A PHARMACOLOGICAL STUDY OF THREE SULPHONES

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PART I—ABSORPTION, DISTRIBUTION & EXCRETION.

INTRODUCTION.

In 1908 Fromm and Wittman synthesised 4,4′ diaminodiphenylsulphone. Not until 1937, when Fourneaux and Buttle, working independently, shewed that this compound possessed a high degree of activity against a hæmolytic streptococcus, was any interest shewn in the chemotherapeutic properties of the new sulphone.

Since 1941 numerous favourable reports have been published concerning the therapeutic activity of the proprietary sulphones. Many of these reports are of a clinical nature. Ross, however (1), confines his report to blood and urine levels produced by the parenteral and oral administration of three proprietary (American) sulphones. A clinical and laboratory study of sulphones, particularly sulphetrone, was carried out during the treatment of nearly 300 patients, the majority suffering from severe lepromatous leprosy, at the British Empire Leprosy Relief Association Research Unit, Uzuakoli, S.E. Nigeria. This report will consider only the aspects of absorption, distribution and excretion. Other pharmacological aspects including the specific toxic phenomena will be reported in subsequent papers.

THE SULPHONES STUDIED.

Sulphetrone, a complex derivative of diaminodiphenylsulphone, is the subject of reports by Brownlee *et al* (2). The parent substance constitutes about one quarter of the molecule.

Diasone, the formaldehyde sulphoxylate derivative of diaminodiphenylsulphone is the subject of reports by Raiziss (3). The parent substance constitutes about one half of the molecule. Owing to currency difficulties the supply of this drug was very limited.

Diaminodiphenylsulphone is not a proprietary substance. Because of early reports of its toxicity in animals it has never been widely used as a therapeutic agent in man. With the doses required for the treatment of acute infections, severe haemolytic anaemia and methaemoglobinaemia are encountered.

Pharmacologically the most desirable sulphone is one that is least toxic, is absorbed completely from the gut, and excreted slowly in the urine. Blood and tissue levels of a therapeutic order may then be maintained on small, widely spaced doses, thus

reducing the cost of therapy, and making possible mass oral treatment with the minimum of staff.

The attempt was made to compare the three sulphones from the points of view mentioned above. To this end the faecal and urinary excretions of the three sulphones were studied at different blood levels produced by variations in the amount of drug intake. Oral and parenteral routes of administration were used.

METHODS.

1. **Determination of sulphones in blood.** The method of Bratton and Marshall as modified by Brownlee (4) was used for all three sulphones. Blood extractions were performed between 0900-1000 hours every morning, the morning dose of sulphone having been administered at 0800 hours. At the commencement of the study it was found that twice daily administration of any of the sulphones used produced reasonably steady blood levels throughout the day; the difference between minimum and maximum levels being 1mg%. For all practical purposes therefore the blood levels recorded hereafter may be considered as minimal levels.

2. **Determination of sulphones in urine.** Twenty-four hour specimens were collected, over a period of three days. Dilutions of each 24 hour sample of from 1-5 to 1-20 were found satisfactory with sulphetrone and diasone; dilutions of from 1-10 to 1-50 were necessary with diaminodiphenylsulphone. 1ml. of the diluted sample was estimated as for blood. The mean of the total three days' excretion was calculated.

3. **Determination of sulphones in stool.** As soon as possible after the passage of the specimen, the total weight of the sample was estimated. Portions of the stool were removed from various sites of the mass and placed in a tared litre beaker. The weight of sample taken was recorded, an amount approximating to 100g. was found satisfactory. This was thoroughly emulsified in c. 500ml. of $N/_1$ HCl, the whole mass was poured into a litre graduated cylinder and made up to 1000ml. A few hours were allowed for the particles to sediment, and 1ml. of the supernatent fluid was estimated as for blood. Further dilution was sometimes necessary, in this case 50ml. of the supernatent fluid were diluted 1-2, 1-4 and so on as necessary.

With stool suspension it was sometimes difficult to obtain a perfectly clear filtrate, neither centrifuging nor double filtration being effective. (In these cases attempts must not be made to clear the liquid by absorption on charcoal, since the sulphones are absorbed themselves on activated charcoal).

Visual estimation in a block comparator with a blank consisting of 1ml. of the *undiazotised* filtrate, gave better and more reliable results than other methods. A colour falling within range of from 4-9mg% for sulphetrone, 1-6mg% for diasone, and 0·4-2·0mg% for diaminodiphenylsulphone was aimed at, and dilutions of the original suspension was affected to achieve this.

