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SULPHETRONE*: A CHEMOTHERAPEUTIC AGENT FOR TUBERCULOSIS: PHARMACOLOGY AND CHEMOTHERAPY

BY

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The discovery and evaluation of the chemotherapeutic activity of diaminodiphenylsulphone in these laboratories (Buttle, Stephenson, Smith, Dewing, and Foster, 1937) inaugurated a search which has gone on ever since for a drug which combined the activity of the sulphone with freedom from toxicity to the host. A number of derivatives proved to be active antibacterial agents and were subjected to a comprehensive pharmacological study. The sulphone derivatives include in their range high antibacterial activities to streptococci, pneumococci, and *M. tuberculosis*.

One derivative, 4 : 4′-bis(γ-phenyl-*n*-propylamino) diphenylsulphone-tetrasodium sulphonate, given the registered name "sulphetrone," attracted attention by reason of its freedom from toxicity and its high antibacterial activity. A pharmacological and therapeutic study of this derivative has been made and details are given of the structure, chemical and physical properties, pharmacology, experimental therapy, and possible clinical uses of the drug.

CHEMISTRY

Preparation and properties of sulphetrone

The chemical preparation has been described by Gray and Henry (1936), and by Buttle, Dewing, Foster, Gray, Smith, and Stephenson (1938). The final stage is precipitation with alcohol which yields an amorphous material containing, when air-dried, 5–7 per cent water which it loses when heated to 110° C. *in vacuo*. Obtained crystalline, the compound contains 5–7 per cent of water; the calculated percentage for monohydrate is 1.98. Its probable constitution is:

$$C_6H_4CH.CH_2CH.NH.C_6H_4SO_2C_6H_4NH.CH.CH_2CH.C_6H_5$$
$$\quad\quad|\quad\quad\quad|\quad\quad\quad\quad\quad\quad\quad|\quad\quad\quad|$$
$$\quad SO_2Na\ SO_2Na\quad\quad\quad\quad\quad SO_2Na\ SO_2Na$$

Molecular weight = 892.5

Typical migrating boundaries may be obtained by electrophoresis, and analysis by this means has enabled Mr. P. A. Charlwood, of these laboratories, to say that the drug as prepared is not homogeneous but consists of a major component of some 94 per cent and a minor component of 6 per cent.

The compound is insoluble in alcohol and other organic solvents, but is exceedingly soluble in cold water to give a syrup. Twenty and forty per cent (w/v) solutions are stable when neutral or slightly alkaline and may be autoclaved. Boiling with normal acid yields a brown coloured complex of high molecular weight and only by more drastic hydrolysis is it possible to recover a small proportion of the whole as diaminodiphenylsulphone.

Estimation of sulphetrone

Sulphetrone was estimated in blood, urine, cerebrospinal fluid and tissues by diazotization and coupling to N-(1-naphthyl)-ethylenediamine hydrochloride (Bratton and Marshall, 1939) and estimating the pigment colorimetrically or, preferably, absorptiometrically. Although the facility with which derivatives of diaminodiphenylsulphone are adsorbed by undenatured protein is not shared by sulphetrone, admixture with precipitated proteins is intimate, and therefore the conditions governing the optimum recovery of drug were determined experimentally. When the following conditions are rigidly adhered to, 90 per cent recoveries of drug are obtained; the over-all dilution of 1 in 15 and the concentration of acid are critical: 0.5 c.c. blood, or other body fluid, is added to 5 c.c. *N*/1 HCl and mixed well; 2.0 c.c. 12 per cent (w/v) trichloroacetic acid are added, and the well-mixed solution filtered immediately through

* The development of sulphetrone is part of the programme of work on antituberculous compounds carried out by the Therapeutic Research Corporation of Great Britain, Limited.

a No. 5 Whatman paper and repassed until brilliant; 3 c.c. of filtrate are mixed with 0.05 c.c. 0.3 per cent fresh (weekly) sodium nitrite and left 3 min.; 0.05 c.c. 1.5 per cent ammonium sulphamate is now added and the solution left 2 min.; finally, 0.05 c.c. 0.1 per cent N-(1-naphthyl)-ethylenediamine hydrochloride is added and mixed well. The colour should be allowed to develop for 30 min. before being read colorimetrically or absorptiometrically with a Wratten 61 filter.

The visible absorption spectra of the naphthylethylenediamine derivatives of diaminodiphenylsulphone and sulphetrone are shown in Fig. 1. The colour intensity of the sulphetrone-dye complex is much less than that of the parent substance, although the optimum conditions for colour production were found experimentally and the concentrations were adjusted to be approximately equivalent; this suggests that sulphetrone may not be readily hydrolysed, a suggestion borne out by the pharmacological evidence. The ultra-violet absorption spectra of the derivative and the parent compound are included in Fig. 1.

All estimations of sulphetrone are given in terms of the anhydrous compound.

By collaboration with The Tintometer, Ltd., a standard Lovibond colour disc is available for the rapid reading of blood and body-fluid estimates falling within the range of 0 to 9 mg. per 100 c.c.

Toxicity

Acute toxicity

A 10 per cent (w/v) solution of sulphetrone is isotonic with 0.91 per cent sodium chloride, and hypertonic solutions up to 60 per cent (w/v) are readily obtained. The need to administer large volumes of these grossly hypertonic solutions, together with poor absorption from the gut and low intrinsic toxicity, make the determination of acute toxicity in precise figures of questionable significance.

The results of an estimate of oral toxicity are shown in Table I. All mice receiving 2.0 g. per kg. as 60 per cent solution, and those which died after 1.75 and 1.50 g. per kg., died quickly, after collapse and circulatory failure. *Post mortem* there was gross dehydration and collapse of major blood vessels, the stomach and gut were distended and the contents contained blood. The opinion was formed that the hypertonicity of the solutions made a major contribution to the death of the animals. For example, the blood concentrations at death after the three higher doses appear to be related to hypertonicity and time of death, and are in marked contrast to the concentration found at the fifth hour in the fourth group.

TABLE I

Toxicity for mice of sulphetrone given orally in solution

Dose g. per kg.	Dead/Total	Time of death in hours	Mean blood concentration at death; mg. per 100 c.c. ± S.D.
2.00	20/20	0.75	960 ± 330
1.75	12/20	1.50	2700 ± 874
1.50	4/20	within 5.00	240 ± 19.9
1.25	0/20	—	Mean blood level of all survivors at 5 hours: 14 ± 2

Intravenous administration of a 20 per cent (w/v) solution caused deaths in groups of mice at

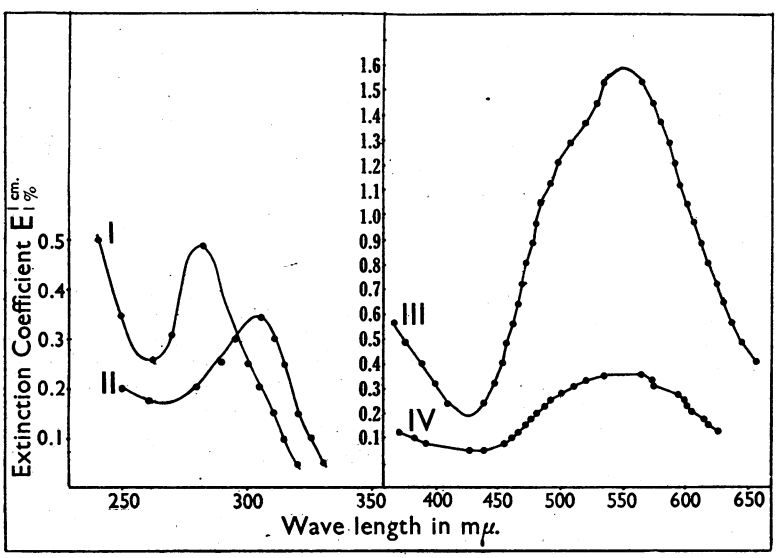

FIG. 1.—Spectrometic measurements of diaminodiphenylsulphone I and sulphetrone II in the ultra-violet, and of diaminodiphenyl-sulphone-naphthylethylenediamine dye III and sulphetrone-naphthylethylenediamine dye IV in the visible wavelength. Ordinates, extinction coefficient, $E^{1cm}_{1\%}$. Abscissae, wavelength, in mμ. I, 1.018 mg. per 100 c.c. *N*/10 HCl; II, 1.022 mg. in 100 c.c. H_2O; III, 0.25 mg. per 100 c.c. *N*/1 HCl; IV, 0.8 mg. per 100 c.c. *N*/1 HCl.

