THE INTRAMUSCULAR ADMINISTRATION OF SULPHETRONE IN THE TREATMENT OF LEPROSY

DHARMENDRA, M. B., B. S., D. B.
N. C. DEY, B. Sc., M. B.
R. BOSE, B. Sc.
AND P. L. KAPUR, M. B.
From the Leprosy Department
School of Tropical Medicine, Calcutta

INTRODUCTION

The sulfone drugs have made a great advance in the treatment of leprosy. Although the use of the parent substance, diaminodiphenyl sulfone, is of late being investigated, the most widely used sulfones so far have been the derivatives promin (given intravenously) and diasone and sulphetrone (both given orally). All three have been found effective in lepromatous cases, although all have their limitations. In our work promin was found to be more toxic than the other two, and thereafter attention was concentrated on the latter.

When given by mouth large proportions of these drugs pass out unabsorbed and are thus wasted.[1] This makes the treatment uneconomical, and the cost puts it beyond the reach of large numbers of patients in countries where leprosy is endemic. Moreover, we have found (6) that after they have been administered for a few months the same dose gives rise to lower blood concentrations than before. Brownlee (1) has reported that the fixation of elementary iron by sulphetrone, and the alteration of the intestinal flora produced by it, are partly responsible for the anemia resulting from its use. We consider it probable that the parenteral administration of these sulfones, as has been done by Cochrane (4, 5) on a small scale for other reasons, would eliminate these deficiencies and make the treatment more economical and effective. This matter has been investigated, and the results are reported here.

The first objective was to study in detail the absorption and elimination of sulphetrone after intramuscular injections in different vehicles, in order to ascertain a suitable preparation, dosage and time interval for that route. We are also investigat-

[1] Recently Smith (7) has reported that after oral administration diasone is recoverable from the feces in amounts varying from 20 to 67 per cent and in sulphetrone from 70 to 100 per cent of the daily intake.

ing the advantages, if any, of a combined treatment with that sulfone and hydnocarpus oil; both have their limitations, and it is possible that by combining the two better results may be obtained than with either alone.

The study has been made in 80 advanced lepromatous cases, all bacteriologically positive and lepromin negative, all inpatients of the leprosy hospital in Calcutta. Many of them were suffering from chronic ulcers or eye complications, or were subject to frequent reactions.

PREPARATIONS USED

Using the fine levigated sulphetrone powder obtained from the manufacturer,[2] six different preparations were made in order to ascertain which one would produce the highest blood concentrations and maintain them longest. All contained 30 per cent of sulphetrone substance. They are:

(1) Aqueous solution.
(2) Suspension in hydnocarpus oil without wax.
(3) Suspension in hydnocarpus oil with 1 per cent beeswax.
(4) Suspension in arachis oil without wax.
(5) Suspension in arachis oil with 4 per cent beeswax.
(6) Emulsion of hydnocarpus oil and water.

The aqueous solution was included because sulphetrone is highly soluble, and this preparation claimed first attention. The oily suspensions were included to see if they had any repository effect i.e., if they would release the drug gradually and over prolonged periods. Hydnocarpus-oil preparations were included for the combined therapeutic effect, as stated. The addition of wax was to enhance the repository effect. With the hydnocarpus oil, which is very viscid, only 1 per cent of wax was added, while 4 per cent was added to the arachis oil suspension. The emulsion with hydnocarpus oil was made to produce an easily injectable preparation.

METHODS OF PREPARATION

Aqueous solution.—Because weaker solutions are unstable when autoclaved a 60 per cent solution was first prepared, and at the time of use this syrupy product was diluted with an equal volume of pyrogen-free distilled water. The original solution was adjusted to pH 7.4 with sodium carbonate and filtered into ampules which were sealed and autoclaved.

Emulsion in hydnocarpus oil.—A 30 per cent emulsion was made by

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[2] We are grateful to Messrs. Burroughs Wellcome & Co., London, for the free and generous supply of the fine levigated powder of sulphetrone used in this study.

mixing equal volumes of the 60 per cent solution and sterilized hydnocarpus oil.

Suspension in hydnocarpus oil.—The sulphetrone powder was put into a glass mortar and, under aseptic conditions, the quantity of sterilized oil required to make a 30 per cent suspension was gradually added to make a uniform product. This preparation was stored in ampules.

Suspension in arachis oil.—This was prepared in the same way as the hydnocarpus suspension. There is a tendency for the drug to disintegrate in this oil, as shown by the appearance of a yellow color on the surface when kept for a few days.

Suspensions containing beeswax.—The wax was first incorporated in the oil by melting, and the mixture was sterilized. The powder was then incorporated as usual.

PRELIMINARY TESTS FOR SUITABILITY

The oils used were of low acid value, but before those preparations were injected into patients they were tested to make sure that they would not cause local pain and irritation. Tests were made to find out whether the addition of sulphetrone and subsequent autoclaving raised the acid values, and whether the preparation would cause necrosis when injected intradermally and intramuscularly in guinea-pigs.

The acid values.—No indication was found that the addition of water produced any hydrolysis of the oil and consequent increase of acidity. On the contrary, it appeared that the addition of water actually lowers the acid value of the hydnocarpus oil-sulphetrone mixture, from around 8 to 10 down to 5 to 6, and that this value is not appreciably raised after autoclaving.[3] Dr. Brownlee, chief chemist of the Wellcome Physiological Research Laboratories (2) found these results surprising but verified them in detail. He made the following comment:

> It is at first sight difficult to appreciate why the addition of water to hydnocarpus oil should lower and not increase the acid value. However, we know from other studies ... [(3)] ... that in certain circumstances sulphetrone hydrolyses to set free alkali and indeed this appears to be the reason why the period of physiological adjustment is necessary.

Intradermal injection tests.—The effects of the various preparations in intradermal injections of 0.1 cc. in a guinea-pig were determined after removal of the hair. The 30 per cent aqueous solution caused slight necrosis, while the other preparations produced no such effect.