The majority of the balance experiments were conducted upon patients who had been upon continuous sulphone treatment for over one month. Many observations were upon patients who had been taking a standardised dose for over six months without a break, whilst for the very high dosage levels studied, patients were used

who had been on continuous treatment for over nine months. No estimations were performed upon patients who had not been standardised upon *the dosage level under investigation* for at least 14 days.

RECOVERY OF DRUGS.

Using the techniques detailed above, the method of Brownlee (4) was found to give excellent results with all the sulphones studied. It was not found necessary to use sulphatoethyl-m-toluidine (5) as a coupling component for diaminodiphenylsulphone; thus the one method of estimation was applied to all sulphones, resulting in a considerable saving of time.

As a test of the accuracy of the method and technique of estimation, the following *recoveries* of the sulphones were obtained, measured amounts of the sulphones were added to faeces, urine and blood, (Fig. 1) estimations being made against standard tubes.

FIG. 1.

DRUG.	FÆCES.			URINE.			BLOOD.		
	Added.	*Recovered.*	*%age.*	*Added.*	*Recovered.*	*%age.*	*Added.*	*Recovered.*	*%age.*
DADPS*	0·1g.	0·11g.	110	0·1g.	0·1g.	100	10mg.	10mg.	100
DIASONE	0·5g.	0·5g.	100	0·5g.	0·48g.	96	10mg.	10mg.	100
SULPHETRONE	1·0g.	0·9g.	90	1·0g.	0·92g.	92	10mg.	10mg.	100

* Diaminodiphenylsulphone.

ABSORPTION.

Excretion of sulphones in the fæces.

Twentyfour-hour stool collections were made over a period of three days, and the mean daily excretion was calculated. Many analyses of single specimens of stool have been carried out here; they are unreliable as an indication of the *total* sulphone excreted daily in the stool, wide variations occurring between morning and evening specimens. Only the results of the three day collections are given in Fig. 2.

Fig. 2.

Excretion in Stool.

Drug.	*Daily Dose in Grammes.*	*No. of 3 day observations.*	*Mean Faecal Excretion in Grammes.*	*% Intake.*
*DADPS orally. Single administration daily.	0·1	8	0·001	1
	0·2	5	0·005	3
	0·3	13	0·008	3
	0·1	2	0·030	7·5
			Mean	4
DIASONE orally twice daily	0·3	8	0·08	27
	0·6	8	0·3	50
	0·9	6	0·4	44
	1·2	6	0·8	67
	2·7	3	0·1	41
			Mean	46
SULPHETRONE orally twice daily administration.	2	3	1·9	95
	3	8	2·4	80
	4	16	3·5	87
	5	20	4·0	80
	6	15	4·2	70
	8	15	7·7	96
			Mean	85

* Diaminodiphenylsulphone.

The restricted range of the observations with diaminodiphenylsulphone was necessitated by the fact that higher doses tend to be toxic.

Discussion.

Diaminodiphenylsulphone in the dosage employed is absorbed from the gut almost completely. In none of the analyses here has the amount of diaminodiphenylsulphone present in the faeces been greater than 10% of the daily oral intake, and it is usually much lower.

Diasone is recoverable from the faeces in amounts varying from 20-67% of the daily oral intake.

Of the daily oral intake of sulphetrone 70-100% may be recovered from the faeces. Wide variations in day to day output are found; individual stool samples containing more than the day's intake are encountered if a tendency to constipation is encountered.

It is believed that the amounts of sulphone recovered from the stool represent substantially the portion remaining unabsorbed from the gut. Excretion of sulphones via the bile and ileum do not at the most amount to more than 10% of the daily oral intake. This is shewn by the following experiment.

Parenteral Sulphetrone.

Three patients were given 4g. of sulphetrone intramuscularly, and one patient 2g. intramuscularly, as a single dose. The following table shews the faecal excretion after these doses (Fig. 3).

FIG. 3.

FÆCAL SULPHETRONE IN GRAMMES

(*Parenteral Administration*)

Patient.	Dose in Grammes.	Day 1.	Day 2.	Day 3.	TOTAL.	% age Dose Excreted.
1	4	0·2	0·08	—	0·3	7·5
2	4	0·1	0·1	0·08	0·28	7·0
3	4	0·15	0·2	0·05	0·4	10·0
4	2	0·05	0·05	—	0·1	5·0

DISTRIBUTION.

Owing to a lack of post-mortem material it was not possible to determine the concentration of the sulphones in the internal organs.