doses of 2.5, 2.75, and 3 g./kg. but not at 1.5 g./kg., at which dose level the mean blood concentration was 650±160 mg. per 100 c.c. Most deaths took place within one minute. The mice became cyanosed, involuntary spasms were overtaken by epileptiform convulsions which passed to extensor hind-limb paralysis; dyspnoea was followed by apnoea. There were a number of late deaths (1–2½ hours). The results are set out in Table II. Computed by the method of Bliss (1938) the LD50 is 2.7 g. with a range of 2.4 to 3 g. per kg.

TABLE II

Toxicity for mice of sulphetrone given intravenously in solution

Dose g./kg.	Dead/ Total	Time of death	Mean blood concentration at death; mg. per 100 c.c. ± S.D.
2.5	6/20	4 in 1 min. 2 in 2.5 hrs.	1000 ± 200
2.75	14/20	10 in 1 min. 4 in 1 hour	1150 ± 240
3.00	14/20	14 in 1 min.	1400 ± 400

A dog was given 1 g. sulphetrone per kg. intravenously in 40 per cent (w/v) solution (a total of 10 g.); the resulting blood concentrations were 61.8 mg. per 100 c.c. at 15 min., 14 mg. at 1 hour, 18 mg. at 2 hours, and 6.8 mg. at 24 hours. It will be seen that urinary clearance was rapid. Apart from lethargy and unsteadiness of gait, no symptoms were observed until after one hour, when the animal had a rigor with tachycardia lasting for 15 min. Two hours after the injection the animal was in high spirits. Intraperitoneal injection of 2 g. per kg. in 40 per cent (w/v) solution was followed by essentially the same symptoms and resulted in a blood level of 96.4 mg. per 100 c.c. at 15 min., 18 mg. at 1 hour, and 2 mg. at 24 hours.

After 2 g. per kg. intravenously the dog collapsed for a brief period 20 min. after the injection; 20 min. later there was a rigor and vomiting and a period of tachycardia for 15 min. The animal was lethargic but recovered, and appeared normal in 2 hours. Blood levels were 160 mg. per 100 c.c. at 15 min., 38 mg. at one hour, and 2.5 mg. at 24 hours.

Experiments on the effect on alkali reserve, described later, suggest that some but not all of the immediate effects after large intravenous doses may be associated with increased plasma alkali. However, since blood concentrations of the simple sulphonamides of half this magnitude cause severe symptoms and death in dogs, it is apparent that sulphetrone has little acute toxicity in the sense in which this term is normally used.

Chronic toxicity

Four adult rabbits of mixed sexes weighing 2.5–3 kg. were kept in metabolism cages for 40 days, during which period they received a diet containing 4 per cent sulphetrone and consisting of bran, sugar-beet pulp, and water. Two additional rabbits were fed upon the diet without drug.

A record was made of the drug intake, together with daily estimations of the concentrations of drug in blood, urine, and faeces. Estimations were made of reticulocytes, erythrocytes, total

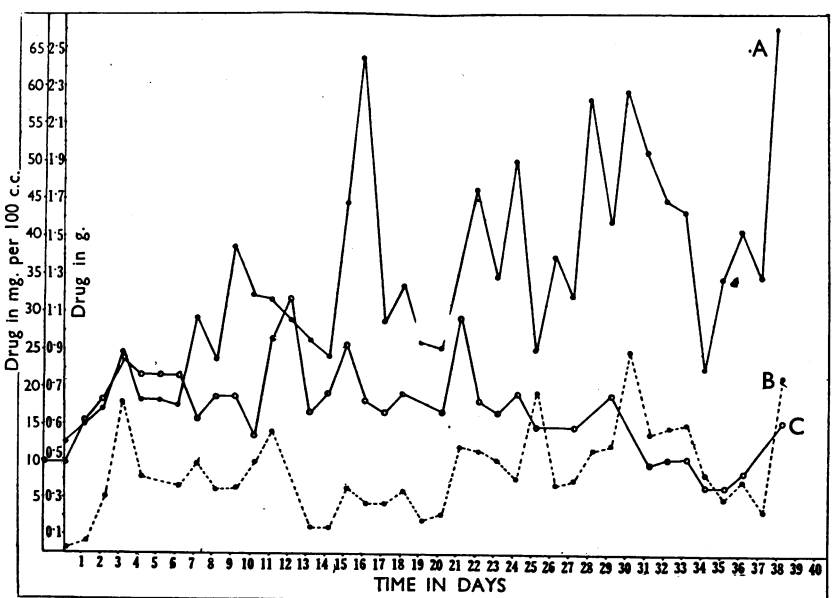

FIG. 2.—Sulphetrone drug-balance experiments in a group of four rabbits receiving 4 per cent of drug in the diet. A, average daily drug in urine and B, average daily drug in faeces, the ordinates being drug in grams. C, average daily blood concentration, the ordinates being drug in mg. per 100 c.c. Equilibrium is established after 3 or 4 days. Between days 6 and 20, about one-third of the drug is unabsorbed; subsequently this figure drops to about one-quarter.

B

white-cell count, and haemoglobin concentration. Although no abnormalities were detected in the white-cell count, progressive hypochromic anaemias were observed in 3 out of 4 rabbits. In one rabbit this was the cause of death at the 15th day. In Fig. 2 are graphed the mean amounts of drug in urine (A) and faeces (B) and the blood level (C) in mg. per 100 c.c. Equilibrium was achieved after about three days. At first about 1/3, but later only about 1/4, was unabsorbed, After equilibrium had been achieved the blood level averaged 18.6 mg. per 100 c.c. ± S.D. 6.0.

Treatment of hypochromic anaemia in rabbits

During the first experiment on rabbits no abnormal excretion of blood pigments was observed. Occasional (8) estimates of methaemoglobin by Zeiss pocket spectroscope were negative. Although the urine was darker in colour than usual no abnormal urobilin excretion was observed. Soon after the administration of the drug there was a high colour index, but this shortly fell, and the anaemia became hypochromic in type. Reduced haematopoiesis rather than abnormal degradation was suspected.

In a second experiment four adult rabbits, two male and two female, of 2–2.5 kg. weight, were fed for 25 days with a diet of bran, sugar-beet pulp, water, and 4 per cent of sulphetrone. In addition to estimates of drug balance, daily estimates were made of reticulocytes, erythrocytes, haemoglobin, and urobilin. Estimates of total urinary porphyrins excreted and of methaemoglobin were also made. The observations are summarized in Fig. 3. A slight though significant increase in urobilin occurs at once and is continued throughout the experiment; reticulocytosis indicates an attempt to make good the deficiency of cells, but an increase in colour index shows this to fail. This state corresponds to a slight but persistent haemolytic anaemia. At this stage a precipitous anaemia with a raised colour index intervenes; this responds to iron, peroral or parenteral, but only to a limited degree.