Intramuscular injection tests.—The hydnocarpus oil suspen-

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[3] The table showing the acid values of several preparations, with and without sulphetrone, before and after autoclaving, has been omitted for considerations of space. These data can be obtained on request to the authors.—EDITOR.

sion and the aqueous solution were further tested by injecting them intramuscularly into guinea-pigs, in doses ranging from 200 to 500 milligrams per kilogram. This work was done for us by Dr. Bishtu Mukerjee, director of the Central Drug Laboratory, Calcutta, and he reported as follows:

(1) Sulphetrone in oil: Dosage up to 300 mg/kg was well tolerated, there being no definite signs of irritation and inflammation. With dosage of 500 mg/kg definite signs of inflammation at the site of injection were noticed after 72 hours—swelling, heat and induration, but the animal was not limping. These signs increased and on the 7th day the animal was limping (?) slightly. The muscle on section on the 8th day showed some exudation and the presence of oil (?) at the site of injection. There appeared to be slight necrosis at the site but this was not definite.

(2) Sulphetrone, aqueous solution: Dosage up to 400 mg/kg did not produce any definite irritation effect when injected intramuscularly. A dose of 500 mg/kg, however, produced some induration and swelling after the 7th day.

Conclusions: Dosage up to 300 mg/kg of both the samples is well tolerated. Higher doses (500 mg/kg) produce some irritation and inflammation which is more marked with the oily preparation.

Since the doses in which the sulphetrone preparations were to be used in cases of leprosy were relatively much smaller than those used in the guinea-pigs, it was concluded that these preparations could be used safely.

NATURE OF THE INVESTIGATIONS IN PATIENTS

EXAMINATIONS MADE

The investigations in patients have included the following subjects:

(1) The blood concentration and its maintenance after injections of varying doses of the various preparations.

(2) The excretion of the drug in the urine.

(3) The concentration in the skin as compared with the blood; excretion in the sweat.

(4) Liver function tests for detecting damage caused by the drugs, including the Vandenberg test, qualitative and quantitative, Schlezenger's test for urobilinogen, and the aldehyde test for urobilin in urine.

(5) Blood examinations, including total red cell counts, hemoglobin estimations, and mean corpuscle volumes, to study the degree of anemia produced.

(6) Repeated bacteriological examinations to determine improvement in that respect.

(7) Frequent clinical examinations to detect clinical progress or complications.

The study has involved about 4,000 estimations of the concentration of the drug in the blood, and about 2,000 urine estimations.

METHODS OF SULPHETRONE ESTIMATIONS

The estimations were made by the Bratton and Marshall method according to the technique recommended by Brownlee (3). A Lovibond comparator was used with a special set of discs calibrated from 2 to 12 mgm. per cent of sulphetrone. For lower concentrations we used a specially devised electric comparator with standard solutions containing 0.5, 1.0, and 1.5 mgm. per cent of sulphetrone.

Blood was used undiluted, 1 cc. being put into 10 cc. of N/1 HCl, to which was added 4 cc. of 12 per cent trichloroacetic acid, 10 cc. of the filtrate being used in the test.

Urine, 0.05 to 1 cc., was diluted as usual according to the expected concentration of the drug, a procedure which has the advantage that it minimizes the interference in the readings sometimes caused by the presence of a chromogenic substance in the urine. The total volume was made up with N/1 HCl without the addition of trichloroacetic acid, which is not necessary unless albumin is present.

The procedure used in determining the concentration in the skin was as follows: The skin specimen, taken without subcutaneous fat, is accurately weighed, cut into small pieces, and thoroughly ground in a glass mortar with glass powder. Adding a few cc. of N/1 HCl at a time, the grinding is continued until the skin is thoroughly broken down, after which enough of the acid is added to bring the volume to 11 cc. Then 4.0 cc. of the trichloroacetic acid solution is added and, after thorough shaking, the mixture is filtered through a Whatman No. 5 filter. Of this filtrate 10 cc. is treated in the same way as the blood filtrate. The calculations are then made from the following formula:

$$\frac{\text{(Reading) mgm. of drug per 100 cc.} \times 1{,}000}{\text{Weight of skin in mgm.}} = \text{mgm. of drug per 100 gm. of tissue}$$

RESULTS OF INVESTIGATIONS IN PATIENTS

CONCENTRATIONS IN THE BLOOD

Smallest dose producing detectable blood concentrations.—(1) Dose of 0.03 gm: This dose (i.e., 0.1 cc. of the 30% aqueous solution), given intramuscularly by means of a tuberculin syringe, was the smallest used for this purpose because of the results obtained with it. These injections were given to 8 patients on 5 consecutive days. The estimations were made two hours after the injections, since previous experience (see later) had shown that the blood concentration is highest at that time.

Detectable amounts of the drug were found in a little over one-half of the samples tested, but in most cases the concentration was less than 0.5 mgm. per cent, too low to be estimated by colorimeter. Because of these findings later estimations were not made.

The concentration in the urine at two hours was considerably higher than in the blood and was measurable, being from 5 to

10 mgm. per cent. The drug was still present in specimens taken after 24 hours, but the concentrations were below 2 mgm. per cent.

(2) Dose of 0.05 gram: This dose (i.e., 0.1 cc. of a 50% solution) was given to the same 8 patients in the same way for the same length of time. Samples of blood were taken after intervals of 2, 4, 8 and 12 hours; samples of urine were collected daily at 24-hour intervals.

The drug was found in almost all of the 2-hour blood specimens in a concentration of 0.5 mgm. per cent; in the 12-hour specimens it was detectable in only one-half of the specimens, and the concentration was below 0.5 mgm.; at the end of 24 hours the blood was free from the drug.

With this dose the concentration in the urine was much higher than before, and it could be detected after 48 to 72 hours although in low concentration.