Estimation of sulphone in 1ml. of the following substances were performed: sweat, saliva, tears, lymph. Results are as shown in Fig. 4.

FIG. 4.

SUBSTANCE	Blood Level mg%	mg% Suphetrone	mg% Diasone.	mg% Diamino-diphenylsulphone.
Sweat ...	1	Trace	1·5	1·8
,, ...	2	2	—	—
,, ...	4	5	—	—
,, ...	6	4	—	—
Saliva ...	1	—	0·5	0·8
,, ...	4	2	—	—
Tears ...	1	—	Trace	Trace
,, ...	4	1	—	—
Lymph ...	1·5	—	—	3·0
,, ...	3·0	—	5·0	—
,, ...	6·0	4	—	—
Skin ...	1·0	Trace	Trace	0·9
,, ...	1·5	Trace	0·5	1·9
,, ...	4·0	4·0	—	—
,,* ...	4·0	4·0	—	—

* Macule..

Comment.

From the very limited results above we can only state that the drugs are found in the tissue fluids examined. We have not as

yet evidence of equal distribution. No evidence for the relative concentration of sulphones by the skin was found in any of 30 analyses performed. No difference in drug levels between macular and normal skin was observed when both types of skin were removed from the same patient at the same time.

Excretion.

Excretion of sulphones in the urine.

Twentyfour-hour urine collections were made over a period of three days. The mean values of these were calculated, and are shewn in Fig. 5.

Fig. 5.

Excretion in Urine.

Drug.	*Daily Dose in Grammes.*	*No. of 3-day Observations.*	*Urine Output in Grammes.*	*Proportion Total Daily Dose.*
Diaminodiphenyl-sulphone orally. Single dose daily.	0·1	24	0·08	80%
	0·2	20	0·18	90%
	0·3	20	0·23	77%
	0·4	4	0·33	83%
			Mean	
Diasone orally. Twice daily administration.	0·3	8	0·20	67%
	0·6	8	0·30	50%
	0·9	6	0·43	48%
	1·2	6	0·55	46%
			Mean	
Sulphetrone orally. Twice daily administration.	2·0	10	0·20	10%
	3·0	20	0·30	10%
	4·0	20	0·48	12%
	5·0	20	0·50	10%
	6·0	15	0·50	8%
	7·5	10	0·60	8%
	8·0	5	1·0	12·5%
	9·0	5	1·3	14·5%
	10·0	5	1·2	12%
	14·0	1	1·5	11%
			Mean	
Sulphetrone parenterally. Single dose.	2·0	1	1·9	95%
	4·0	3	3·3	83%
			Mean	

Discussion.

The assumption that the daily urinary output of sulphone is a measure of the absorption of that sulphone is open to certain objections, that (a) the drug may be degraded in the body into various non-sulphone type compounds and (b) that other channels of excretion, for example, the sweat, the bile etc., are possible.

However, for a related series of sulphones the use of the 24-hour urinary output as a measure of absorption is substantially accurate.

From the above results diaminodiphenylsulphone may be said to be extremely well absorbed, 83% of the daily oral intake being recoverable from the urine. Diasone is well absorbed, 53% of the oral intake being recoverable from the urine. Sulphetrone is poorly absorbed, only 11% of the daily oral dose being recoverable from the urine. When 4g. of sulphetrone was given intramuscularly, however, over 70% of the amount was recovered in the *first* 24 hour urine and single samples of urine were passed containing 1g. of sulphetrone per 100ml. of urine, within five hours of the administration of the drug, indicating an extremely rapid renal clearance.

BLOOD LEVELS OF THE SULPHONES.

The values given in Fig 6 represent the minimal blood levels. Observations upon sulphetrone are too numerous to detail, approximately 2,500 estimations being made with this group. The observations upon diasone are limited to a much smaller number of patients, approximately 80 observations being made. With diaminodiphenylsulphone a group of 10, rising to 50, patients has been studied for periods of from one to five months. Therapy in the majority of these cases has been continuous, and the figures given represent 230 estimations.

FIG. 6.

BLOOD LEVELS OF THE SULPHONES.

DRUG.	*Dose in Grammes.*	*Mean Minimal Blood Levels mg%.*	
Diaminodiphenylsulphone orally.	0·1	0·4	
	0·2	0·6	
	0·3	1·0	
	0·4	1·5	
Diasone orally.	0·3	0·3	
	0·6	0·8	
	0·9	1·2	
	1·2	1·5	
	2·7	4·0	
Sulphetrone orally.	2·0	1·5	
	3·0	2·0	
	4·0	3·5	
	5·0	4·0	
	6·0	4·5	
	7·0	5·0	
	8·0	5·5	
	10·0	6·5	
	14·0	7·5	
		1 hr.	*24 hr.*
Sulphetrone intramuscularly.	2·0	26	4
	4·0	40	4

Discussion.

