Brit. J. Pharmacol. (1950), **5**, 565.

ANTIBACTERIAL ACTION AND METABOLISM OF FIVE SULPHONES

BY

J. FRANCIS AND A. SPINKS

From the Biological and Research Laboratories, Imperial Chemical Industries Limited Hexagon House, Manchester, 9

(Received July 25, 1950)

The therapeutic action of 4:4'-diaminodiphenylsulphone (DDS) was demonstrated by Buttle, Stephenson, Smith, and Foster (1937), and Fourneau, Tréfouël, Nitti, and Bovet (1937). Bauer and Rosenthal (1938) showed that it was thirty times as active as sulphanilamide against streptococci in mice and fifteen times as toxic; that is, its therapeutic ratio was twice as good as that of sulphanilamide. Despite this, DDS was considered to be too toxic for use in man, apparently because it could not be given in the same doses as the sulphonamides. As far as one can tell, no trials were undertaken to assess the effects of doses that were tolerated.

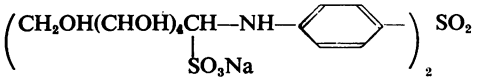
However, McEwen, Pizer, and Paterson (1941) introduced DDS into veterinary medicine. Francis (1947a) and Francis, Peters, and Davies (1947) showed that it was more potent than any of the sulphonamides against *Str. agalactiae in vitro*, in the chick-embryo, and in the mouse (at equal blood concentrations), but that it was less active than the heterocyclic sulphonamides against Gram-negative organisms. DDS was more persistent and produced better blood-concentration curve areas than the sulphonamides in all domestic animals except the pig, in which its place was taken by sulphamezathine (Francis, 1949b). When 10 g. suspended in liquid paraffin were injected into each quarter of non-lactating cows, blood concentrations above 2 mg./100 ml. were present for five days or more, and high " milk " concentrations for much longer periods (Francis, 1947b). This dose cured 79.4 per cent of 63 non-lactating quarters infected with *Str. agalactiae*, and when combined with 50,000 units of penicillin the single injection cured 96.3 per cent of 54 quarters (Francis, 1949a).

Because DDS itself was believed to be too toxic for use in man, attempts were made to develop less toxic derivatives, and promin was the first substance shown to have a striking action against experimental tuberculosis and against human leprosy. It is natural, therefore, that it should have attracted great attention and attempts should have been made to synthesize similar derivatives. This hardly excuses the extraordinary confusion which has existed in the English and American literature concerning these substances. It seems to have been assumed *a priori* that the higher the dose of a compound which could be given the better that compound would be. Promin has been given intravenously over long periods because it is " less toxic " by that route than when given orally. Thus Hinshaw and Feldman (1941) pointed out that, although only 1.2 to 3.2 g. of promin could be given daily by mouth and often produced cyanosis, 16 g. could be given parenterally without producing

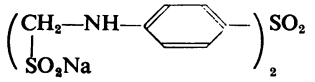
cyanosis. The work of Johnson (1940) provided circumstantial evidence that promin was broken down in the intestinal tract, and Smith, Emmart, and Westfall (1942) showed that it was a tenth as active as DDS in culture medium. Because of this circumstantial evidence that promin was converted to DDS before being absorbed from the intestine, and our own early experimental evidence that the therapeutic ratio of DDS was just as good as that of any of its soluble derivatives, we decided to study its action in rat leprosy, and Dr. R. G. Cochrane, and later Dr. J. Lowe, studied its action in human leprosy. The work on rat leprosy will be published shortly (Francis, 1950) and the clinical results have already been recorded (Cochrane, Ramanujam, Paul, and Russell, 1949; Lowe and Smith, 1949; Lowe, 1950). It is evident that a dose of 200 to 300 mg. a day, or perhaps even less, is at least as effective as much larger doses of the complex sulphones.

It is the purpose of this paper to describe the concurrent experimental observations which have shown beyond reasonable doubt that the action of all the soluble derivatives is due to the liberation of DDS. The derivatives studied are:

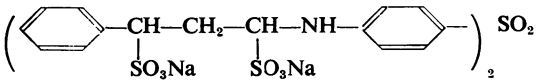
1. Promin



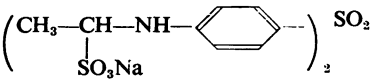
2. Diasone



3. Sulphetrone



4. 2196



The last compound, 2196, was developed by Rose (1942) in these laboratories. It was never tested clinically because of our belief that the soluble compounds had no real advantage over the parent sulphone. However, the present work has shown that its facile conversion to DDS in biological fluids might make it useful in special circumstances.

Experimental Section

The relative activity of various sulphone derivatives against streptococci

Our original assumption was that the antibacterial action of the soluble derivatives was due to the liberation of DDS, and this is now known to be correct (see p. 575). It follows that their relative activity can be tested against any organism, and the results applied to any other organism, because the relative therapeutic response is only a measure of the amount of DDS liberated.

A series of preliminary experiments was carried out, in which various concentrations of drugs were added to food: mice were given this food for two days and then injected intraperitoneally with Lancefield Group A streptococci. A range of concentrations of each drug in the food was chosen which gave approximately the same survival times with different drugs. A series of three experiments was then

carried out using five mice per group in each experiment. The average survival time in each group in each experiment is shown in Table I.

TABLE I

Average survival time in hours* of mice which were fed on food containing various percentages of drugs. Feeding was begun 2 days before injecting the mice with 0.2 ml. of a 1/100 dilution of a 24-hour culture of *Str. pyogenes* (Lancefield Group A)

	4:4'-diaminodiphenylsulphone				2196			
Percentage of drug in food	0.05	0.025	0.0125	0.00625	0.1	0.05	0.025	0.0125
Survival time ..	101.47	92.66	42.13	22.0	98.13	80.0	42.53	22.8
	Promin				Diasone			
Percentage of drug in food	0.4	0.2	0.1	0.05	0.1	0.05	0.025	0.0125
Survival time ..	116.1	77.83	70.1	26.8	61.46	44.8	18.88	17.2
	Sulphetrone							
Percentage of drug in food	1.6	0.8	0.4	0.2				
Survival time ..	62.0	31.6	18.88	18.0				

* The figures for each group represent the average results of three experiments in which there were 5 mice a group. The mice were observed morning and evening and the following survival times given to animals dead at the intervals shown below:

Days	1	2	3	4
Morning (hours)	14	38	62	86
Evening ,, ...	26	50	74	98

The average survival time of the control mice was 15.2 hours.

The detailed results were analysed by Dr. O. L. Davies, who found that the comparison between the drugs was consistent at the various doses. The relative concentrations of the drugs in the food which were required to give the same effect were as follows:

DDS	= 100		
2196	= 224	95% limits of error	171–292
Promin	= 621	,, ,, ,, ,,	470–819
Diasone	= 538	,, ,, ,, ,,	350–825
Sulphetrone	= 9,888	,, ,, ,, ,,	6,198–15,776

Sulphetrone (Brownlee, Green, and Woodbine, 1948) was by far the least potent. Diasone and promin were about one-fifth to one-sixth as potent as DDS but many times more potent than sulphetrone. No. 2196 was half as potent as DDS but significantly more potent than all the other compounds.