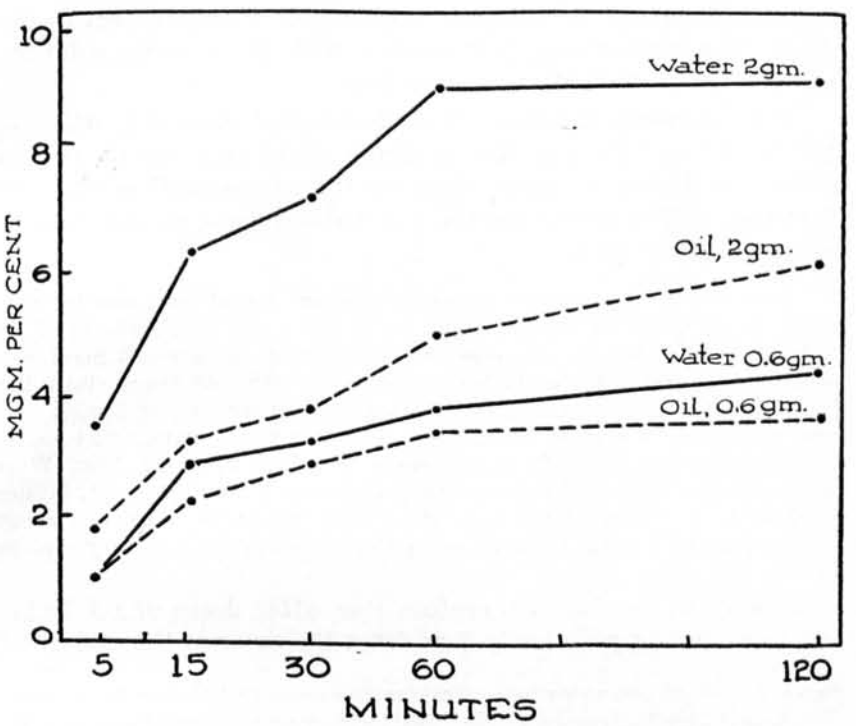
It can, therefore, be concluded that 0.05 gm. (50 mgm.) of sulphetrone given intramuscularly in aqueous solution produces blood concentrations of 0.5 mgm. per cent in 2 hours in all cases, while at the end of 24 hours the blood has cleared. With the smaller dose used the drug could not be detected in about one-half of the specimens. (The concentrations produced by larger doses and the period in which the drug can be found in the blood are discussed later.)

Earliest appearance in the blood.—To find out how early the drug could be detected in the blood after intramuscular injection, samples were taken in a number of cases at intervals of 5, 15, 30 and 60 minutes after the injection of the watery solution and oily suspension, and also after 2, 12, and 24 hours. Two doses were used, a small one of 0.6 gm. and a large one of 2 gm. The results are shown in Text-fig. 1.[4]

It will be seen that with both of the preparations and both of the doses used the drug appeared in measurable amounts within 5 minutes. The concentration continued to increase up to 2 hours, although the rise after 1 hour was not marked. The later values were considerably lower. Dose for dose, the watery solution gave the higher concentrations.

Period of highest concentration.—These findings, and similar ones of decrease after 5 and 8 hours in other trials, made it evident that the peak hour lay between 2 and 5 hours. This point was investigated by making estimations at intervals of

[4] In this graph, and in other succeeding ones, each point represents the average of a number of observations.



TEXT-FIG. 1. The earliest appearance of sulphetrone in the blood, and the increase of its concentration there, up to 2 hours after intramuscular injection of 0.6 gm. and 2.0 gm. in aqueous solution and oily suspension.

1, 2, 3, and 5 hours after injection, using the different preparations in varying doses.

The results showed definitely that, whatever the preparation and dose used, the highest concentration occurred 2 hours after the injection except in the case of the smallest (0.3 gm.) dose in aqueous solution, when the level was slightly higher after 1 hour than after 2 hours. Moreover, with the largest doses (1.5 and 2 gm.) of watery solution or hydnocarpus emulsion the 2-hour readings were only slightly higher than the 1-hour readings. In all instances the concentrations had decreased materially at the third hour, and more at the fifth.

It can be concluded therefore that in most cases the peak is reached 2 hours after the injection, declining thereafter. Hence in all later work, the 2-hour observation was taken to represent the highest blood level produced by a particular preparation or dose.

Longest period of persistence.—The next question was how

long the drug can be found in measurable concentration in the blood. The matter was first studied with the aqueous solution, and later with the other preparations.

(1) Aqueous solution: The doses used were 0.1, 0.2, 0.3, 0.6, 1.0, 1.5 and 2.0 gm. The findings are in part represented in Table 1 and, for the 2-gm. dose, by the corresponding curve in Text-fig. 2. The curves for the lesser doses were, in fair degree, proportionately lower.

With the 0.1 and 0.2 gm. doses the average initial level was below 2 mgm. per cent, falling below 1 mgm. by 12 hours and practically to nil at 24 hours.[5] With the 0.3 gm. dose the initial level was about 3 mgm. per cent, reduced to 1 mgm. at 12 hours and to nil within 48 hours. With the 0.6, 1.0 and 1.5 gm. doses the initial level was between 4 and 6 mgm. per cent, down to 2 to 4 mgm. by 12 hours, and to 1 to 1.5 mgm. by 24 hours. Some specimens taken at 72 hours proved to be free from the drug. With the 2 gm. dose the initial concentration was about 9 mgm. per cent, falling to about 4.5 mgm. by 12 hours, to about 3.5 mgm. at 24 hours, and about 1.6 mgm. at 48 hours. After 72 hours the concentration was very low, or nil.

It may be concluded therefore that after doses of 0.1 to 0.3 gm. the blood is practically free from the drug at the end of 24

TABLE 1.—*Blood concentrations after varying doses of the various preparations injected intramuscularly: (a) the highest concentrations, in milligrams, and (b) the time in hours of maintenance of 1 milligram or more.*

Preparation	Dose									
	0.3 gm.		0.6 gm.		1.0 gm.		1.5 gm.		2.0 gm.	
	Highest concentration	Maintenance, hours	Highest concentration	Maintenance, hours	Highest concentration	Maintenance, hours	Highest concentration	Maintenance, hours	Highest concentration	Maintenance, hours
Aqueous solution	2.8	12	4.4	24	6.5	36	7.5	40	9.2	48
Hydnocarpus emulsion	2.4	12	4.1	24	5.1	36	6.7	40	9.1	48
Hydnocarpus suspension	2.5	8	4.0	24	4.9	36	5.3	40	7.2	48
Arachis suspension	2.5	8	3.8	24	4.9	36	5.4	40	7.8	48

[5] Note that figures such as these in the text, and those given in the tables, represent averages of multiple determinations.

hours; after 0.6 to 1.5 mg. it is practically free at the end of 48 hours, and after 2 gm. it is practically free at the end of 72 hours.

(2) Other preparations: The results with various doses of the other preparations, compared with those just related, are summarized in Table 1.[6]

The preparations containing water gave rise, dose for dose, to slightly higher blood concentrations than the oily suspensions, but the drug was maintained in appreciable amounts for about the same length of time with all preparations. This period naturally varies with the dose, and is approximately 12 hours after a dose of 0.3 gm., 24 hours after 0.6 gm., 36 hours after 1 gm., 48 hours after 1.5 gm., and more than 48 hours after 2 gm.