Absorption of the iron salt of sulphetrone

The fact that iron by mouth stimulated haematopoiesis suggested a direct interference by sulphetrone with iron metabolism. The iron salt was prepared by Mr. W. H. Gray of the Wellcome Laboratories for Research in Tropical Medicine, and its absorption after oral doses was compared with sulphetrone. Given orally the iron complex is completely unabsorbed; parenterally it is distributed like sulphetrone. This finding taken with the evidence of haematopoiesis after oral iron (Fig. 3) indicates that sulphetrone combines with alimentary iron and prevents its absorption.

Modification of intestinal flora by sulphetrone

At this stage, as a result of comparative tests with mice (Brownlee and Tonkin, 1941) the marked ability of sulphetrone to modify the intestinal flora was appreciated. The extent to which many animals, and particularly ruminants, rely on biosynthesis for growth factors, some of which are vitamins, suggested a third experiment with the same diet but containing 10 per cent of dried yeast. Four adult rabbits, male and female, weighing 2.5–3.5 kg. were kept in metabolism cages for 39 days on the modified diet containing 10 per cent dried yeast. The drug balance figures were essentially similar to those of the first experiment. The animals maintained good condition and put on weight; at autopsy on the 45th day, no gross changes were seen in any of them. Histology of spleen, kidney, and red bone-marrow showed a normal picture. Slight cloudy swelling of some peripheral liver cells was the only abnormality observed. The results of blood examination (Fig. 4) justify the conclusion that the gross anaemia previously observed arose directly from a lack of an essential metabolite previously furnished largely by biosynthesis which was prevented by unabsorbed sulphetrone residues and was now furnished by dried yeast. When this form of anaemia is excluded hypochromic anaemia attributable to iron lack is still present. On the third day after withdrawal of drug the residual haemolytic anaemia was made good.

Specific toxic effects

It is known that sulphonamide drugs share with certain thioureas the property of causing hyperaemia and hyperplasia of the thyroid gland in some animals and in man, when they are administered at dose levels and for times similar to those in therapeutic use. The hyperplasia is believed

TABLE III

Goitrogenic effect of drugs fed to groups of five littermate rats as 1 per cent of the diet, for 17 days

Groups	Average body weight, g.		Deviation weight per cent	Thyroid hyperaemia	Thyroid weights mg. per 100 g.
	Initial	Final			
Control	47.2	97.5	103	—	10.1
Sulphetrone ..	45.7	92.0	100.5	+	13.6
Sulphadiazine ..	48.0	88.0	83	+	17.6
Sulphaguanidine	55.0	93.8	70	++	20.1

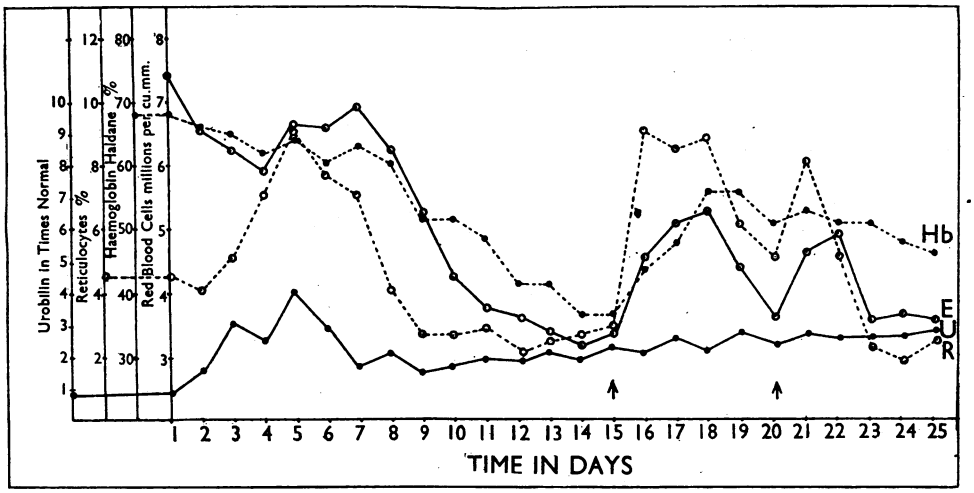

FIG. 3.—Typical response of a rabbit (335 ♂) to a 4 per cent sulphetrone diet. Hb is the haemoglobin estimate (Haldane); E, red blood cells in millions per cu.mm.; R, reticulocytes in per cent of red blood cells; U, spectrometric estimates of urobilin (Watson, 1936) in times normal. At each arrow, 10 mg. of iron was given by mouth, in the form of iron ammonium chelidamate.

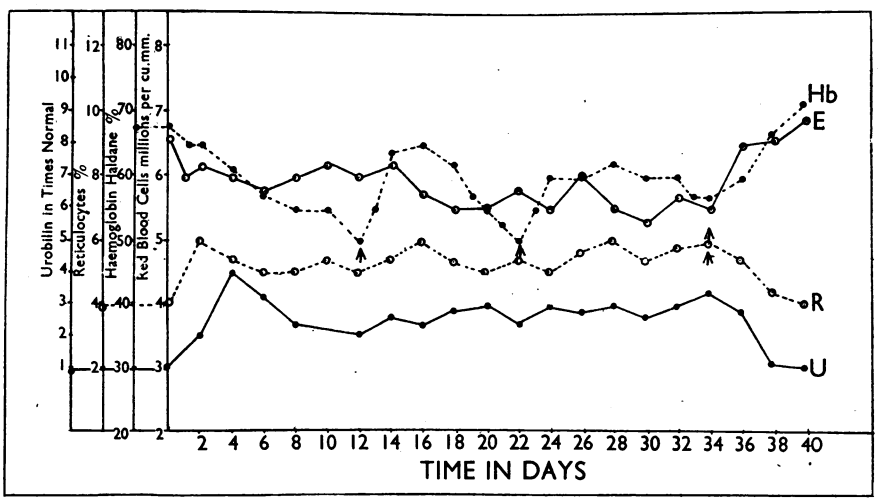

FIG. 4.—Typical response of a rabbit (336 ♂) to a 4 per cent sulphetrone diet, but containing in addition 10 per cent of dried yeast. Hb, is the haemoglobin estimate (Haldane); E, red blood cells in millions per cu.mm.; R, reticulocytes in per cent of red blood cells; U, urobilin by spectrometric estimates of urobilin (Watson, 1936) in times normal. At each single arrow, 10 mg. of iron, in the form of iron ammonium chelidamate was given by mouth. At the double arrow drug was withdrawn.

(Astwood, Sullivan, Bissell, and Tyslowitz, 1943) to result from a hypothyroidism produced by failure to synthesize thyroid hormone. The goitrogenic activity decreases from sulphadiazine, sulphapyridine, sulphathiazole, sulphaguanidine, sulphanilylurea, sulphanilamide to sulphasuxidine (MacKenzie and MacKenzie, 1943).

Sulphetrone was tested for this specific toxic effect together with sulphadiazine and sulphaguanidine in litter-mate groups of five immature female rats of Wistar strain fed on a powdered diet containing 1 per cent of the drugs for a period of 17 days. The thyroids were removed, weighed, and studied histologically. The results summarized

in Table III show a decrease in goitrogenic activity from sulphaguanidine and sulphadiazine to sulphetrone, which shows a slight toxic effect.

Effect on normal growth of rats

The effect of feeding a synthetic diet containing 1 per cent of sulphetrone and 0.01 per cent of iron to litter-mate groups of rats for 78 days is summarized in Table IV. Both the female group and the male group were retarded in growth for the first two weeks, but thereafter increased in weight by normal increments so that graphs of the growth-rates followed parallel courses. The weight increases of the thyroid glands are not significant in comparison with those produced by treatment with sulphonamides.