It was not easy to decide the exact implications of the foregoing results, because the extent to which the various derivatives were absorbed and broken down to DDS had not then been determined. Simple comparisons of the activity of the four drugs *in vitro* were, therefore, carried out.

A 1/500 solution of each of the soluble derivatives was prepared immediately before carrying out the test and sterilized by filtration. Falling threefold dilutions were made, and each was added to an equal volume of sterile separated milk containing 2 per cent glucose and a 1/1,000 dilution of a 24-hour serum broth culture of *Str. agalactiae*. Milk was used as a medium because Brownlee (1945) had shown it to be uniformly low in sulphonamide-inhibiting substances, and in our hands it has given regular results. After 18 hours' incubation the contents of the various tubes were streaked out on blood-agar plates which were read after a further 18 hours' incubation. The lowest dilution of a drug that permitted full growth was taken as the end-point; this criterion gave regular results, except that in one experiment all the end-points were higher than in the others (Table II).

TABLE II

In vitro ACTIVITY OF COMPOUNDS AGAINST *Str. agalactiae* "4" IN SEPARATED MILK CONTAINING 1% GLUCOSE

Falling threefold dilutions were used starting at 1/1,000: the figures given are the reciprocals of the dilution of drugs in the first tube in which there was full growth

Exp. No.	4:4′-diamino-diphenyl-sulphone	2196	Promin	Diasone	Sulphetrone
III/77	729,000	243,000	9,000	81,000	3,000
III/88	19,683,000	6,561,000	27,000	243,000	81,000
IV/2	2,181,000	729,000	3,000	27,000	3,000
IV/11	729,000	243,000	3,000	27,000	3,000
Logarithmic mean	2,187,000	729,000	6,473	58,240	6,473

It will be seen that under the conditions of these experiments sulphetrone and promin were about two hundred and fifty times less active than DDS, whereas diasone was twenty-seven, and 2196 only three, times less active. This test therefore placed the drugs in the same order of activity as the experiments in mice, but they were all considerably less active in relation to DDS than they were *in vivo*; this is consistent with the view that they act by breaking down to DDS because it is reasonable to assume that breakdown would be greater in the gut than under the conditions *in vitro*.

Some other workers have found the derivatives of DDS relatively more active, but this clearly depends on the care taken to prevent breakdown. More tests were carried out, similar to those already described, except that one series of dilutions was autoclaved before inoculation. The unautoclaved series of tubes gave practically the same results as those recorded above, but, after autoclaving, the activities of promin and sulphetrone were greatly increased: they were only nine and twenty-seven times respectively less active than DDS. It must be assumed that they formed DDS in considerable amount during the autoclaving, and this probably explains the high activity reported by some previous workers (cf. Brownlee, Green, and Woodbine, 1948).

The foregoing results showed that the soluble derivatives were less active, some much less active, than DDS, but it was necessary to develop direct methods of estimating free DDS in the presence of its derivatives in order to establish that the only active substance was DDS.

Estimation of free 4 : 4'-diaminodiphenylsulphone in blood in the presence of its soluble derivatives

At the time this work was begun soluble derivatives had been estimated only by treating the blood with an acid protein-precipitant, which also hydrolysed the soluble compounds partly or wholly to diazotizable amines (e.g., Brownlee, Green, and Woodbine, 1948). Such a method did not allow detection of the conversion of soluble compounds to the parent amine, DDS. We have been able to estimate DDS in the presence of the soluble derivatives by the following method.

Reagents

1. Benzene, AR.
2. 0.2 M-disodium hydrogen phosphate.
3. 0.1 N-hydrochloric acid.
4. 100 mg. sodium nitrite/100 ml.
5. 1 g. N-β-sulphatoethyl-*m*-toluidine/100 ml.
6. Redistilled ethanol.
7. Standard aqueous solution of DDS, 1 mg./100 ml.

Procedure

Pipette 1 ml. of blood into 2 ml. of 0.2 M-disodium hydrogen phosphate in a 30 ml. glass-stoppered bottle. Add 20 ml. of benzene and shake for 5 min. Allow the upper layer to separate, clarify it by filtration, and transfer an aliquot of 15 ml. to a dry bottle. Add 4 ml. of 0.1 N-hydrochloric acid and shake for 5 min. Transfer 3 ml. of the lower layer to a test-tube and add 0.5 ml. of the sodium nitrite solution. Mix, wait 5 min., and add 0.5 ml., of the N-β-sulphatoethyl-*m*-toluidine solution. Mix, wait 20 min., and add 3 ml. of redistilled ethanol. Mix, transfer to a 2 cm. spectrophotometer or colorimeter cell, and read the optical density (*a*) at 520 mμ against a blank. Prepare the blank and a standard at the same time as the unknown by substituting 1 ml. of water and 1 ml. of standard DDS solution for blood in the above procedure. Read the optical density (*b*) of the standard against the blank. Then the concentration of DDS in the blood is Fa/b mg./100 ml., where F is a recovery factor derived from experiments similar to those summarized in Table III. Normal blood gives no colour.

Notes

The graph of concentration against optical density was a straight line: a single standard was therefore adequate. The only other solvents we have examined were light petroleum (b.p. 100–120°) and chloroform, neither of which was as good as benzene. While this paper was being prepared for publication Titus and Bernstein (1949) described a similar method in which methyl*iso*butylketone was used as solvent. The information on which our selection of benzene was based is contained in Fig. 1, which shows the partition coefficient of DDS between 0.1 M-citrate-hydrochloric acid buffers of varying *p*H and benzene. The extraction of the standard involved a small loss of DDS during each of the two stages (cf. Fig. 1). The loss during the extraction with hydrochloric acid could be lowered almost to zero by increasing the concentration of the latter to 0.5 N, but the rate of coupling of tetrazotized DDS was then much reduced, and this modification was not adopted. Apart from these losses during extraction from aqueous solution there was an additional loss during extraction from blood (Table III). The volumes given above cannot therefore be altered without influencing the recovery. Disodium hydrogen phosphate was added to increase the stability of the soluble compounds by making the blood weakly alkaline. It is shown on p. 579 that sulphetrone, promin, and 2196 are readily hydrolysed to DDS on

TABLE III

RECOVERY OF DDS FROM BLOOD, ALONE AND IN THE PRESENCE OF SOLUBLE DERIVATIVES

DDS added (μg.)	Soluble derivative added (μg.)	μg. DDS found	Percentage recovery
0	0	0	—
2.5	0	2.15	86
7.5	0	6.31	84
12.5	0	10.8	86
17.5	0	14.0	80
25.0	0	21.7	87
0	50 Diasone	0.052*	(0.10)
5	50 ,,	4.51	89
0	500 ,,	2.66	(0.53)
10	500 ,,	11.4	87
0	50 Promin	0.34	(0.68)
5	50 ,,	4.75	88
0	500 ,,	1.54	(0.31)
10	500 ,,	10.7	92
0	50 Sulphetrone	0.018	(0.04)
5	50 ,,	4.80	96
0	500 ,,	1.14	(0.23)
10	500 ,,	9.80	87
0	100 2196	1.03	(1.03)
5	100 ,,	4.84	76
10	100 ,,	8.60	76
		Mean	86±3.2

* All the soluble compounds appeared to contain free DDS. The results in parentheses give the amount of free DDS found as a percentage of the amount of soluble compound present.