This matter has been studied in greater detail with 2-gram doses of the various preparations, with the same results. The average blood levels at different intervals are shown in Table 2 and Text-fig. 2.

TABLE 2.—*Blood concentrations after injection of 2 grams of the drug in various vehicles, in milligrams per 100 cc. after different periods of time.*

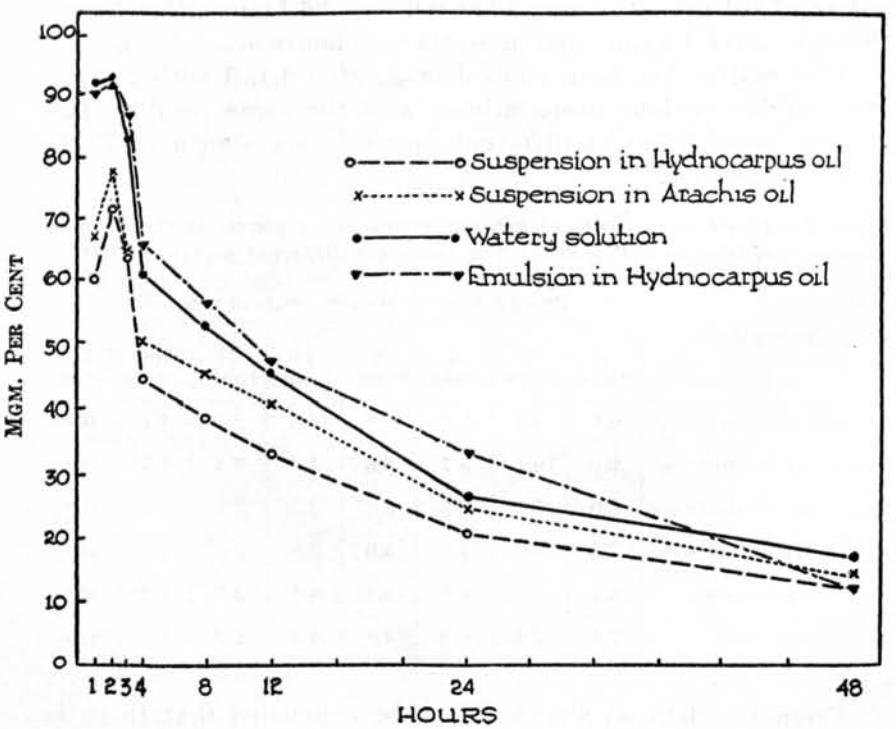
Preparation	Blood concentrations, by hours in mgm. %							
	1 hour	2 hours	3 hours	4 hours	12 hours	24 hours	48 hours	72 hours
Aqueous solution	9.1	9.2	8.3	6.1	4.6	2.7	1.7	0.5
Hydnocarpus emulsion	9.0	9.1	8.7	6.6	4.8	3.4	1.2	0.5
Hydnocarpus suspension	6.0	7.2	6.5	4.5	3.3	2.1	1.2	0.0
Hydnocarpus with wax	7.5	8.0	7.3	5.0	3.8	3.0	1.4	0.5
Arachis suspension	6.7	7.8	6.6	5.1	4.1	2.5	1.4	0.2
Arachis with wax	7.1	7.4	6.9	4.8	4.2	2.7	1.3	0.5

From the data so shown it can be concluded that there is some delay in absorption from the oily suspensions as compared with the watery solution. For this reason the watery preparations give rise to higher initial blood levels. However, the delay of absorption from the oily suspensions is not marked enough for blood levels to be maintained longer than with the watery preparations. For example, with all preparations, 2 gm.

[6] The preparations with wax have been excluded from this table because the numbers of observations were not sufficient, although the results obtained were more or less similar. Comparative figures for the wax preparations are included later in a consideration of the 2 gm. doses of the drug in various preparations.

doses give blood concentrations around 1.5 mgm. per cent at the end of 48 hours.

Comparison of blood concentrations after oral and intramuscular administration.—The average blood concentration of 8 to 10 mgm. per cent after an average daily dose of 3 gm. of sulphetrone by mouth reported by Wharton (8) is much higher than has been reported by other workers or found in this country (6), where the maximum was 4 mgm. per cent, and the average was lower. That finding has been further confirmed



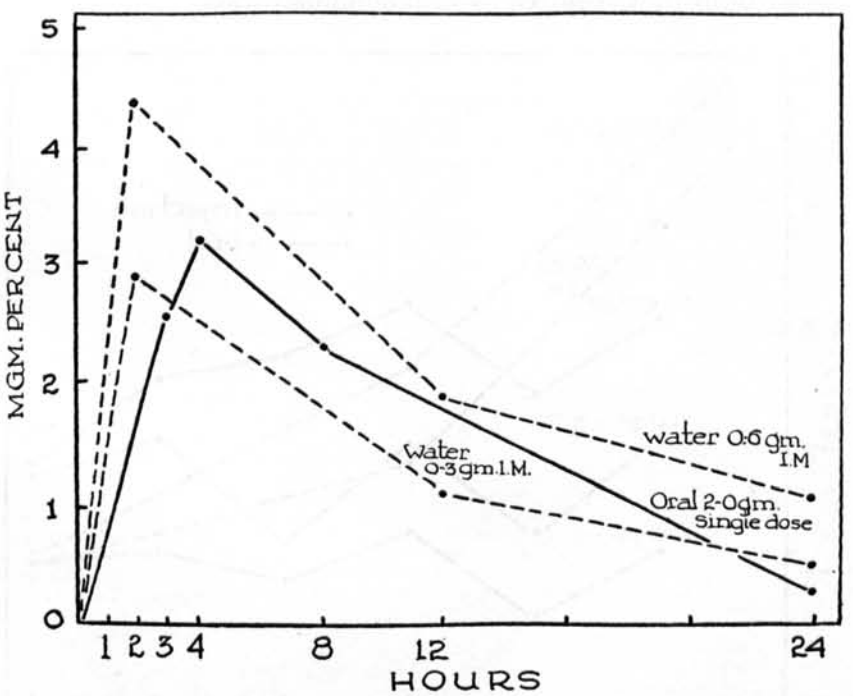
TEXT-FIG. 2. The sulphetrone blood levels after intramuscular injection of 2 gm. sulphetrone powder in different vehicles.

in the present study; the maximum concentration on 6 gm. per day has been only 5 mgm. per cent. The blood concentrations after intramuscular injections have been compared with those after oral administration of both a single daily dose of 2 gm. and fractional doses given 3 times a day at 8 hours intervals.