TABLE IV

The effect on the growth-rate, and thyroid weights, of a 1 per cent sulphetrone diet fed to two litter-mate groups, male and female, of rats, for 78 days. Male and female control groups are included

Rats Litter-mate groups of eight	Average weight in g.		Thyroid weights mg. per 100 g. mean ± S.D.
	Initial	After 78 days	
Drug 1% ♂	34.0	180.6	9.2 ± 0.8
Controls ♂ ..	33.9	192.1	8.9 ± 0.7
Drug 1% ♀	30.9	138.0	11.5 ± 1.1
Controls ♀ ..	32.8	149.1	9.9 ± 0.8

Effect on alkali reserve

The evidence of acute and chronic toxicity, together with the spectrometric evidence of the difference between the dye-coupled products of sulphetrone and of diaminodiphenylsulphone, shows that sulphetrone is not hydrolysed to diaminodiphenylsulphone in the body. Since large amounts of the drug pass through the blood stream, the probable effects of hydrolysis of one or more of the sodium sulphonate radicles on the alkali reserve were investigated. The results, which were unexpected, are discussed later.

Groups of adult rabbits of mixed sex were maintained on a diet of bran and oats containing 50 per cent of water for one week to stabilize their metabolism, and were then given a single oral or parenteral dose of 1 g. sulphetrone per kg. as a 50 per cent (w/v) solution. At intervals blood was withdrawn from the heart into an oxalated syringe, immediately centrifuged under paraffin, and the CO_2 capacity determined by the volumetric method of Van Slyke. The results (Table V) show a rise in the alkali reserve after an oral dose of sulphetrone in one of two experiments, and after an intraperitoneal injection in two of three experiments.

TABLE V

Plasma CO_2—combining capacity, in volumes per cent, after oral and intraperitoneal doses of 1 g. per kg. sulphetrone in rabbits

Rabbit	CO_2 capacity of plasma (vols. %) after						Mean blood concentration mg. per 100 c.c. ± S.D.
	0 hr.	1 hr.	2 hr.	4 hr.	6 hr.	24 hr.	
1 intraperitoneal	32.9	39.6	45.4	52.1	44.3	—	75 ± 25
2 intraperitoneal	32.0	40.6	42.5	42.5	47.4	41.3	40 ± 32
3 intraperitoneal	40.6	39.0	36.0	40.3	40.6	—	—
4 oral	40.6	39.0	36.0	40.3	40.6	—	6.0 ± 6
5 oral	38.1	41.0	47.0	48.5	40.3	—	2.5 ± 1

The effect of parenterally administered drug is the same in the dog (Table VI) and it is interesting that the effect of 1 g. sulphetrone per kg. is approximately matched by 94 mg. sodium bicarbonate per kg. injected intravenously. This equivalence corresponds to the sodium of one of the four sodium sulphonate radicals being set free as sodium hydroxide. Dogs injected intravenously with this dose of sodium bicarbonate are subdued and

TABLE VI

Plasma CO_2—combining capacity, in volumes per cent, after parenteral sulphetrone or sodium bicarbonate in dogs

Dog	CO_2 capacity of plasma (vols. %) after						Mean blood concentration mg. per 100 c.c. ± S.D.
	0 hr.	1 hr.	2 hr.	4 hr.	6 hr.	24 hr.	
A. Sulphetrone 2g./kg.I.P.	53.1	—	56.7	57.1	57.1	57.3	17.7±12
B. Sulphetrone 1g./kg.I.V.	54.6	62.0	68.5	52.5	61.5	65.0	8.5±4
C. Sulphetrone 1g./kg.I.V.	43.5	46.5	49.0	51.0	52.0	—	22.0±16
D. Sodium bicarbonate 94 mg./kg. I.V.	40.9	48.9	50.4	48.1	—	—	—
E Sodium bicarbonate 188 mg./kg. I.V.	43.6	55.1	52.0	53.5	51.7	—	—

lethargic for about 30 minutes but show no other signs of toxicity.

The alkali reserve after repeated doses of sulphetrone

For the purpose of a "chronic" test a total daily dose of 0.5 g. sulphetrone per kg. was given orally in 2 portions as a 5 per cent (w/v) solution. Five adult rabbits fed on a diet of bran, sugar-beet pulp, and water were divided into groups, one of which received drug and the other water for a period of 7 days. Eighteen hours after the last treatment the CO_2 capacity of the plasma was estimated. Treatment was now resumed so that rabbits which had been given water now received sulphetrone and vice versa. The plasma CO_2 capacity, together with the blood concentrations of sulphetrone at the end of four weekly periods, are given in Table VII, which shows that when repeated doses are given from day to day equilibrium is achieved.

TABLE VII

The CO_2 capacity of the plasma of rabbits, as volumes per cent, after repeated doses of sulphetrone in a "cross-over" test

Rabbits	CO_2 capacity of plasma as volumes per cent after		Blood concentrations of drug, mg. per 100 c.c.
	Water 5 c.c./kg. twice daily	Sulphetrone 5 c.c. of 5% twice daily	
4	55.0	33.0	10.7
5	28.5	44.5	6.0
6	46.0	48.0	8.0
7	47.0	35.0	8.8
8	41.5	59.0	9.0
9	46.0	51.0	5.2
10	37.0	39.5	8.8
11	50.0	—	—
12	53.0	51.5	3.0
13	49.5	41.0	9.0
Mean value ± S.D.	45.3 ± 8.1	44.7 ± 8.4	7.6 ± 2.5

Absorption and Excretion

When sulphetrone in solution was given by mouth to groups of mice, the blood concentration–time curves were about the same for large and small doses. Typical results are given in Fig. 5, curves for intravenous and intraperitoneal injections being also included; curves for oral doses of diaminodiphenylsulphone and sulphanilamide are included for comparison.

A closer relation between large and small doses is seen in dogs after single doses given orally.

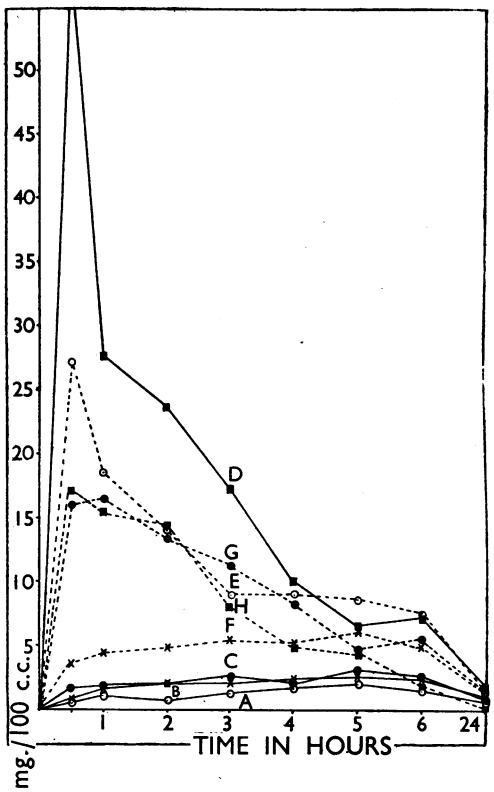

FIG. 5.—Blood concentration-time curves after sulphetrone, diaminodiphenylsulphone and sulphanilamide. Each curve is the mean of determinations on five fasting mice. A, sulphetrone, 0.1 g./kg. by mouth. B, same, 0.5 g./kg. C, same, 1.0 g./kg. D, same, 0.1 g./kg. as 40 per cent solution intravenously. E, same, 0.1 g./kg. as 40 per cent solution intraperitoneally. F, diaminodiphenylsulphone, 0.1 g./kg. suspended in acacia by mouth. G, same, 0.25 g./kg. H, sulphanilamide, 0.1 g./kg. suspended in acacia by mouth.