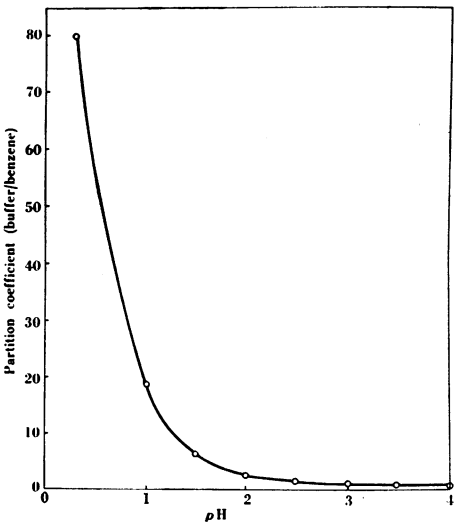


FIG. 1.—Effect of *p*H on the partition coefficient of DDS between 0.1 M-citrate-hydrochloric acid buffer and benzene.

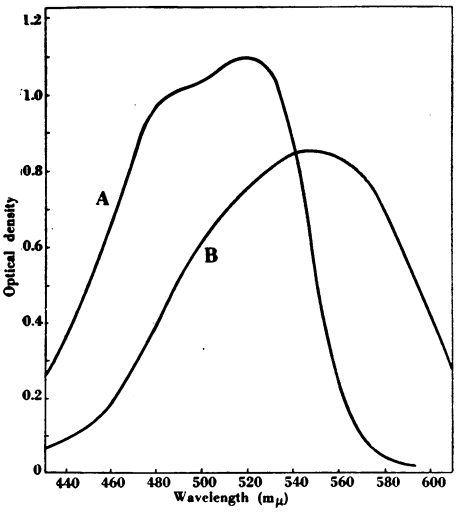


FIG. 2.—Absorption spectra of dyes from DDS (15 μg.) and N-β-sulphatoethyl-*m*-toluidine (A) or N-1-naphthylethylenediamine (B) in aqueous ethanol (10 ml., 2 cm. cells).

standing in the cold with acid, and that the last is readily hydrolysed in incubated blood. We have therefore been careful to analyse blood immediately after withdrawal. The choice of coupling component lay between N-β-sulphatoethyl-*m*-toluidine (Rose and Bevan, 1944) and N-1-naphthylethylenediamine (Bratton and Marshall, 1939) which we have found to be the most suitable among those commonly used. The curves in Fig. 2 show that the former gave slightly higher sensitivity, as it did when used for the estimation of several other aromatic amines (Spinks and Tottey, 1946). It has the further advantage that its use does not necessitate the destruction of excess nitrite. However, dyes are formed from it more slowly than from N-1-naphthylethylenediamine and are usually less soluble. Ethanol was added for the latter reason.

Recovery of DDS from blood, alone and in the presence of soluble compounds, is shown in Table III.

Estimation of sulphetrone and diaminodiphenylsulphone in urine

We wished to examine the excretion rate of a soluble compound and its conversion to free DDS *in vivo*. DDS could be readily estimated in urine by the procedure given above for blood, and recovery was quantitative when referred to that of an aqueous standard extracted under the same conditions. We have used three methods for the estimation of sulphetrone, based on: (A) its ultra-violet absorption spectrum, (B) the change in the absorption spectrum which accompanies hydrolysis to DDS, and (C) the colorimetric estimation of the DDS formed. The first method only indicates the approximate amount of sulphetrone present, because of the strong absorption of normal urine in the ultra-violet. The three methods need only a single sample of urine.

Reagents

1. All the reagents needed for the estimation of DDS in blood.
2. N-sodium hydroxide.
3. Concentrated hydrochloric acid (AR).
4. Freshly prepared aqueous standard solution of sulphetrone, 2 mg./100 ml.

Procedure

1. Estimate DDS exactly as described for blood using 1 ml. of urine if sulphetrone was administered intravenously, 0.1 ml. if it was administered orally.

2. (*Method A*) Dilute the urine with distilled water to a concentration which has an optical density (a) of about 0.6 to 1.2 when read against distilled water in a 2 cm. cell at 307 mμ. Also read the optical density (b, about 1.22) of a 2 mg./100 ml. solution of sulphetrone. Then the approximate concentration of sulphetrone in the urine is $2a/b$ mg./100 ml.

3. (*Method B*) Take 9 ml. of the diluted solution used in (2) above. Add 1 ml. of concentrated hydrochloric acid, mix rapidly, transfer to a 2 cm. cell, and read the optical density at 307 mμ against distilled water exactly 1, 2, 3, and 4 min. after adding the acid. Plot the readings against time, preferably on semi-logarithmic paper, and extrapolate to zero time. Let the density thus obtained be a. Read the density (b) again after 2 hours. Obtain the corresponding values a' and b' for a 2 mg./100 ml. solution of sulphetrone. Then the concentration of sulphetrone in the unknown is $2(a-b)/(a'-b')$ mg./100 ml. The value $(a'-b')$ is usually about 0.94.

4. (*Method C*) Take 1.8 ml. of the final acid solution from (3) above, that has stood for 2 hours, and add 1.2 ml. of N-sodium hydroxide. Mix and tetrazotize and couple exactly as described for the estimation of DDS in the acid aliquot of 3 ml. obtained after

the extraction of blood. Let the optical density at 520 mμ of the final solution be *a*, and that from the standard 2 mg./100 ml. solution of sulphetrone similarly treated be *b*. Then the concentration of sulphetrone in the urine is $2a/b$ mg./100 ml. The value *b* is usually about 0.74.

Notes

We used all three methods in experimental work because we had not developed a technique for confirming our identification of sulphetrone in urine, and wished to increase the specificity of analysis. It is probable that paper chromatography (Boyer, Troestler, Rist, and Tabone, 1950) or countercurrent distribution (Titus and Bernstein, 1949) could be used instead.

Identical methods can be applied to promin, using its absorption maximum at 302 mμ. Recovery of promin was quantitative as was that of sulphetrone (Table IV). We were not able to apply the method to 2196, which decomposes slowly in dilute aqueous solution at room temperature, and we have not examined the behaviour of diasone. The ultraviolet absorption spectra of sulphetrone, its acid decomposition product, and DDS are shown in Fig. 3. At *p*H 7 (not shown) DDS has an absorption maximum at 291 mμ, and the amount of sulphetrone found by method (A) is increased by about 1.6 mg. for every mg. of DDS present. The amount found by method (C) is increased by 4.6 mg. for every mg. of DDS present. The amount found by method (B) is not affected. The conversion of sulphetrone to DDS was not quantitative under our conditions (Fig. 3, see also p. 579), and the absorption spectra given by Brownlee, Green, and Woodbine (1948) suggest that it was not under their conditions. They considered that sulphetrone was not readily hydrolysed to DDS by acid, but the absorption spectra given in Fig. 3 and the results described on p. 579 show that this was incorrect. Titus and Bernstein (1949) have described a method of estimating the soluble compounds which is almost identical with our third. They reported quantitative conversion of sulphetrone to DDS under their conditions.