(1) Single oral dose of 2 grams: The blood levels after this dose by mouth have been compared with those after injec-

tions of 0.3 gm. and 0.6 gm. in aqueous solution. The results are shown in Text-fig. 3.

It will be seen that the single 2-gm. dose produced an average concentration of 3.2 mgm. per cent 4 hours after administration, falling to 2.3 mgm. at 8 hours, to below 2 mgm. at 12 hours, and to 0.5 mgm. at 24 hours. The results of the 0.3 gm. injections compare favorably with the single oral dose of 2 gm., and those



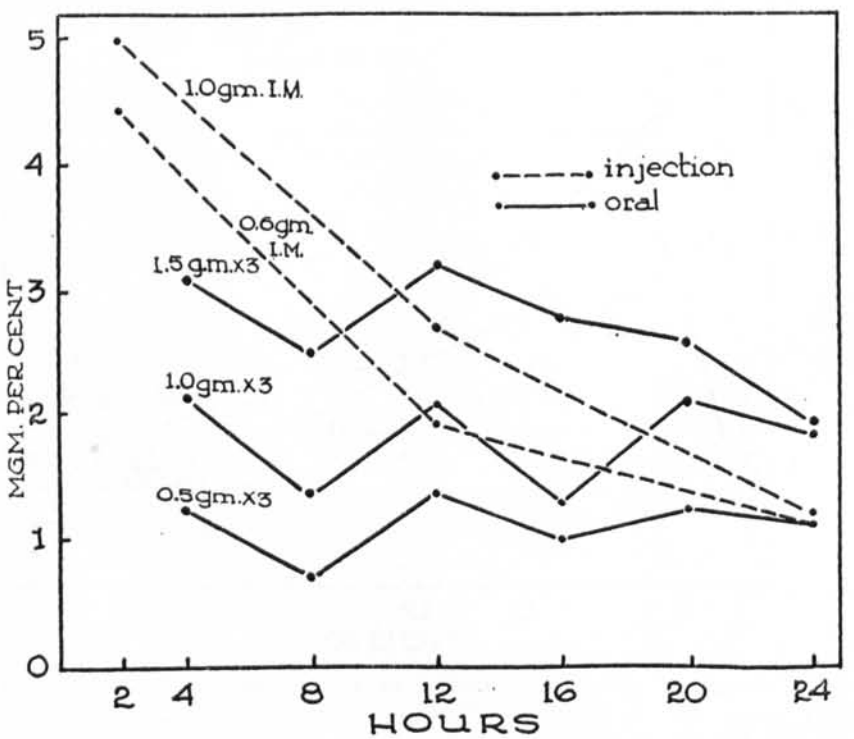
TEXT-FIG. 3. Blood concentration of sulphetrone after a single dose of 2 gm. by mouth, compared with those after intramuscular injections of 0.3 and 0.6 gm. in aqueous solution.

of the 0.6 gm. injections are definitely superior as regards both highest levels and maintenance of the concentration.

(2) Repeated oral doses: The comparison of the results of fractional dosage—which is the way the drug is given in practice —with those of a single dose given intramuscularly is made in Text-fig. 4. By the oral route doses of 0.5, 1 and 1.5 gm. were given 3 times a day, while the doses given in single intramuscular injections were 0.6 gm. and 1 gm.

It will be seen that with 0.5 gm. given 3 times a day (i.e., 1.5 gm. daily) the blood concentration remained about 1 mgm. per cent; that with 1 gm. given 3 times a day it was between

1.5 and 2 mgm. per cent; and that with 1.5 gm. given 3 times a day it was between 2.5 and 3 mgm. per cent. The single dose of 1 gm. given intramuscularly in the aqueous solution was definitely superior to the total 4.5 gm. oral dose, and 0.6 gm. given intramuscularly was far superior to the 3 gm. oral dose. It would thus appear that, to maintain the same blood concentration, the dose required by intramuscular injection is about one-fifth of that required by oral administration.



TEXT-FIG. 4. Blood concentrations of sulphetrone after repeated oral doses, of 0.5, 1.0 and 1.5 gm. three times a day, and those after single intramuscular injections of 0.6 and 1.0 gm. in aqueous solution.

EXCRETION IN URINE

Being freely soluble in water, sulphetrone is rapidly excreted by the kidneys. Its excretion was studied with respect to its earliest appearance in urine, the time of highest concentration, the maintenance of concentration, the total excretion, and the relation between the concentrations in blood and urine. Specimens were examined at intervals of 1, 3, 5, 8, 12, 24, 48, 72 and 96 hours after the injection of varying doses (0.3 to 2.0 gm.) of

different preparations. In the case of the 2-gm. doses, further specimens were examined in a number of instances up to 7 days at 24-hour intervals.

Time of appearance.—With all the doses and all the preparations, the drug appeared in the urine in fair amounts in the first hour. With doses of 0.3 and 0.6 gm. the concentration after 1 hour was usually between 15 and 30 mgm. per cent; with the higher doses it was usually between 50 and 100 mgm. per cent.

Highest concentration.—The excretion continues to increase after the 1-hour period and is at its height after 2 to 3 hours, which corresponds to the peak hour in the blood. The concentration in the urine shows marked variations, however, since it depends on several factors other than the blood concentration, such as the amount of urine passed, the fluid intake, and the extent of sweating. With the smaller doses (0.3 and 0.6 gm.) the highest concentration is below 100 mgm. per cent; with larger doses it is usually above that level, but varies from 50 to 300 mgm. per cent.

How long found.—After 3 hours, the concentration of the drug in the urine begins to decline gradually; the end-point when it can no longer be detected varies with the dose. On the average a concentration of 2 to 5 mgm. per cent is found at the end of 24 hours with the 0.3 gm. dose, at the end of 48 hours with higher doses up to 1 gm., and at the end of 72 hours with still higher doses up to 2 gm. With the 1.0 and 2.0 gm. doses, traces of the drug were detected up to 96 and 120 hours, respectively.

Total excretion.—The question of what percentage of the drug given intramuscularly could be accounted for in the urine was investigated with the higher doses only. Several patients were injected with 2 gm. in aqueous solution, hydnocarpus suspension or hydnocarpus emulsion, and total 24-hour specimens were collected for five consecutive days, when the urine was found to contain little or no drug. From the concentrations found the amount of the drug excreted on each day was calculated. The results are shown in Table 3.