Blood and urine concentrations after intravenous doses are also included in Table VIII.

The total quantity excreted in the urine is given in per cent of the amount administered. It is interesting that although drug given intravenously is cleared from the blood stream rapidly and excreted almost completely in the urine in 24 hours, only 75 per cent of an oral dose is excreted in the same time. Evidence is given later that poor absorption from the bowel, and in particular from the large intestine, may account for the remainder.

Blood concentration–time curves obtained in a dog after an oral dose, and after the same dose given in three divided portions at three-hourly in-

TABLE VIII

ABSORPTION AND EXCRETION OF SULPHETRONE IN DOGS

Dog	Dose g./kg.	Blood concentrations, in mg. per 100 c.c. blood, at hours:							Excreted in per cent of total dose at hours:			
		0.25	1	2	3	4	6	24	2	3	6	24
Terrier 1	0.1	1.4	2.8	4.1	3.8	2.8	1.2	0.1	20.4	50.1	62.7	76.0
	0.2	0.9	3.3	—	3.2	1.6	1.4	0.3	19.4	39.2	49.6	67.4
	0.5	1.8	7.0	7.2	4.5	4.2	2.2	0.0	23.2	29.8	64.4	71.2
	1.0	1.6	7.2	9.4	7.2	5.2	4.1	0.2	24.3	49.7	59.4	73.9
Greyhound 2	0.2	0.4	2.8	4.6	4.5	3.9	2.7	0.1	19.8	37.4	49.2	67.8
	0.5	0.2	6.2	6.3	4.8	4.1	1.2	0.2	18.2	39.4	62.4	78.4
Mongrel 3	0.5	1.0	4.2	3.9	2.6	1.9	2.1	0.4	17.2	28.4	42.6	66.4
	1.0	0.2	4.7	4.9	6.2	5.3	4.0	0.2	24.6	33.2	47.6	75.2
Terrier 1*	0.1	12.1	4.4	3.2	1.8	1.4	1.0	0.6	69.7	69.9	82.6	98.6
	0.2	20.5	6.0	4.5	3.4	2.4	1.8	0.8	64.2	68.4	78.4	96.4

* Intravenously

tervals (Fig. 6), suggested that absorption took place high in the alimentary tract. An experiment in which the drug was injected first into the large and then into the small intestine supports the view that absorption is largely confined to the small intestine (Fig. 7).

Renal clearance in the rabbit

The essential preliminary to a study of the elimination of sulphetrone from the body is a knowledge of its mode of excretion by the kidney. In a study in the dog, Marshall, Kendall, and Cutting (1937) found the clearance of sulphanilamide to be 20 to 30 per cent of a simultaneously determined creatinine clearance. In an experiment in which a group of three rabbits was used the clearance of sulphetrone was 58 per cent that of creatinine, or two to three times as fast as sulphanilamide. The result was the same whether the plasma concentration of the drug was produced by prolonged administration for 40 days or by a single dose. A typical estimate based on single doses is shown in Table IX. Each ratio is a mean of six periods of one experiment, thus Rabbit X, 0.62 ; Y, 0.63 ; and Z, 0.5 ; giving a grand mean of 0.58.

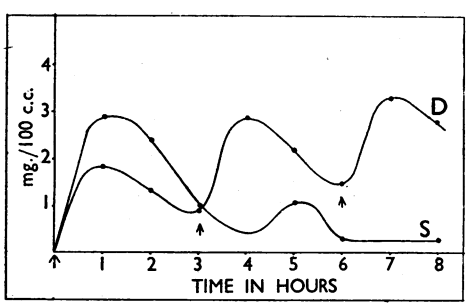

FIG. 6.—Blood concentration-time curves in a dog after sulphetrone by mouth. S, a single dose of 0.2 g. per kg. in 60 per cent solution at first arrow. D, a divided dose of 0.1 g. per kg. given at each arrow.

Renal clearance in the dog

Renal clearance for the dog has been calculated from data, some of which are given in the last

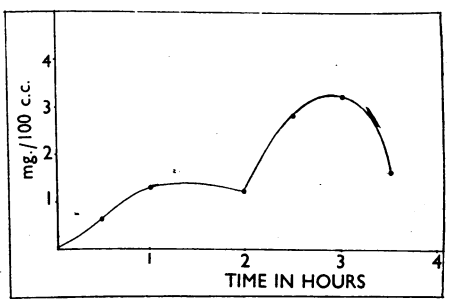

FIG. 7.—Blood concentration-time curves showing absorption from large and small intestines. Dog anaesthetized with pentobarbitone-sodium. Tube passed through oesophagus and pylorus into duodenum, and pylorus tied; a second tube passed through anus into the descending colon, the tube being tied at the anus to prevent leakage and the ileocaecal junction tied. Sulphetrone 0.1 g. per kg. in 40 per cent solution introduced by the oral tube and washed out after two hours; 0.1 g. per kg. then introduced into the duodenum.

TABLE IX

SULPHETRONE AND CREATININE CLEARANCE IN THE RABBIT

The rabbit weighed 2.2 kg. and received 1 g. of creatinine and 3 g. of sulphetrone dissolved in 25 c.c. of water. This quantity represented the third of three volumes of water given at 20 minute intervals

Period	Mins.	Urine A c.c./min.	Creatinine mg./100 c.c. B plasma	Creatinine mg./100 c.c. C urine	Sulphetrone mg./100 c.c. B plasma	Sulphetrone mg./100 c.c. C urine	Clearance $\frac{C \times A}{B}$ Units per min. Creatinine	Clearance $\frac{C \times A}{B}$ Units per min. Sulphetrone	Ratio Sulphetrone / Creatinine
1	27	0.57	9.2	350	4.1	102.0	2.15	1:4	0.65
2	37	0.39	11.6	133	7.5	56.6	4.45	2.94	0.66
3	30	0.25	12.0	160	8.5	59.2	3.35	1.75	0.52
4	23	0.83	11.2	265	8.9	46.7	19.5	4.3	0.22
5	60	0.34	10.1	240	4.0	171.5	8.2	6.44	0.78
6	60	0.37	8.3	270	8.9	232.4	11.5	9.6	0.98

experiment in Table VIII. Six determinations gave a mean clearance ±S.D. of 85.2±14.3 units ; six sulphanilamide clearances on the same animal gave a mean clearance ±S.D. of 17.9±3.1 units. Accepting creatinine clearance as a rate of glomerular filtration it seems that some 40 per cent of sulphetrone is resorbed in the passage of glomerular filtrate along the tubules in the rabbit, while in the dog the quantity resorbed is very slight.

Distribution of sulphetrone in tissue

The penetration of sulphetrone into the tissues, with the exception of brain and cerebrospinal fluid, is rapid and complete. The distribution and concentration is the same whether the drug is given in the diet for prolonged periods or acutely by one intravenous dose (Table X).