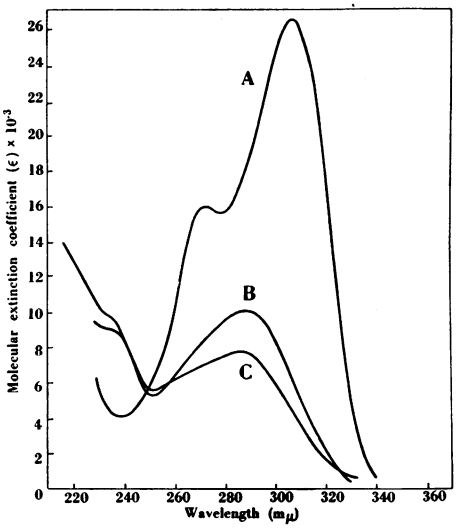


FIG. 3.—Absorption spectra of sulphetrone in water (A), DDS in 0.1 N-hydrochloric acid (B), and sulphetrone after standing for 4 hours in 0.1 N-hydrochloric acid (C).

Concentration of free diaminodiphenylsulphone in blood after the administration of soluble compounds

1. *Oral administration to rabbits.*—Sulphetrone, promin, diasone, and 2196 were administered orally to rabbits in doses of 1 g./kg. and free DDS was estimated at intervals in blood drawn from a marginal ear vein. DDS was administered orally in doses of 50 and 100 mg./kg. It was estimated by the extraction method, as in the other experiments.

Mean concentrations obtained with three rabbits for each drug are shown in Fig. 4. The concentrations of DDS resulting from administration of all the

TABLE IV

RECOVERY OF SULPHETRONE FROM URINE

Added mg./100 ml.	Found mg./100 ml. Method			% Recovery Method		
	A	B	C	A	B	C
0	23.9	0.84	0.81	—	—	—
50	73.7	50.7	57.0	100	100	112
100	122	101	107	98	100	106
0	60.9	2.83	1.89	—	—	—
10	70.7	13.0	11.0	98	102	91
20	82.2	21.8	20.8	107	95	94
40	103	41.3	39.2	105	96	93
70	136	66.2	69.2	107	91	96
100	160	92.0	99.0	99	89	97
0	55.1	1.07	0.53	—	—	—
10	65.8	12.6	10.9	107	115	104
20	76.2	22.6	21.2	106	108	103
30	86.6	32.6	30.4	103	105	100
50	105	52.5	50.1	100	103	99
70	124	74.6	70.2	99	105	100
100	149	105	97.3	94	104	97
			Mean	102±2.5	101±3.2	99±3.5

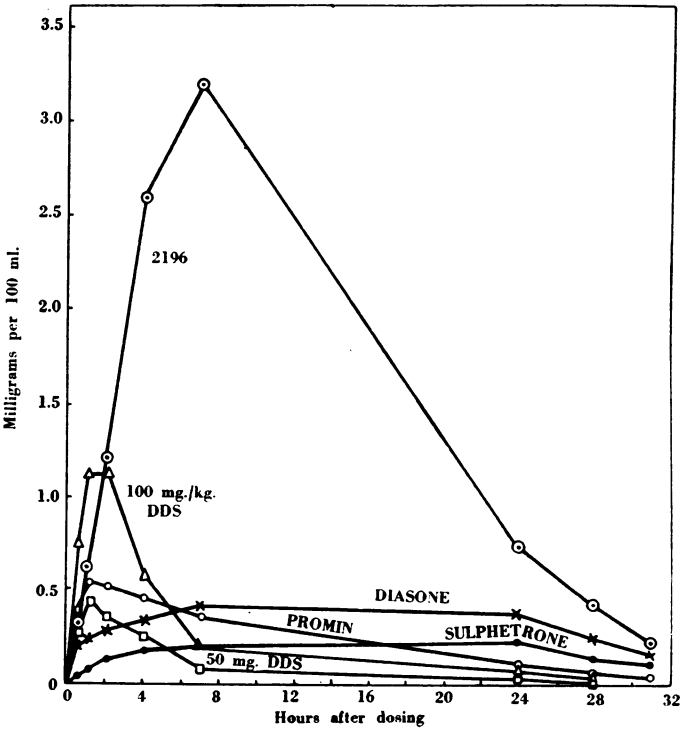


FIG. 4.—Blood concentrations of DDS in rabbits after oral administration of 1 g. promin (O), 1 g. diasone (×), 1 g. sulphetrone (●), 1 g. 2196 (⊙), and 50 (□) and 100 (△) mg. of DDS per kg.

compounds can only be compared by allowing for the dose, which was not constant. The ratio of maximum concentration (mg./100 ml.) over dose (mg./kg.) varied as follows: DDS, 11.3, 9.0; 2196, 3.2; promin, 0.54; diasone, 0.44; sulphetrone, 0.23. These corrected concentrations tally well with the activities of the compounds in the mouse (see p. 567); although the positions of promin and diasone are reversed they lie close to each other in both series. There is no doubt that all the compounds are converted at least in part to the parent amine, as assumed by nearly all workers in this field and proved very recently by others independently (Smith, 1949; Titus and Bernstein, 1949; Boyer, Troestler, Rist, and Tabone, 1950).

2. *Intravenous administration to rabbits.*—Promin, sulphetrone, and 2196 were administered intravenously in doses of 1 g./kg. No toxic symptoms were observed. Concentrations of DDS found in single rabbits are shown in Fig. 5. The amounts

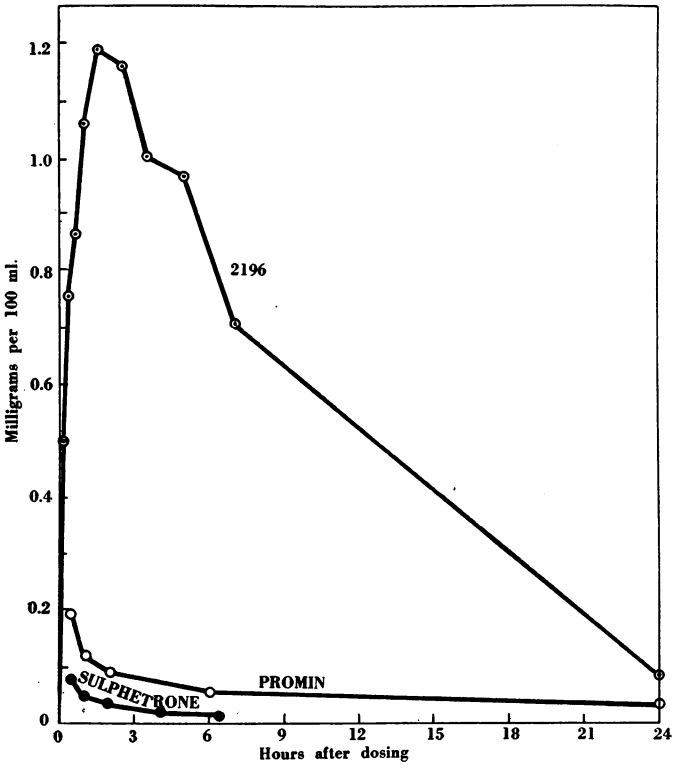


FIG. 5.—Blood concentrations of DDS in rabbits after intravenous administration of 1 g. promin (O), 1 g. sulphetrone (●), and 1 g. 2196 (◎) per kg.

