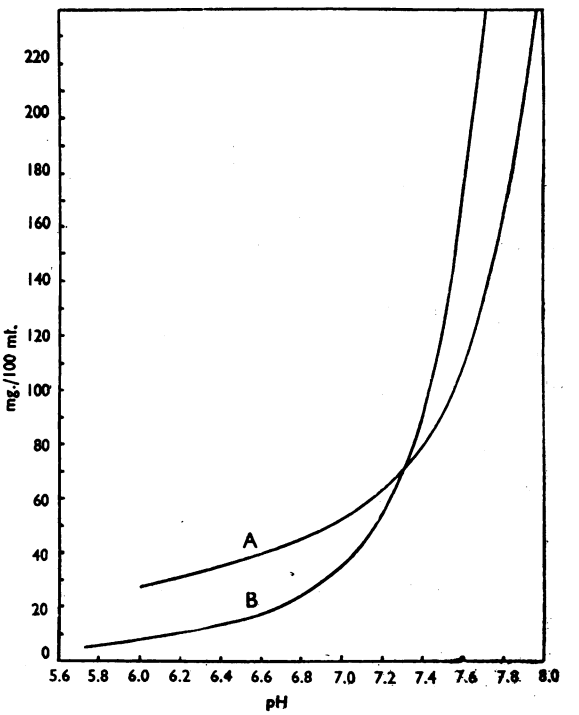


FIG. 7.—Solubility in water of sulphadimethoxypyrimidine (A) and of the acetyl derivative (B).

The acid dissociation constant.—The acid dissociation constant has been measured by potentiometric titration of the saturated aqueous solution and

TABLE XI

PROTEIN BINDING OF SULPHADIMETHOXYPYRIMIDINE

Plasma or serum	Percentage of drug bound to protein	
	Ultrafiltration	Dialysis
Rat plasma	85	82
Rat serum	87	80
Human plasma	—	80
Mouse plasma	—	78
Cat plasma	79	—
Sheep serum	63	—
Rabbit serum	71.5	—
Horse serum	63	—

the pK_a value found to be 7.00. A solvent partition method gave the value 7.02. The pK_a values for other sulphanilamide derivatives of pyrimidine are known—e.g., sulphadiazine 6.48; sulphamerazine 7.06; sulphamezathine 7.37 (Bell and Roblin, 1942).

Protein binding.—The association of the drug with blood proteins has been estimated by the equilibrium dialysis method of Davis (1943) and by ultrafiltration through collodion. Table XI shows the results obtained with various species.

It appears that sulphadimethoxypyrimidine is among the more highly bound sulphonamides, though a precise comparison is not possible, owing to the wide variation in values quoted for other drugs in the literature. The results are of the same order as the figure of 78 per cent quoted by van Dyke *et al.* (1945) for the same compound.

Summary and Conclusions

1. The properties of 2-*p*-aminobenzenesulphonamido-4:6-dimethoxypyrimidine (sulphadimethoxypyrimidine) are described, and include water solubility data for the free amine and the acetyl derivative over a range of *p*H.

2. The drug is relatively non-toxic in mice and rats, rather more slowly absorbed than sulphadiazine, sulphamerazine, or sulphamezathine when given orally to mice, but markedly more persistent in the blood-stream than these three drugs. Absorption data are given for other experimental animals.

3. A high percentage of the absorbed drug is excreted by the mouse in conjugated form; but feeding the acetyl derivative (or injecting intraperitoneally) gives rise, after a few hours, to a concentration of the free amine in the blood almost equal to that attained by initial administration in the latter form.

4. The antibacterial activity *in vitro* of the drug against *Streptococcus pyogenes* is intermediate between that of sulphamezathine and sulphadiazine, but therapeutic activity against this organism in the mouse is, in general, at least equal to or slightly better than that shown by any of the other sulphapyrimidine derivatives, in conformity with the high persistence of the drug.

5. Acetylsulphadimethoxypyrimidine given orally or intraperitoneally to infected mice exerts an appreciable therapeutic effect, greater than that shown by the acetyl derivatives of the several other heterocyclic sulphonamides examined.

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