The similar tissue concentrations in animals receiving drug by one intravenous dose, and in the diet for 10 weeks, point to the importance of renal clearance as one major controlling factor. In this connection the low concentration in plasma compared with liver and kidney, but not bone-marrow or lung, indicates that the liver also is functioning as an organ of concentration and excretion. The high concentration in bile supports this view. That neither the rate of excretion nor the route are the sole controlling factors may be deduced from Table XI, where the distribution in the tissues of the nephrectomized rabbit is given. In spite of striking increases in the plasma concentration

TABLE X

The penetration of sulphetrone into various tissues in the rabbit and the dog, expressed as mg. per 100 c.c. of fluid and mg. per 100 g. of tissue

Tissue	Rabbit (2 kg.) 1% sulphetrone in diet 10 weeks	Rabbit (1.75 kg.) 100 mg. sulphetrone per kg. intravenously. Estimates at 2 hrs.	Dog (12 kg.) 100 mg. sulphetrone per kg. intravenously. Estimates at 2 hrs.
Blood	3.3	3.8	4.1
Plasma	6.3	7.4	6.7
Corpuscles	0.8	1.2	1.1
C sternal fluid	1.1	0.4	1.5
Bile	80.0	92.0	79.0
Liver	14.0	12.9	11.5
Kidney	13.1	15.3	20.4
Spleen	7.1	6.2	9.8
Lung	4.8	4.1	6.2
Bone-marrow	3.1	2.4	0.8
Striated muscle of thigh	1.6	2.0	6.3
Ileum, empty	8.6	1.6	4.5
Cerebral hemispheres	0.9	0.5	1.9
Vitreous humour	0.8	0.7	0.4
Fat	0.9	1.1	1.2

TABLE XI

The distribution of sulphetrone in the tissues of the nephrectomized rabbit at 2 hours after 100 mg. per kg. intravenously. Kidneys were removed under pentobarbitone-sodium, 30 mg. per kg.

Tissue	Rabbit (1.65 kg.) mg./100 g. or c.c.	Rabbit (1.60 kg.) mg./100 g. or c.c.	Rabbit (1.70 kg.) mg./100 g. or c.c.
1 Hour			
Blood	20.5	22.9	19.5
Plasma	42.6	53.8	35.5
Corpuscles	—	12.4	5.5
2 Hours			
Blood	30.8	—	21.2
Plasma	39.2	43.0	35.0
Corpuscles	—	6.2	4.6
Cisternal fluid	1.3	trace	trace
Bile	136.0	—	170.0
Liver	10.3	15.0	11.9
Spleen	6.5	7.5	8.1
Lung	11.8	18.6	13.8
Bone-marrow femur	11.0	12.5	8.1
Striated muscle of thigh	4.4	3.0	2.6
Ileum, empty	8.8	11.1	8.5
Cerebral hemispheres	1.8	1.2	0.9
Vitreous humour	1.3	—	—

(about five times) little change is reflected in the concentrations in the viscera. Biliary excretion is significantly high and concentration of drug in the ileum indicates that this, too, is an organ of excretion.

Other pharmacological properties

Examined by the usual pharmacological methods, sulphetrone is inert. Thus, a solution of 1 in 1,000 has no action on the isolated guinea-pig uterus or on the rabbit intestine *in vitro* at 37.5° C., or on the frog heart perfused through the vena cava. A cat anaesthetized with phenobarbitone-sodium (30 mg. per kg.) and given 0.2 g. sulphetrone per kg. intravenously showed no demonstrable effect on blood pressure, heart rate, respiratory frequency, or volume.

Experimental Therapy

Antibacterial activity

Our procedure for measuring antibacterial activity of sulphonamide-type compounds *in vitro* consists of observing the effect of exposing constant inocula of selected pathogens to successive dilutions of known concentrations of test and standard drug in media containing 10 per cent of blood. Results of tests to determine the limiting inhibitory concentrations of sulphetrone, promin, and diaminodiphenylsulphone in Wright's broth containing 10 per cent of blood against Gram-positive and Gram-negative organisms, and in Long's medium containing 10 per cent of blood against *M. avium* and *M. tuberculosis* var hominis and var bovis, are summarized in Table XII. Initial drug concentrations of 2.0 g. per litre were made and diluted out in the medium to obtain a series of drug concentrations which decreased by multiples of two. Drug solutions in the test medium were autoclaved, mixed with blood, and inoculated with 0.2 c.c. of a suitably diluted culture. Size of inoculum and conditions of test were such as to demonstrate antibacterial activity under conditions which permitted turbid growth in the control tubes in less than 24 hours. Initial bacterial viable counts in test mixtures were made by roll-tube counts.

TABLE XII

The limiting inhibitory concentrations of diaminodiphenylsulphone, sulphetrone, and promin against different organisms *in vitro*

Organisms	Diaminodiphenylsulphone	Sulphetrone	Promin
Str. pyogenes CN. 10	2^{-9}	2^{-7}	
D. pneumoniae CN. 33	2^{-6}	2^{-4}	
S. aureus CN. 491	2^{-3}	2^{-1}	
S. salivarius CN. 30	2^{-3}	2^{-1}	
,, ,, CN. 199	2^{-11}	2^{-9}	
,, ,, CN. 13	2^{-3}	2^{-6}	
E. typhosa CN. 512	2^{-4}	2^{-1}	
Sh. sonnei CN. 188	2^{-3}	2^{-2}	
Sh. dysenteriae CN. 191	2^{-4}	2^{-4}	
Sh. paradysenteriae CN. 185	2^{-6}	2^{-4}	
E. coli CN. 150	2^{-4}	2^{-2}	
V. comma CN. 249	2^{-6}	2^{-4}	
Cl. perfringens CN. 1491	2^{-3}	2^{-2}	
Cl. septicum CN. 368	2^{-8}	2^{-7}	
Cl. novyi CN. 735	2^{-2}	2^{-1}	
M. avium CN. 281	2^{-7}	2^{-6}	2^{-5}
,, ,, CN. 280	2^{-6}	2^{-5}	2^{-4}
M. tuberculosis var hominis			
CN. 844	2^{-4}	2^{-3}	2^{-2}
CN. 271 (H37)	2^{-6}	2^{-5}	2^{-4}
CN. 1877	2^{-6}	2^{-5}	2^{-4}
CN. 1878	2^{-4}	2^{-3}	2^{-2}
M. tuberculosis var bovis			
CN. 858	2^{-4}	2^{-3}	2^{-3}
CN. 868	2^{-6}	2^{-5}	2^{-4}

Reversal by p*-aminobenzoic acid*

Titrations in a semi-synthetic reinforced gelatine-hydrolysate medium containing added cystine and tryptophane, together with the essential growth factors, and inoculated with 1,300 viable organisms of *S. pyogenes* N.10, enabled the concentration of *p*-aminobenzoic acid which just reversed the antibacterial action of sulphetrone to be found. The figure for eight estimates, derived from tenfold dilutions of *p*-aminobenzoic acid from 10^{-3} to 10^{-10}, was $1 : 5{,}000 \pm 500$.

Streptococcus and pneumococcus mouse infections

The chemotherapeutic activity of sulphetrone in mice against a β-haemolytic streptococcus infection (CN.10) and against a Type 1 pneumococcus infection (CN.33) was assessed in a survival test experiment in which groups of 30 mice receiving a diet containing 2 per cent of sulphetrone or of other sulphonamides were inoculated with a number of lethal doses of the test organisms. Drug diet intakes and blood concentrations were measured at 14 days after infection, at which time treatment was terminated. Deaths were recorded daily and the cause of death ascertained by blood culture; survivors were observed for a further seven days. The data from these experiments are recorded in Table XIII for the streptococcus test in which sulphanilamide was used for comparison. Sulphetrone is as good an antistreptococcal drug as sulphanilamide and in terms of a therapeutic factor *F*, obtained by dividing the mean free drug concentration in blood by the mean drug intake, it is more efficient. Rather surprisingly, in view of the efficiency of diaminodiphenylsulphone, sulphetrone has no antipneumococcal activity.