of DDS found after giving promin and sulphetrone were low, and the first concentrations measured were the highest: they could perhaps be attributed mainly to the presence of traces of free DDS in the material administered (cf. Table III). These two compounds are obviously much more readily converted to DDS when given orally, possibly by acid hydrolysis in the stomach (see pp. 579 and 580), in conformity with their higher toxicity and activity after oral administration. No. 2196 on the other hand was converted to DDS in considerable amount, as

shown by the high concentrations detected and the occurrence of a maximum ninety minutes after administration.

3. *Oral administration to mice.*—(*a*) *By stomach tube.*—The soluble compounds were administered as solutions and DDS as a dispersion, each mouse receiving 0.5 ml./20 g. The doses were: promin and sulphetrone, 1 g./kg.; diasone, 500 mg./kg.; 2196, 250 mg./kg.; DDS, 100 mg./kg. A group of three mice was killed with chloroform, and pooled heart blood was analysed to obtain each point on the blood-concentration time curve. The results are shown in Fig. 6. The

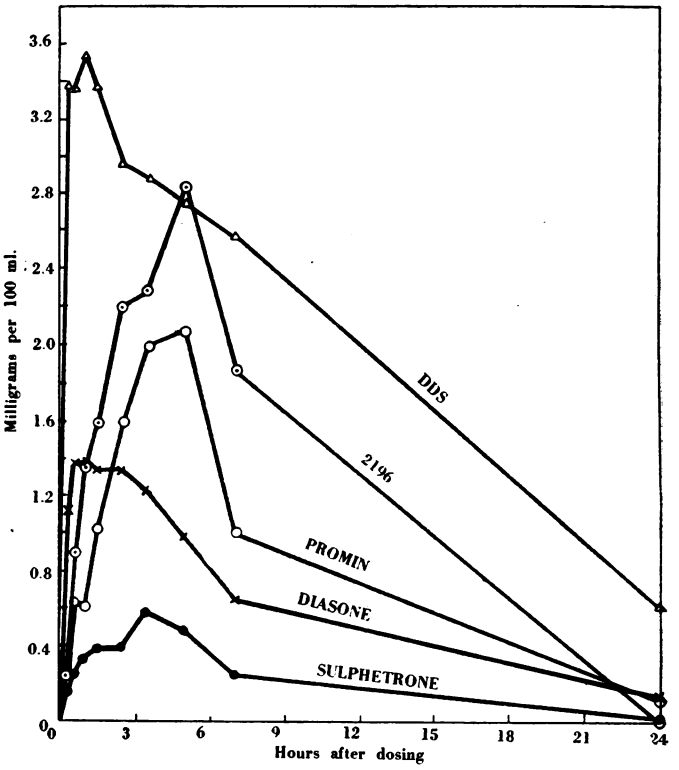


FIG. 6.—Blood concentrations of DDS in mice after oral administration of 1 g. sulphetrone (●), 1 g. promin (O), 0.5 g. diasone (×), 0.25 g. 2196 (⊙), and 0.1 g. DDS (△) per kg.

concentrations were three to five times higher than those obtained in the rabbit when allowance was made for the different doses, but fell in almost the same order, diasone and promin being reversed as compared with their positions in the rabbit. Fig. 7 shows the relation of blood concentration in the mouse to activity against a streptococcal infection in the mouse (see p. 567), expressed as the reciprocal of the amount of drug needed to give a constant degree of activity. The points fall on or near a smooth curve, and it is therefore concluded that the activity of all the compounds, at least against this infection, is due to the free DDS present in the blood stream. As suggested in the introduction the results can probably be applied to other infections.

Titus and Bernstein (1949) obtained similar results to ours in mice with four of the drugs; they did not examine 2196.

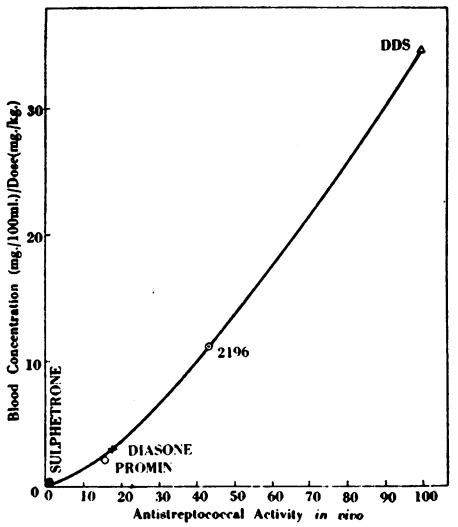


FIG. 7.—Maximal concentrations (adjusted to a dose of 1 g./kg.) of DDS in the blood of mice receiving various sulphones by stomach tube plotted against antistreptococcal activity of the sulphones in mice (● sulphetrone, O promin, × diasone, ⊙ 2196, △ DDS).

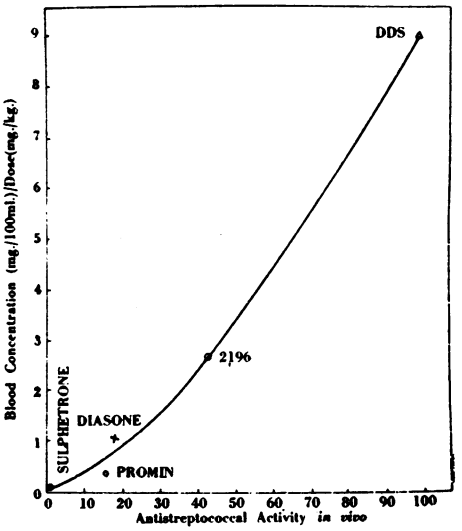


FIG. 8.—Mean concentrations (adjusted to a daily intake of 1 g./kg.) of DDS in the blood of mice receiving various sulphones in the food plotted against antistreptococcal activity of the sulphones in mice (code as in Fig. 7).

(*b*) *In the food.*—Table V shows the concentration of free DDS found in the blood of mice receiving the compounds in the food. Fig. 8 shows the relation of blood concentration to activity against streptococci. The results were very similar to those obtained by giving the drugs by stomach tube.

4. *Administration to rats in the food.*—Table VI shows the concentrations of free DDS in the blood of rats receiving the compounds in the food. The results suggest that the conversion of diasone to DDS is relatively lower, and that of promin higher, than in the mouse. The rats were infected with rat leprosy, and the therapeutic results will be described elsewhere (Francis, 1950).