Blood levels of 0·4 to 1·0 mg.% can be obtained with doses of diaminodiphenylsulphone up to 300 mg. daily. Approximately three times as much diasone, and about five times as much sulphetrone are needed to give comparable levels with DADPS.

The range of observations with diasone is very narrow, due to the fact that supplies of diasone were restricted owing to dollar difficulties. It should be kept in mind that these concentrations do not so far as is at present known indicate clinical solidarity, and the drugs are not compared at the same levels.

Blood level/time curves.

Fig. 7 shews the rate of fall of blood sulphone after cessation of therapy. The figures for suphetrone were compiled from five cases who had been on continuous therapy for over a year, the

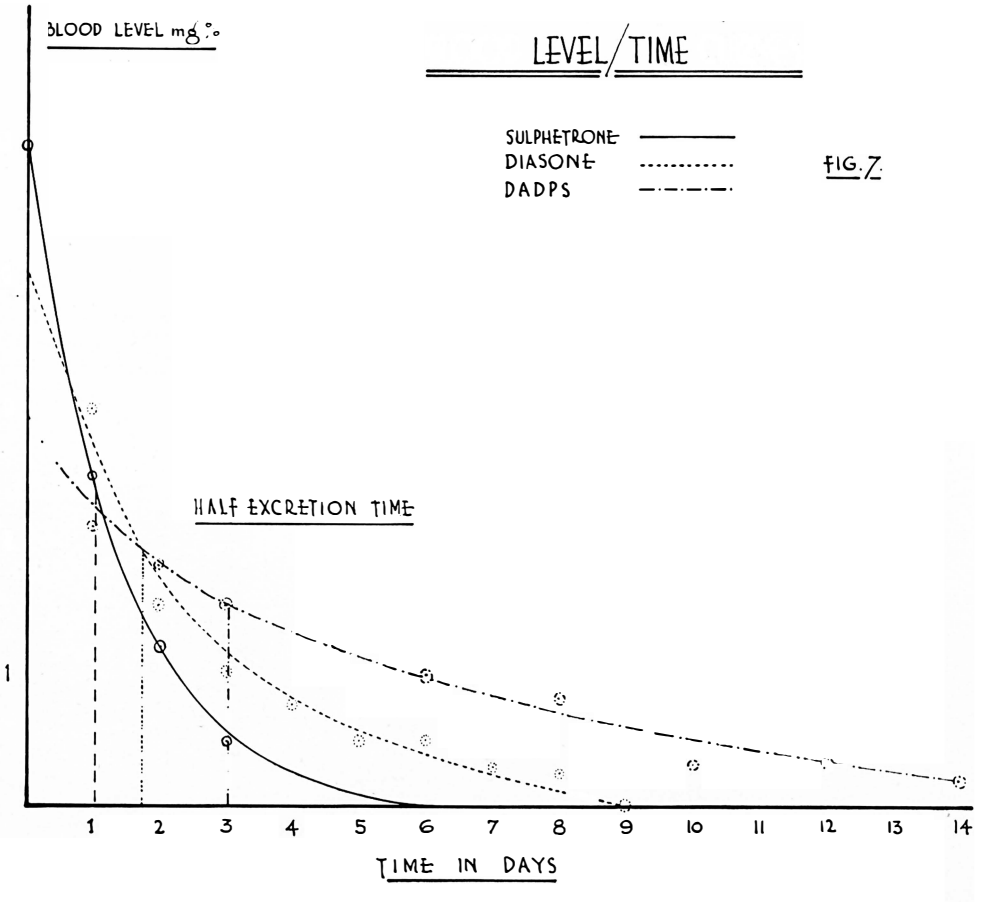

FIG. 7.

figures for diasone were from four cases who had been on continuous therapy for three months, the figures for diaminodiphenylsulphone were from ten cases on continuous treatment for six weeks.

Discussion.

The area enclosed by the curve for diaminodiphenylsulphone is much greater than that for either of the other two sulphones. The half excretion time of each of the drugs demonstrate clearly that sulphetrone is not retained in the body for any considerable period in appreciable quantities. Traces of diaminodiphenylsulphone are still detectable in blood 14 days after the cessation of a six weeks' period of administration.