It will be seen that with the aqueous solution and the hydnocarpus emulsion, about 70 to 80 per cent of the drug could be accounted for in the urine in the five days following intramuscular injection; in the case of the hydnocarpus suspension the total accounted for is slightly less. About one-half of the drug is excreted in the first 24 hours, and the quantity passed during the successive 24-hour periods is progressively less. The marked

TABLE 3.—*Total excretion in the urine after intramuscular injection of 2-gram doses of various preparations, as determined from successive 24-hour specimens.*

Preparation	Case No.	Daily excretion (mgm.)					Total excretion (mgm.)	Percentage excreted
		First 24 hrs.	Second 48 hrs.	Third 72 hrs.	Fourth 96 hrs.	Fifth 120 hrs.		
Aqueous solution	74	972	348	60	10	....	1390	69.5
	75	1008	525	100	40	....	1673	86.6
	76	1170	400	90	15	....	1675	88.7
Hydnocarpus suspension	1	750	355	100	25	....	1230	61.5
	37	1105	365	60	20	....	1550	77.5
	38	800	350	110	25	....	1280	64.0
Hydnocarpus emulsion	27	1000	400	37	30	....	1467	73.8
		1000	220	100	44	10	1374	68.7
	40	1056	320	45	40	....	1461	73.5
		1000	300	105	30	20	1455	72.7
		1100	220	100	50	10	1480	74.0

variations referred to earlier are seen mostly in the first 24 hours; as the excretion in urine decreases these variations become less marked.

Relation between the blood and urine concentrations.—This relationship was studied in a group of cases for 5 days after single intramuscular doses of three different preparations. Blood and urine concentrations were estimated at 2, 8, 12, 24, 48, 72 and 120 hours. Although the urine concentrations varied markedly in the first 24 hours, the following conclusions can be drawn:

1. At every stage the urine concentration is much higher than the blood concentration, on the average about 10 times as high. With barely 10 mgm. per cent in the blood after 2 hours, the urine concentration was on the order of 120 mgm. or more; and after 24 hours the corresponding figures were around 3 mgm. as against 20 to 30.

2. The drug can be found longer in the urine than the blood. With a 2 gm. dose only traces are found in the blood after 72 hours and none after 96 hours, whereas the urine concentration is from 3 to 5 mgm. per cent at 72 hours and in some cases traces were found at the end of 120 hours.

3. In general the relation between the blood and the urine concentrations is the same regardless of the preparation used. With the oily suspensions, the figures of excretion at 8 and 12

hours are closer together than in the case of watery solution and the emulsion, where the 12-hour figures are considerably lower than the 8-hour figures. This indicates a slight lag in the absorption and excretion of the drug from the oily suspension, as does the lower blood concentration with the oily suspension.

What has been said of the 2-gm. dose is also true for the smaller doses. The urine concentration is several times higher than that of the blood, and the drug can be detected longer. The data on these points are summarized in Table 4.

CONCENTRATIONS IN THE SKIN

It has been suggested by some workers that the sulfones concentrate in the skin more than in other tissues, including the blood. Later it was found that the findings were fallacious

TABLE 4.—*Relation between blood and urine concentrations following intramuscular injection of different doses, after various intervals.*

Dose	Concentration (mgm.)					
	24 hours		48 hours		72 hours	
	Blood	Urine	Blood	Urine	Blood	Urine
0.3 gm.	0.5	5	Nil	2	Nil	Nil
0.6 gm.	1.5	8	0.4	3	Nil	1

because procaine (novocaine) had been used as the local anesthetic, a defect which cocaine does not have. We have found that a 2 per cent solution of novocaine produces a distinct color reaction in the Bratton-Marshall test, whereas a 4 per cent solution of cocaine gives none. However, in control experiments carried out in some patients before they were put on sulphetrone, skin removed after injection of cocaine produced a slight color reaction. It was negligible, being equivalent to about 0.3 mgm. per cent of sulphetrone, but this amount has been taken into consideration when calculating the skin concentrations of patients on sulphetrone treatment.

Comparative tests have been made with blood and skin at different intervals after injection of 2 gm. of sulphetrone in various vehicles. For obvious reasons skin specimens could not be removed from the same set of patients at various intervals after the injections, and consequently four different sets of patients have been used for the estimations made at intervals of 2, 3, 8 and 24 hours after injection. The results are shown in Table 5.

TABLE 5.—*Comparison of concentrations in blood and skin after different intervals following intramuscular injections of 2 gm. of the drug in various vehicles.*[1]

Preparation	Concentration (mgm.)							
	2 hours		3 hours		8 hours		24 hours	
	Blood	Skin	Blood	Skin	Blood	Skin	Blood	Skin
Aqueous solution	9.0	9.2			6.0	5.3	2.5	1.9
	8.0	7.0			6.5	5.6		
	8.5	7.6			6.0	5.4		
	9.5	9.0			6.5	5.8		
Hydnocarpus emulsion	8.0	9.5					2.5	1.7
Hydnocarpus suspension	7.4	6.4	5.0	8.3			2.0	1.5
Arachis suspension	4.0	4.7						
Hydnocarpus with wax			7.5	6.6				
Arachis with wax			7.0	3.4			2.0	1.6

[1] The four different time periods relate to different groups of cases. The data are arranged as they are for economy of space, without implication of repeated examinations of the same cases.

It has been found that, at all stages and with the different blood levels, the blood and skin concentrations of the drug are very similar. It follows that the higher concentrations in the skin reported by other workers can be attributed to the use of novocaine.

Concentration in the sweat.—The concentration of the drug in the sweat has also been investigated. It was found to vary considerably with the variations in the amount of sweat secreted. It was usually between 0.1 to 2 mgm. per cent, but in highly concentrated sweat it was sometimes much higher, up to 5 mgm.

CHOICE OF PREPARATION FOR INTRAMUSCULAR INJECTIONS

These investigations were originally started with six preparations with a view to selecting one or more which are suitable for regular use. In determining that matter the following points were considered: (*a*) The height of the blood concentrations produced after a particular dose given in the various vehicles; (*b*) the maintenance of the blood levels and the longest periods during which the drug can be detected; (*c*) ease of injection; (*d*) local irritation and induration; and (*e*) the degree of

anemia produced. The further question, that of whether or not combined treatment with hydnocarpus oil and sulphetrone would give better therapeutic results than sulphetrone alone, needs a long-term study, and its answer will have to wait until a later date.