5. *Excretion of sulphetrone and DDS in the rabbit.*—Male rabbits were catheterized without anaesthesia, and urine was collected in half-hourly or hourly samples.

TABLE V

MEAN CONCENTRATIONS OF FREE DDS IN THE BLOOD OF MICE RECEIVING SULPHONES ORALLY IN THE FOOD

Each value is a mean of four observations, each on the pooled heart blood of a group of three mice

Sulphone	DDS	2196	Diasone	Promin	Sulphetrone
Dose (% in food)	0.05	0.10	0.10	0.40	1.60
Approximate dose (g./kg./day)	0.1	0.2	0.2	0.8	3.2
DDS mg./100 ml. blood ..	0.906	0.530	0.218	0.269	0.348
Concentration/dose	9.06	2.65	1.09	0.34	0.11

TABLE VI

MEAN CONCENTRATIONS OF FREE DDS IN THE BLOOD OF RATS RECEIVING SULPHONES ORALLY IN THE FOOD

Each value is a mean of six observations, each one on the heart blood of a single rat

Sulphone	Dose (% in food)	Dose (g./kg./day)	DDS (mg./100 ml.)	Concn./Dose
DDS	0.025	0.025	0.130	5.2
DDS	0.1	0.1	0.635	6.35
Diasone	0.1	0.1	0.053	0.53
Diasone	0.4	0.4	0.194	0.48
Promin	0.25	0.25	0.804	3.22
Promin	1.00	1.0	1.55	1.55
Sulphetrone	1.00	1.0	0.477	0.477
Sulphetrone	4.00	4.0	1.59	0.40

After 6–7 hours the catheter was removed and urine was collected overnight from a metabolism cage. After the collection of the first sample sulphetrone was administered orally or intravenously and sulphetrone and free DDS were estimated in all samples. Typical results in single rabbits are shown in Tables VII and VIII.

The figures have been corrected for the apparent concentrations of the two compounds in the sample collected before dosing, and the figures for sulphetrone by methods A and C have been corrected for the contribution made by the free DDS present. Acetylated, or similarly conjugated, DDS was not estimated accurately, but in some experiments an attempt was made to obtain an approximate estimate by taking part of the final solution from method B (urine that had been allowed to stand in the cold with strong acid for two hours) and heating it at 100° for two hours. The amount of DDS found by subsequent analysis was corrected for the amount found by method C (analysis after standing with acid in the cold) to give an approximate figure for conjugated DDS; some was present in each sample, but the amount was lower than, or of the same order as, that of free DDS,

TABLE VII

Excretion of sulphetrone and DDS in the urine of a rabbit (♂, 2.6 kg.) after the intravenous administration of 1 g. sulphetrone per kg.

Period (hours)	Sulphetrone excreted % of dose by method			DDS excreted % of theory
	A	B	C	
0–½	22.2	22.7	21.9	0.029
½–1	15.0	15.1	14.8	0.056
1–1½	12.9	13.0	12.8	0.058
1½–2½	7.45	8.04	7.70	0.018
2½–3½	5.06	5.25	5.18	0.048
3½–4½	2.06	2.28	2.59	0.008
4½–5½	2.15	2.39	2.61	0.023
5½–6½	2.76	2.77	3.21	0.029
6½–24	4.95	5.03	7.14	0.122
Total ..	74.5	76.6	77.9	0.391

and would therefore not greatly affect the degree of conversion of sulphetrone to DDS shown in Tables VII and VIII. Smith, Jackson, Chang, and Longenecker (1949) concluded that DDS was excreted unchanged to a large extent in the rabbit (but cf. below, this page).

TABLE VIII

Excretion of sulphetrone and DDS in the urine of a rabbit (♂, 2.3 kg.) after the oral administration of 1 g. sulphetrone per kg.

Period (hours)	Sulphetrone excreted % of dose by method			DDS excreted % of theory
	A	B	C	
0–1	0.28	0.19	0.27	0.01
1–2	0.56	0.47	0.72	0.11
2–3	0.62	0.82	0.62	0.14
3–4	0.62	0.64	0.87	0.07
4–5	0.39	0.20	0.45	0.11
5–6	0.48	0.24	0.11	0.14
6–7	0.55	0.19	0.55	0.14
7–24	1.84	1.57	4.74	1.20
24–48	10.6	1.76	14.6	0.64
Total ..	15.9	6.08	22.9	2.56

Table VII shows that after intravenous administration most of the sulphetrone is rapidly excreted. The excellent agreement between results obtained by the three methods of analysis suggests that most of the material measured is unchanged sulphetrone. Traces only of free DDS were excreted, in conformity with the very low blood concentrations observed after intravenous administration (Fig. 6). This DDS may have been derived directly from traces present in the material administered, or indirectly by conversion from sulphetrone either in the blood stream or after excretion into the gut. The results of oral administration (Table VIII) were very different. Only 6 per cent of the drug was excreted unchanged as judged by method B (fall of optical density at 307 mμ on adding acid). The other two methods indicated larger amounts, although the figures were corrected for DDS. It must be concluded that a third compound was present. The nature of this compound is unknown, but some properties can be deduced. Its detection by methods A and C shows that it must absorb ultra-violet light of a similar range to that absorbed by DDS and sulphetrone, and that it is hydrolysed to DDS or a related diazotizable amine in the cold, or is itself diazotizable. It was observed that when acid was added to diluted urine containing the compound the optical density fell instantaneously, this fall being distinct from that which subsequently occurred slowly as sulphetrone present was hydrolysed. A very slight fall only is observed with solutions of pure sulphetrone before hydrolysis begins. Therefore, the compound either is an amine, or is hydrolysed immediately on adding acid. Its apparent absence from the urine after intravenous administration of sulphetrone suggests either that it is formed by partial breakdown of sulphetrone in the gut, e.g., by hydrolysis at one end of the molecule only, or that it is formed from DDS; the latter was itself formed in much larger amount after oral administration. Pressure of other work has so

far prevented further investigation, but Titus and Bernstein (1949) have reported the conversion of DDS in the dog into a derivative highly soluble in water and very readily hydrolysed by acid; it is possible that this is identical with our unknown compound. The conversion of sulphetrone to DDS after oral administration was not very great, as judged by the amount of free DDS detected; this is in agreement with the blood concentrations observed after oral administration. A dose of 50 mg. DDS/kg. gave about twice as high a concentration of DDS as a dose of 1 g. sulphetrone. The relative efficiency of giving sulphetrone was therefore found to be about 2.5 per cent in both experiments. These results are in general agreement with those obtained by Smith (1949), who showed that large amounts of sulphetrone (or other material that could be hydrolysed to DDS) appeared in the faeces after oral administration, and by Titus and Bernstein (1949), who found about 40 per cent of unchanged sulphetrone in dog urine after intravenous, and 10 per cent after oral, administration, and only traces of DDS after either.