TABLE XIII

Three groups of 30 mice infected with 2 lethal doses (LD100) of *Streptococcus pyogenes* CN.10. All controls were dead within 48 hours and the survivors are shown after 21 days' treatment with 2 per cent drugs in the diet. /When the factor *F*, derived from groups of 25 mice on the 14th day, is considered, sulphetrone is the more efficient

Drug 2% in diet	Survivors	Mean drug intake mg.	Mean free drug. Blood conc. in mg. per 100 c.c.	$F = \frac{\text{blood conc.}}{\text{drug intake}}$
Sulphetrone	25/30	69.2 ± 9.5	11.4 ± 3.4	0.161 ± 0.031
Sulphanilamide	27/30	54.6 ± 3.8	18.4 ± 4.2	0.336 ± 0.057

In vivo–in vitro antituberculous test

Before proceeding to a full-scale guinea-pig protection trial it is our custom to exact the following minimum therapeutic requirement from a potential antituberculous drug. Guinea-pigs of 550–600 g. are injected intraperitoneally with 2 g. of the substance suspended in 10 per cent gum acacia and, after two hours or before if symptoms of acute toxicity are seen, they are anaesthetized, the thorax is opened and they are bled aseptically from the heart by Pasteur pipette. By drawing the specimen while the heart still beats, no difficulty is experienced in obtaining more than 3 c.c. of blood, which is stored in a sterile citrated bottle. The blood is diluted in serial increments with equal volumes of 0.5 c.c. of Long's agar contained in previously sterilized and stoppered Lambeth tubes. The tubes are sloped in the usual fashion and sown with 0.01 c.c. of a uniform suspension of *M. tuberculosis* containing 0.5 mg. per c.c. The tubes are incubated at 37.5° C. and inspected at intervals; the inhibition of growth is compared with the inhibition produced by diaminodiphenylsulphone tested in the same way at the same time. Avian strains are read at six days, and bovine and human strains at 21 days. A comparison of sulphetrone and diaminodiphenylsulphone is given in Table XIV.

TABLE XIV

Minimum effective drug blood concentrations in mg. per 100 c.c. blood (from serially diluted blood of a guinea-pig given drug parenterally) which just inhibit strains of mycobacteria

Concentration of drug in guinea-pig blood mg. per 100 c.c.	Diaminodiphenylsulphone	Sulphetrone
M. avium CN. 281	0.14	0.22
M. avium CN. 280	0.14	0.22
M. tuberculosis var bovis CN. 858	75	110
,, ,, ,, ,, CN. 868	19	55
M. tuberculosis var hominis CN. 844	75	110
,, ,, ,, ,, CN. 271 (H37)	19	55
,, ,, ,, ,, CN. 1877	19	110
,, ,, ,, ,, CN. 1878	75	110

Discussion

The preparation in these laboratories of diaminodiphenylsulphone and its characterization as a potent antibacterial and chemotherapeutic agent resulted in many attempts here and elsewhere to discover an equally potent but less toxic derivative. With the knowledge of its chemotherapeutic antituberculous effect the search was intensified. Sulphetrone was described in 1938 (Buttle *et al.*, 1938), when its chemotherapeutic antistreptococcal effect was appreciated, but it was not until 1941 that its chemotherapeutic antituberculous action was first realized. While sulphetrone was still under test, preliminary reports of promin (Feldman, Hinshaw, and Moses, 1941), the first diaminodiphenylsulphone derivative to be used in man for the treatment of tuberculosis, appeared. This drug, and the derivatives which have appeared since, diasone and promizole, are very toxic drugs when administered orally and may be given only for short intermittent periods. The fact that sulphetrone proved to be so relatively non-toxic, coupled with the hopes that clinical trials of this kind have always engendered, led us to the conclusion that all publication should be suspended until a mature appreciation could be given; with the publication of clinical reports this can now be done.

The antibacterial activity of sulphetrone approaches closely that of its parent substance, diaminodiphenylsulphone, and suggests that it may prove equally effective in the treatment of experimental tuberculosis in the laboratory animal.

The acute toxicity of sulphetrone when given by mouth is so slight that it cannot with certainty be determined, but the studies are complicated by the soluble nature of the drug which necessitated the administration of grossly hypertonic solutions. On the basis of blood concentrations sulphetrone would seem to be many times less acutely toxic than sulphanilamide. This is in marked contrast to the studies on acute toxicity undertaken with promin, diasone, and promizole.

The fact that sulphetrone is not acetylated, or conjugated in any other way, throws an interesting light on this lack of toxicity. The implication of this finding is that the drug is not hydrolysed to diaminodiphenylsulphone, an inference which is implicit in the lack of acute toxic symptoms observed in the dog with a very high blood level of

sulphetrone. Since in its suggested use, the problem of dosage is to maintain an effective consistent level in blood and other tissue for very long periods of time, we have been interested in the chronic toxicity of long-maintained blood levels.

Chronic toxicity experiments indicate that very large doses can be given by mouth to mice and dogs without producing symptoms or pathological changes in the tissue. With similar doses in rabbits, very interesting haematological changes are seen. First, there is a small but continuous haemolytic anaemia which is marked by a concurrent reticulocytosis. Secondly there is a progressive hypochromic anaemia of iron-lack. This anaemia is due to competition for alimentary iron by sulphetrone which forms an insoluble iron salt, which is not absorbed. It may be prevented or cured by the administration of iron, parenterally or orally.

The third anaemia which is seen is slower to appear but is then precipitous ; it is of nutritional origin and may be prevented or cured by the administration of dried yeast. Its probable cause is the limitation and alteration of the bacterial flora of the gut by the concentration of sulphetrone which is present.

A specific toxic effect which sulphetrone shares with all sulphonamides is the property of causing hyperaemia and hyperplasia of the thyroid gland after courses of treatment similar to those in therapeutic use. The hyperplasia is believed to result from hypothyroidism owing to failure to synthesize thyroid hormone.

Experiments in groups of rats maintained on a diet containing 1 per cent of the drugs for 17 days showed sulphetrone to be the least, and sulphaguanidine the most, toxic, while sulphadiazine was intermediate in this respect.

In a further study of chronic toxicity, it was observed that 1 per cent concentrations of drug in the diet of groups of rats retarded normal growth for an initial period of 14 days, but thereafter increases in weight followed the curve of normal increments during a 78-day period of observation. After this long period of treatment, the increases in the weight of the thyroids were not significantly greater than those in the control groups.

The lack of acute toxicity and the low chronic toxicity, with complete freedom from the toxic sequelae seen after administration of diaminodiphenylsulphone, make it clear that sulphetrone is not degraded to diaminodiphenylsulphone in the body. However, the possible hydrolysis of one or more of the four sulphonated side chains could not be overlooked and alkali reserve experiments were made to study this point. Simple hydrolysis resulting in the liberation of one or more molecules of sodium acid sulphate seemed the most likely event. Experiments in rabbits receiving oral and parenteral sulphetrone and experiments in dogs with the drug administered parenterally showed, however, that alkali was liberated in the blood stream, resulting in consistent increases in alkali reserve. Comparison with parenteral sodium bicarbonate makes it possible to say that the increase corresponds to one molecule of sodium hydroxide being liberated from each molecule of sulphetrone. A theoretical equation, involving the intervention of one molecule of water for the condensation of two molecules of sulphetrone through an $-SO_2$-O-SO_2- linkage with the liberation of two molecules of sodium hydroxide, may be proposed. $2R.SO_3Na + H_2O = R.SO_2OSO_2R + 2NaOH$. Experimental evidence in man points to the existence of a complex condensation product of this kind. When chronic tests were made on groups of rabbits on a " cross-over test " basis, no significant increases in alkali reserve were recorded eighteen hours after the last dose, at which time the average blood levels were 7.0 mg. per 100 c.c. It seems that under these conditions the normal animal is able to re-establish an equilibrium in its plasma alkali balance.