FINAL DISCUSSION.

From the results presented in these studies it is apparent that diaminodiphenylsulphone meets the pharmacological criteria stated earlier in this paper better than either of the two proprietary sulphones.

Diaminodiphenylsulphone is the active radicle from which both the proprietary compounds are synthesised. Absorption from the gut is of a very high order, excretion in the urine is slow thus enabling blood levels of 1-2mg% to be maintained on oral doses of 0·3g. daily. However, the " parent " sulphone has the reputation of being too toxic a drug for use in human infections, and no reports of its therapeutic trial in leprosy are available. In the treatment of acute infections with diaminodiphenylsulphone when doses similar to those of sulphonamides are administered, it may be predicted that severe toxic effects will be manifested. When the treatment of a chronic disease such as leprosy is considered the dosage of diaminodiphenylsulphone may be adjusted (in the light of the treatment of leprosy with proprietary sulphones) to give a blood level comparable with that obtained when using the proprietary compounds, " comparable " that is in terms of chemical equivalents.

Diasone and sulphetrone are both incompletely absorbed from the gut; the absorption of diasone compares with that of some of the earlier sulphonamides, and if the oral treatment of leprosy with *proprietary* sulphones is being considered it would appear to be the drug of choice. It is suggested that parenteral administration of sulphetrone is the most suitable method of administering this compound, only 10% of the drug being recoverable from the faeces by this route.

Earlier in this paper the theoretical evidence for the breakdown

of the proprietary sulphones was considered. From the fact that the activity of both sulphones and sulphonamides is reversed by para-aminobenzoic acid a similar mode of action may be postulated. Diaminodiphenylsulphone can be isolated from the urine of patients on both diasone and sulphetrone therapy.

The importance of this lies in that if the proprietary substances are not active *per se* then a " desirable " proprietary sulphone should be degraded to a high degree, since the non-degraded compound is therapeutically inert. Further evidence may be obtained regarding this subject by a perusal of the literature upon the therapeutic trials of the proprietary sulphones. Diasone, about 50% of which is diaminodiphenylsulphone, is reported as being a fairly toxic compound—doses of the order of 1g. being advocated. If complete *in vivo* hydrolysis of the absorbed diasone occurs, about 0.25g. of diaminodiphenylsulphone is available to the body (i.e., approximately the daily dose advocated in this paper). Sulphetrone, about 30% of which is diaminodiphenylsulphone is regarded as being virtually non-toxic—doses of the order of 3g. daily being advocated. If complete hydrolysis of *the absorbed* sulphetrone occurs, about 0·1g. of diaminodiphenylsulphone is available to the body (i.e., a dose well within the toxic limits of the " parent " compound). The degree of degradation of the different sulphones is obviously a point of importance, and it is hoped to publish further work on this subject in the near future.

An aspect of great practical importance must now be considered. The treatment of leprosy with drugs of the sulphone class is a long term project, therapy of 4 years' duration and above being necessary in some severe lepromatous cases. Most countries with a leprosy problem are poor countries. It is therefore important to reduce the cost of treatment to the minimum. A proprietary sulphone poorly absorbed from the gut, rapidly excreted in the urine, and only degraded to a very small extent to the active compound would therefore not be the sulphone of choice from economic reasons alone. The sulphone of choice must be cheap as well as pharmacologically suitable: it is probable that diaminodiphenylsulphone, being a non-proprietary compound, not subject to patent restrictions, and being a by-product of several manufacturing processes will be cheap: it is certainly pharmacologically suitable.

Summary.

A study of the absorption, distribution and excretion of the sulphones used in the treatment of leprosy has been attempted.

Estimations of sulphones in the faeces shew that diaminodiphenylsulphone is extremely well absorbed from the gut, diasone is well absorbed, whilst sulphetrone is poorly absorbed.

Estimations of urinary excretion shew that diaminodiphenylsulphone is slowly but almost completely excreted, diasone is more rapidly and less completely excreted, whilst sulphetrone is very rapidly excreted, only 10-20% of the daily oral dose is recoverable from the urine, however.

The sulphones are fairly equally distributed throughout the body fluids. No evidence for the concentration of sulphones by the skin has been found.

Parenteral administration of sulphetrone is advocated, oral administration being uneconomic.

From a pharmacological point of view diaminodiphenylsulphone has great advantages in that given orally it is well absorbed, slowly excreted, and thus only a small amount is needed to establish and maintain a blood level comparable with that obtained by other sulphones.

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