Decomposition of sulphetrone, promin and 2196 in vitro

1. *Decomposition by acid.*—One per cent (w/v) solutions of the three compounds were diluted with water and N-hydrochloric acid to give 20 mg./100 ml. solutions in 0.1 N-hydrochloric acid. The solutions were allowed to stand at room temperature (16–20° C.), and 1 ml. was withdrawn for analysis at intervals and immediately neutralized by adding it to 1 ml. of 0.1 N-sodium hydroxide. Free DDS present was estimated immediately by the method described above for the analysis of blood. The results are shown in Fig. 9. None of the compounds was hydrolysed quantitatively under the conditions used. The ease of hydrolysis increased in the order sulphetrone, promin, 2196, the same order which was observed for blood

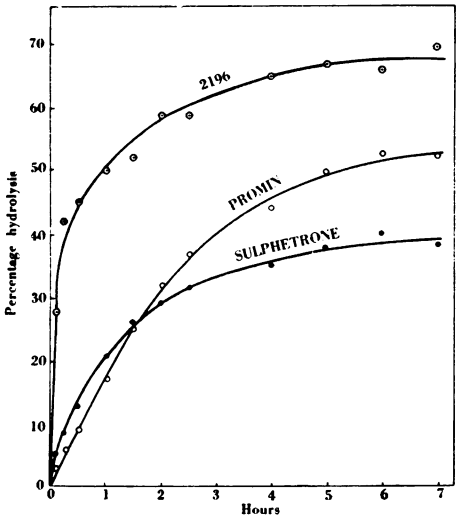


FIG. 9.—Hydrolysis of sulphetrone (●), promin (O), and 2196 (⊙) to DDS in 0.1 N-hydrochloric acid at 16–20° C.

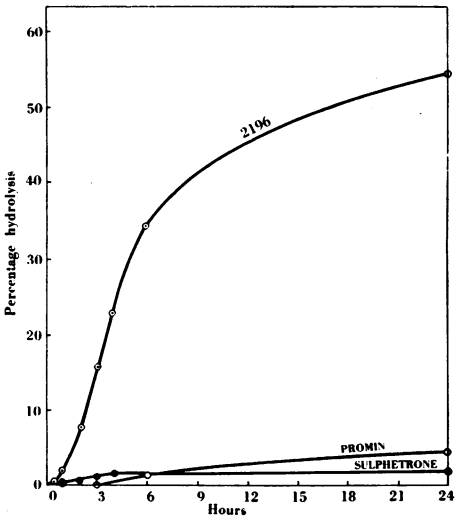


FIG. 10.—Hydrolysis of sulphetrone (●), promin (O), and 2196 (⊙) to DDS in blood incubated at 37° C.

concentrations in mouse and rabbit after oral administration. This supports the view that the formation of DDS after oral administration of the compounds occurs mainly in the stomach.

2. *Decomposition in incubated blood.*—One per cent (w/v) solutions of the three compounds were diluted to 10 mg./100 ml. with fresh rat blood. The blood was incubated at 37° and analysed for DDS at intervals. The results are shown in Fig. 10. Promin and sulphetrone were hydrolysed to a slight extent only; 2196 was hydrolysed almost as rapidly as by 0.1 N-hydrochloric acid. The results tally with those of intravenous administration of the three compounds (pp. 574 and 577) which showed that only 2196 was readily hydrolysed after absorption.

Toxic effects of diaminodiphenylsulphone

The toxicity of DDS has attracted considerable attention, but published observations have been practically confined to descriptions of changes in the blood. We have observed toxic effects on the nervous system.

Doses of 100 mg. DDS/kg. were tolerated by sheep, but two of four sheep given 200 mg./kg. died and one of the others showed severe toxic symptoms but eventually recovered. In sheep the first symptoms of toxicity are excitement and paraplegia; there may be a nervous nodding of the head. The animal soon falls to the ground and shows typical opisthotonos. Both pairs of limbs may be extended in a forward position. At other times the animals exhibit clonic convulsions. Breathing is rapid, there may be pyrexia, trismus, and twitching of the lips. In a sheep that eventually recovered the symptoms were most acute after seven hours when the blood concentration was 4 mg./100 ml. Thirty-two hours after dosing it was 6.6 mg./100 ml. and, after 72 hours, 4.4 mg./100 ml. By this time the sheep was quiescent and it recovered 96 hours after dosing, when the concentration was 2 mg./100 ml.

Similar but less marked nervous symptoms to those described in the sheep have been observed in the dog and in the goat after the intramammary injection of about 5 g. DDS. The symptoms described by Davies (1950) in a child are not dissimilar, although it is surprising that a blood concentration of 15 mg./100 ml. did not produce death more rapidly. No nervous symptoms have been observed in the bovine even after doses of 400 mg./kg.

The production of cyanosis was an alarming feature when DDS was first given to man. Davies (1950) confirmed the occurrence of cyanosis and pigmentation of the blood, but no methaemoglobin could be detected. The production of methaemoglobin by DDS in various animals has been described by Francis (1949a). It is readily produced in the horse and the pig, to a less extent in the dog, hardly at all in the sheep, and not in the bovine. A full spectrographic examination on the blood of one horse indicated that the pigment was methaemoglobin. The concentration of methaemoglobin in the horse fell sooner than that of DDS, and it appears from the results in the sheep, given above, that the nervous system also develops a tolerance to DDS. It is well recognized (Lowe, 1950) that man develops a tolerance to DDS.

There is nothing highly specific in the toxic effects of DDS. Identical nervous symptoms have been described in the goat after the administration of sulphanilamide

(Bower, 1947) and similar symptoms in a child (Reed, 1944). The fact that sulphanilamide may produce haemolytic and other changes in the blood of man similar to those produced by DDS is well known, although the heterocyclic sulphonamides are less liable to produce these effects.

Discussion

Whilst this paper was being prepared for publication the papers of Boyer, Troestler, Rist, and Tabone (1950) and Titus and Bernstein (1949) were seen. Titus and Bernstein obtained similar results to our own with promin, diasone, and sulphetrone, and some other derivatives. Taken together, these and our studies show that the soluble derivatives are partly converted to DDS in the body, and that the therapeutic action depends on the degree of conversion to DDS. When the derivatives were given intravenously we found that only 2196 was extensively hydrolysed to DDS. Sulphetrone was very rapidly excreted in the urine, mostly unchanged. 2196 was also the only one extensively hydrolysed to DDS in the presence of blood *in vitro*. All the derivatives were hydrolysed by 0.1 N-hydrochloric acid, and the degree of hydrolysis tallied with their therapeutic activity after oral administration. All these facts indicate that hydrolysis occurs chiefly on the stomach, and they explain why larger doses of promin can be given parenterally than orally (Hinshaw and Feldman, 1941). After oral administration the relative potencies of the drugs were:

DDS	100
2196	43
Diasone	18
Promin	16
Sulphetrone	1

2196 was the only one which had almost the activity to be expected from its content of DDS and it is, therefore, probably completely converted to DDS *in vivo*. Nevertheless, strong solutions have been kept for years without depositing DDS. It may, therefore, be useful when parenteral administration is necessary. We are at present studying its administration to monkeys intracisternally, and have found it to be tolerated in doses of 5 mg./kg.; twice this dose produced temporary paraplegia.