The matter of the blood concentration and its maintenance has already been considered in detail. Information on these and other points may be summarized as follows:

1. On the same dose given in different vehicles, the watery solution and the emulsion produce higher blood levels than do the oily suspensions, with or without wax.

2. Although there is a slight lag in the absorption of the drug from oily suspensions, the blood levels are not maintained longer than with the watery preparations. In other words, the oily suspensions have no repository effect.

3. As regards ease of injection, the watery solution is the best; the oily suspensions with wax are the most difficult to inject. The emulsion offers no difficulty in injection, but because it is not stable it has to be made at the time of use, or, if previously made, it must be shaken well when used.

4. Regarding local irritation, the watery solution is again the best. The oily suspensions produce induration, especially when wax is added, and they have caused abscess formation in some cases. The emulsion is intermediate; although as a rule there has been no difficulty with it, abscess formation has occurred in a few cases.

5. No appreciable differences have been observed regarding anemia.

Thus the best preparation is the watery solution, and the emulsion in hydnocarpus oil comes next. The choice between them will depend upon whether or not combined hydnocarpus-sulphetrone treatment is found to have any advantage over sulphetrone alone. In further work, therefore, these two preparations are being used. We are now using a 50 per cent stock aqueous solution undiluted, though it still has to be mixed with hydnocarpus oil to prepare the emulsion.

DOSE AND INTERVAL FOR INTRAMUSCULAR INJECTIONS

Selection of a suitable dose and time interval for the injections involves consideration of several points, such as: (*a*) the optimum blood concentrations of the drug for therapeutic effect; (*b*) the concentrations produced by different doses; (*c*) a com-

parison of the concentrations produced by the oral and intramuscular routes; (*d*) the time in which elimination occurs after a previous injection; and (*e*) the degree of anemia and other toxic symptoms produced by the various doses.

There is no information available regarding the optimum blood concentrations of sulphetrone for the treatment of leprosy, although reference has been made to the concentrations reported by some workers after oral administration. In a chronic disease whose treatment has to be prolonged for years, and with drugs which are potentially toxic, the minimum effective concentration should be the optimum concentration. What this is can be decided only by a long-term correlation of clinical and bacteriological improvement with varying concentrations of the drug maintained over long periods, and that study is being attempted.

The matter of toxic effects and tendency to produce anemia is considered later in this paper, but in general there have been no marked toxic effects with the doses used. Anemia is more likely to occur with the higher doses given twice a week than with the smaller doses given daily, and doses of 0.3 and 0.6 gm. are tolerated well even by patients with initial low red cell counts and hemoglobin values.

As has been shown, the 1-gm. dose when injected produces blood concentrations similar to those produced by 4.5 gm. taken by mouth (3 tablets 3 times a day). Further observations have been made on the concentrations resulting from repeated injections of small doses, i.e., 0.1, 0.2, 0.3, 0.6 and 1.0 gms. The two smallest doses were given daily, while with the others both daily and alternate-day injections were tried. It will be seen from Table 6 (in which have also been included figures for the 1.5 and 2 gm. doses) that with 0.1 and 0.2 gm. daily doses the blood concentration is very low and is maintained for a short period, and that with daily doses of 0.3, 0.6 and 1.0 gm. fairly good blood concentrations are obtained and maintained. When injections are given on alternate days (not shown in the table), no dose less than 1 gm. can maintain a concentration of 0.5 mgm. per cent at the end of 48 hours.

These findings suggest that, in patients whose poor blood condition does not demand smaller amounts, the most suitable dosage may be 1 gm. given daily. In case daily injections are not feasible, higher doses may be given less frequently, e.g., 1.5 gm. on alternate days or 2.0 gm. twice a week. Groups of cases are now under treatment with the various suggested dosages,

TABLE 6.—*Blood concentrations after intramuscular injection of varying doses, after different intervals of time.*

Dose	Highest concentration	Concentration after		
		24 hours	48 hours	72 hours
0.1 gm.	Below 1	0	0	0
0.2 gm.	1.5	0.5	0	0
0.3 gm.	3	0.75	0	0
0.6 gm.	4	1.0	0.2	0
1 gm.	5	1.0	0.5	0
1.5 gm.	6	2.0	1.0	0
2 gm.	9	3.0	1.5	0.5

from 0.3 gm. daily to 2.0 gm. twice a week, in order to correlate the clinical and bacteriological improvement with them.

THERAPEUTIC RESULTS OBTAINED

The longest period of treatment by intramuscular injection of the patients included in this study has, so far, been about 9 months. It is yet too early to compare the progress made by them with that generally obtained by oral administration of the drug. However, the following general remarks may be made.

CLINICAL IMPROVEMENT

Within as short a period of treatment as about a fortnight, the patients report that they feel lighter, perhaps because of healing of ulcers when they were present, otherwise perhaps because of some subsidence of the leprous infiltration. Persistent temperature due to the presence of septic ulcers responds rapidly. The reactions of leprosy are gradually controlled; at first their frequency, severity and duration is decreased, and finally they are almost eliminated. Leprous ulcers heal rapidly, apparently more so than with oral administration. A few patients had ulcerating axillary and inguinal glands of long standing which have healed quickly.

Nodules and thick infiltrated areas of the skin begin to shrink after about a month's treatment. Subsidence is slow but appreciable, and in several cases the nodules have now been replaced by scar tissue. In some cases fresh crops of small nodules appeared during the treatment, but they subsided in a few days. In other cases nodules burst to form ulcers which healed quickly.

Leprous eye conditions and eye reactions respond very favorably. More than one-third of the cases had some sort of eye trouble, but after three months' treatment most of the cases with mild symptoms showed considerable improvement, there being no or slight trouble left. In the severer cases treatment for this period was effective in reducing the frequency of pain and duration of eye reactions. A few patients reported that, while the reactions had been controlled, the vision had become slightly more dim.

Several advanced cases suffered from edema of the feet and legs. With the general improvement in their condition the edema begins to subside and disappear.

BACTERIOLOGICAL IMPROVEMENT

Repeated bacteriological examinations of each case are being made. Multiple smears are used to find out whether the expression of the bacteriological results as an "index" has any advantages over the usual method of expressing the degree of positivity by words such as "slight," "moderate" or "high."