Reviewing all the evidence of toxicity in the experiments described, it seems safe to say that when given by mouth not only is sulphetrone the least toxic of the sulphones, but it is also less toxic than any of the sulphonamide drugs.

Experiments in mice and dogs in which single small or large doses are given show that after a certain point is reached large increases in dose do not result in either a higher blood concentration or increased absorption. This is not an unusual finding with sulphonamide drugs, but it seems to occur with sulphetrone at a lower level of dosage. The rabbit is anomalous in that increase in dosage increases the absorption and blood concentration of sulphetrone. The same total amount of drug given in divided doses at intervals of a few hours results in a higher blood concentration than when given as a single dose (dog). The explanation seems to be that this very soluble drug is slowly absorbed, and mainly from the small intestine. In addition, the rate of excretion of sulphetrone by the kidney is very fast. In rabbits the clearance of sulphetrone was 58 per cent that of creatinine or two to three times as fast as sulphanilamide, while the clearance in the dog was five times as fast as sulphanilamide. If creatinine clearance is accepted as a measure of the rate of glomerular filtration it seems that in the rabbit some 40 per

cent of sulphetrone is resorbed in the passage of glomerular filtrate along the tubules while in the dog the quantity resorbed is very slight. Some part, therefore, of the higher blood concentrations found in the rabbit is contributed by slower clearance.

Sulphetrone penetrates all tissues with extreme rapidity, with the exception of brain, but it enters the cerebrospinal fluid rather more slowly than do other sulphonamides.

The tissues of animals receiving the drug intravenously show similar concentrations to those receiving the drug in the diet for 10 weeks. In the normal animal concentration of drug in the liver, kidney, and spleen is always greater than in the plasma. The relation between absorbed drug, drug in transport, and drug in kidney clearance does not appear to be a simple one, and there appears to be an additional factor limiting drug concentrations in tissue such as liver, spleen, and kidney. The plasma levels in the nephrectomized rabbit make an interesting comment on this observation since they are many times the plasma level in the normal animal and many times the level in the other tissues. In both normal and nephrectomized animals the concentrations in bile are very high; in the normal they are some 12 times the plasma levels and may be considered as drug in transport, thus making a significant contribution to the clearance of the drug. This view receives support from the fact of the slight decrease in the plasma concentrations in the nephrectomized rabbits between the first and second hours; the average fall in plasma concentration is some 5 mg. per 100 c.c. which, in a rabbit of 1.7 kg., containing by our records 200 c.c. of plasma, corresponds to a total in bile of 10 mg. of sulphetrone. The recorded bile flow of these anaesthetized rabbits averages 10 c.c per hour, which is equivalent to a sulphetrone loss of 15 mg. It may therefore be argued that in the nephrectomized animal sulphetrone reaches equilibrium within one hour, and the subsequent fall in plasma concentration is due to biliary excretion and not to metabolic destruction.

Sulphetrone has no action on smooth muscle, heart, blood pressure, or respiration in concentrations usual in pharmacological studies.

In considering the data provided by the antibacterial activity of sulphetrone *in vitro* little significance is to be attached to the absolute values recorded for effective inhibitory concentrations. On the other hand, the comparative values for diaminodiphenylsulphone, sulphetrone, and promin are of high significance. In the many comparisons of this kind which have been made in these laboratories the inhibitory concentrations of sulphetrone against mycobacterial strains have always been found to be lower than those observed with promin, and this although, weight for weight, promin contains more diaminodiphenylsulphone. The implication is that the potential cinnamylidene linkages of sulphetrone contribute a significant antibacterial function. No support for this claim can be derived from reversal experiments with *para*-aminobenzoic acid, which reverses the antibacterial rates of sulphetrone at a concentration of 1 : 5,000.

It is surprising, in view of the known efficiency of diaminodiphenylsulphone in pneumococcus infections in mice, that sulphetrone is inactive; however, in streptococcus infections in mice, sulphetrone is more effective than sulphanilamide.

We have introduced the *in vivo–in vitro* antituberculous test in order to bridge the gap in the difficult transition between antituberculous *in vitro* tests and animal protection experiments with strains of mycobacteria. Whenever the drug can be estimated, and it is seldom that it cannot, either chemically or microbiologically, it is possible to express the inhibition of the organism in a quantitative fashion in terms of a standard such as diaminodiphenylsulphone. When the drug cannot be estimated, the method depends on assessing the inhibition of the organisms produced by the quantity of drug existing in the blood after a dose sufficient to produce toxic signs in the animal, and therefore at an optimum level.

An assessment of the efficiency of sulphetrone in the treatment of experimental infections with strains of *M. tuberculosis* in the guinea-pig is the subject of a separate communication. A study has also been made of the toxicity of the drug when used in man. Certain advantages and disadvantages of sulphetrone as a potential chemotherapeutic agent in man may be deduced from the present study. Absorption is slow and excretion is so rapid that limitation of fluids may be essential to maintain an adequate blood level. However, the fact that the drug is not acetylated means freedom from kidney complications and freedom from the toxic manifestations associated with the use of diaminodiphenylsulphone.

The record of clinical trials concurrently appearing in the press should enable a preliminary assessment of the toxicity and therapeutic status of the drug to be made.

Summary

1. The chemical and physical properties of 4 : 4′-bis(γ-phenyl-*n*-propylamino) diphenylsulphone-

tetrasodium sulphonate, given the trade name of "sulphetrone," together with its absorptiometric estimation in body fluids, are described.

2. The acute toxicity of the drug has been investigated in mice and dogs and its chronic toxicity in rabbits.

3. A haemolytic anaemia, an anaemia of iron lack, and an anaemia of nutritional origin arising in the course of chronic sulphetrone administration in rabbits have been investigated and the conditions for their successful treatment indicated.

4. The specific goitrogenic effect, shared by all sulphonamide drugs, has been investigated and shown to be of slight degree.

5. In acute experiments sulphetrone given orally or parenterally raises the alkali reserve of the plasma in the rabbit and the dog. The probable mechanism is discussed. When the drug is administered over a period of time equilibria are established.

6. On the basis of these experiments, together with studies of the influence of the drug on normal growth of rats, it is concluded that sulphetrone is virtually non-toxic in acute experiments, and has low toxicity in prolonged ones.

7. Although exceedingly soluble in water, sulphetrone is slowly absorbed from the intestinal tract; most from the small intestine, little from the large.

8. The drug is not conjugated in the experimental animal or in man.

9. Sulphetrone penetrates all tissues, with the exception of brain, very rapidly, and is present in them to about the same concentration as in blood. It passes into the cerebrospinal fluid much more slowly than the simple sulphonamides do.

10. Sulphetrone has no action on smooth muscle, heart, blood pressure, or respiration.

11. Antibacterial *in vitro* studies, in the presence of blood, show sulphetrone to approach the efficiency of its parent substance diaminodiphenylsulphone against two strains of *M. avium*, two strains of *M. tuberculosis* var bovis, and three strains of *M. tuberculosis* var hominis.

12. The drug is rather more effective against β-haemolytic streptococcus infection in mice than sulphanilamide, but, unlike the parent substance, diaminodiphenylsulphone, it is ineffective against a pneumococcus infection.

13. In an *in vivo-in vitro* test, blood from a guinea-pig previously given parenteral sulphetrone inhibits, *in vitro*, strains of virulent mycobacteria.

14. The pharmacological properties of sulphetrone suggest that it may prove effective in the treatment of experimental tuberculosis in the laboratory animal, and that its administration to man, in large doses for protracted periods, is a practical possibility.

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