Smith (1949) has examined the absorption of solubilized drugs in man, using methods which did not discriminate between the drugs and DDS. However, his results give a useful picture of the proportions of the various compounds that are absorbed after oral and parenteral administration. A single daily dose of 0.3 g. of DDS in man gave minimal blood concentrations of 1 mg./100 ml. The sulphones were fairly equally distributed throughout the body fluids, and no evidence was found for their localization in the skin. Smith points out that from a pharmacological point of view DDS has great advantages, in that given orally it is well absorbed and slowly excreted, so that only a small amount is needed to establish and maintain blood concentrations comparable with those attained by soluble sulphones.

Cochrane, Ramanujam, Paul, and Russell (1949) and Molesworth and Narayanaswami (1949) have obtained good results in the treatment of leprosy by injecting an

oily suspension of DDS subcutaneously once or twice a week. The total weekly dose should not be more than 1 to 1.5 g. Molesworth and Narayanaswami noted few toxic symptoms and found that the drug was particularly well tolerated by children. The last observation has been confirmed by Rist and Cottet (1949).

Lowe and Smith (1949) and Lowe (1950) have given DDS orally for the treatment of leprosy. Lowe (1950) concludes that, contrary to general belief, DDS is not too toxic for use in man. A regime of oral administration of small doses, rising very slowly from 100 mg. a day to the standard 300 mg. a day in five weeks, is recommended. This regime has produced excellent results in lepromatous and tuberculoid leprosy, and the small doses of DDS used have proved to be at least as effective as much larger doses of the soluble sulphones. DDS treatment in severe cases " is very slow but amazingly certain." Francis (1950) has shown that a concentration of only 0.004 per cent of DDS in the food produces as good an effect on rat leprosy as 0.02 or 0.1 per cent. The good action of such small doses is surprising, but Summers (1949) has shown that 0.006 per cent of DDS in the food is active against toxoplasmonia in mice. It is possible that doses of DDS even smaller than those at present being used may be fully effective for the treatment of human leprosy. Even if this possibility is discounted it must be concluded from the experimental and clinical evidence so far available that the complex soluble compounds have no proved advantage over DDS itself and that the latter should be preferred for routine treatment.

SUMMARY

1. It is necessary to incorporate nearly a hundred times as much sulphetrone as 4:4′-diaminodiphenylsulphone (DDS) in the food of mice in order to produce a given therapeutic effect against streptococcal septicaemia. Smaller amounts of diasone and promin are required, and the acetaldehyde bisulphite derivative of DDS, 2196, has nearly the activity one would expect from its content of DDS.

2. Differences in antibacterial potency among these five sulphones are even greater in culture media when care is taken to prevent breakdown of the soluble derivatives of DDS: if the medium is autoclaved after the drugs have been added their " activity " is greatly increased, owing probably to liberation of DDS.

3. A method of estimating free DDS in the presence of the soluble derivatives is described, and it is shown that the therapeutic effect produced by any of the soluble derivatives is closely related to the blood concentrations of free DDS.

4. The chief breakdown of the soluble derivatives probably occurs in the stomach: 2196 is the only one studied that liberates DDS to an appreciable extent in the presence of blood or after parenteral administration. Sulphetrone was rapidly excreted, mostly unchanged, when given parenterally. Others have shown that when DDS is given parenterally it is very slowly excreted.

5. The results of concurrent studies carried out in man are reviewed. It is evident that doses of only 200–300 mg. a day of DDS produce as good, or better, effects in the treatment of leprosy than much larger doses of the soluble derivatives. It is concluded that DDS is to be preferred for the treatment of leprosy.

6. The nervous symptoms produced by DDS in some animals are described.

REFERENCES

Bauer, H., and Rosenthal, S. M. (1938). *Publ. Hlth Rep., Wash.*, **63**, 40.
Bower, D. G. (1947). *Vet. Rec.*, **59**, 618.
Boyer, F., Troestler, J., Rist, N., and Tabone (1950). *Ann. Inst. Pasteur*, **78**, 140.
Bratton, A. C., and Marshall, E. K. (1939). *J. biol. Chem.*, **128**, 537.
Brownlee, G. (1945). *Vet. Rec.*, **57**, 229.
Brownlee, G., Green, A. F., and Woodbine, M. (1948). *Brit. J. Pharmacol.*, **3**, 15.
Buttle, G. A. H., Stephenson, D., Smith, S., and Foster, G. E. (1937). *Lancet*, **1**, 1331.
Cochrane, R. G., Ramanujam, K., Paul, H., and Russell, D. (1949). *Leprosy Rev.*, **20**, 4.
Davies, R. (1950). *Lancet*, **1**, 905.
Fourneau, E., Tréfouël, J., Nitti, F., and Bovet, D. (1937). *C.R. Acad. Sci., Paris*, **204**, 1763.
Francis, J. (1947a). *Vet. Rec.*, **59**, 131.
Francis, J. (1947b). *Vet. J.*, **103**, 193.
Francis, J. (1949a). *J. comp. Path.*, **59**, 225.
Francis, J. (1949b). *J. comp. Path.*, **59**, 245.
Francis, J. (1950). To be published.
Francis, J., Peters, J. M., and Davies, O. L. (1947). *J. comp. Path.*, **57**, 153.
Hinshaw, H. C., and Feldman, W. H. (1941). *J. Amer. med. Ass.*, **117**, 1066.
Johnson, R. M. (1940). *J. Amer. med. Ass.*, **114**, 520.
Lowe, J. (1950). *Lancet*, **1**, 145.
Lowe, J., and Smith, M. (1949). *Int. J. Leprosy*, **17**, 181.
McEwen, A. D., Pizer, N. H., and Paterson, J. D. (1941). *Vet. Rec.*, **53**, 429.
Molesworth, B. D., and Narayanaswami, P. S. (1949). *Int. J. Leprosy*, **17**, 197.
Reed, H. (1944). *Lancet*, **2**, 535.
Rist, N., and Cottet, J. (1949). *Pr. méd.*, **157**, 743.
Rose, F. L. (1942). Brit. Pat. 556,901.
Rose, F. L., and Bevan, H. G. L. (1944). *Biochem. J.*, **38**, 116.
Smith, M. (1949). *Leprosy Rev.*, **20**, 78.
Smith, M. I., Emmart, E. W., and Westfall, B. B. (1942). *J. Pharmacol.*, **74**, 163.
Smith, M. I., Jackson, E. L., Chang, Y. T., and Longenecker, W. H. (1949). *Proc. Soc. exp. Biol., N.Y.*, **71**, 23.
Spinks, A., and Tottey, M. M. (1946). *Ann. trop. Med. Parasit.*, **40**, 101.
Summers, W. A. (1949). *Amer. J. trop. Med.*, **29**, 889.
Titus, E., and Bernstein, J. (1949). *Ann. N.Y. Acad. Sci.*, **52**, 719.