So far no case has become bacteriologically negative, but in most of them there has been a decrease in the numbers of bacilli, and the bacilli show definite morphological changes. They become less acid-fast, and some break down or become granular. It appears that the drug has a definite action on the bacilli, although it takes a long time to eliminate them from the body.

TOXIC EFFECTS

General effects.—There have been no marked toxic effects, as said. In some of the cases the first few injections were followed by a rise in temperature to 100°F. or more, but as the treatment was continued this manifestation disappeared. Nausea, weakness and giddiness were reported by a few early in the treatment, but later these symptoms also disappeared. Cyanosis was noted in one patient, a child of 11 years who was getting 2 gm. twice a week. It subsided after a day's withdrawal of the drug, and he was later put on a lower dose (1.5 gm. twice a week). The cyanosis has not recurred. Exfoliative dermatitis was seen in two cases, necessitating the temporary withdrawal of the drug and the administration of calcium gluconate. No sign of liver damage has so far developed in any of the cases.

Effect of the blood.—The patients are given iron and yeast as a routine, since the sulfone drugs tend to produce anemia. Even with this precaution there is a tendency to lowering of the red cell count and the hemoglobin value. There is also a tendency

for the mean corpuscular volume to increase. However, deterioration in the blood picture has generally been slight; in only a few cases has it been serious enough to necessitate temporary withdrawal of the drug, and this was usually in cases whose initial blood picture was poor. It has been observed that small daily doses are well tolerated even by patients with a low blood count—with about 50 per cent red-cell count and hemoglobin.

SUMMARY AND CONCLUSIONS

After oral administration the absorption from the intestines of the sulfone drugs like diasone and sulphetrone is very defective, and they produce certain changes in the intestine which are partly responsible for the anemia resulting from their use. Parenteral administration, by eliminating these factors, was considered likely to be more economical and more effective.

A detailed study has been made of the absorption, the resulting blood levels, and the excretion of sulphetrone after intramuscular administration with the object of finding out a suitable preparation, dose and interval for that route. The study was made with advanced lepromatous cases, all bacteriologically positive and lepromin negative, all inpatients of a leprosy hospital.

Using the fine levigated sulphetrone powder, six different preparations were made: an aqueous solution, suspensions in hydnocarpus and arachis oils with and without wax, and an emulsion of hynocarpus oil and the watery solution.

Regarding the smallest amount of injected sulphetrone capable of producing detectable blood concentrations, 0.05 gm. in aqueous solution gave, on the average, 0.5 mgm. per cent in 2 hours in all cases, while with the 0.03 gm. dose it was found at that time in only one-half of the specimens and usually in less than 0.5 mgm. per cent concentration.

The drug is absorbed rapidly, and is found in the blood in measurable amounts within 5 minutes after the injection. Thereafter it is present in increasing amounts up to 2 hours, after which the concentration begins to decline.

The length of time that the drug can be found in the blood varies with the dose. With doses of 0.1 to 0.3 gm. the blood is practically free from it at the end of 24 hours; with 0.6 to 1.5 gm. it is practically free after 48 hours; and with 2 gm. it is practically free after 72 hours.

The preparations containing water give rise, dose for dose, to slightly higher initial blood concentrations than the oily suspensions, but despite this lag with the suspensions the drug is maintained in appreciable amounts for about the same period

with all of the preparations. The suspensions have no repository effect.

A comparison of oral and intramuscular administration showed that, to maintain the same blood concentration, the dose required by the intramuscular route is about one-fifth of that required by the oral one.

Being freely soluble in water, sulphetrone is rapidly excreted by the kidneys. With all doses and all preparations, it appeared in urine in fair amounts in the 1-hour specimens and reached its peak at 2 to 3 hours, corresponding to the peak hour in the blood, after which it gradually declined. The end-point varied with the dose: 2 to 5 mgm. per cent was found at the end of 24 hours with a dose of 0.3 gm., at the end of 48 hours with higher doses up to 1 gm., and at the end of 72 hours with still higher doses up to 2 gm.

Calculating the proportion of the injected drug which could be accounted for in the urine within five days, this was about 70 to 80 per cent in the case of the watery preparations and slightly less with the others. About one-half of the drug is excreted in the first 24 hours, and progressively less thereafter.

Comparing the blood and urine concentrations, the latter is very much the higher at every stage (about 10 times higher), and it is detectable longer. This is the case regardless of the type of preparation used.

Comparing the concentrations in the blood and skin at different intervals after the injection, and therefore with different blood levels, it was found that they are very similar. We have therefore not confirmed reports of higher skin concentrations, which can be attributed to the diazotization value of procaine, a defect not possessed by cocaine, which was used in this work.

Considering the various factors involved in the choice of the best preparation, the watery solution appears to be the one of choice, the hydnocarpus emulsion being the next best. The choice between the two will depend upon whether or not the combined hydnocarpus-sulfone treatment has any advantages over treatment with the sulfone alone. The suspensions have no advantages; on the other hand they have certain disadvantages since they are more difficult to inject and are more likely to cause local irritation.

Considering the factors involved in the choice of the preferred dosage, the most suitable one is perhaps 1 gm. injected daily. If daily injections are not feasible, higher doses may be given less frequently, i.e., 1.5 gm. on alternate days or 2 gm. twice a

week. Patients whose blood condition is poor may tolerate only 0.5 gm. daily or even less.

The patients under this treatment have shown marked clinical and appreciable bacteriological improvement. The results cannot yet be compared with those of oral administration, except that the healing of leprous ulcers has been more rapid.

No marked toxic effects have been seen, although in some cases the first few injections were followed by a rise in temperature, and in a few others nausea, weakness and giddiness, but these symptoms soon disappeared. Cyanosis was noted in one patient on the 2-gm. dose, but it did not reappear after the dose had been reduced. No sign of liver damage has developed so far in any case.

The anemia which has occurred has generally been of a slight degree, and only in a few cases did it necessitate temporary withdrawal of the drug. Small daily doses are well tolerated even by patients with low blood counts and hemoglobin.

It can be concluded therefore that the intramuscular administration of sulphetrone is safe, and that it offers several advantages over its use by mouth. It eliminates both the defective absorption and the untoward changes in the intestine following oral administration. Treatment by this method is therefore the more economical, requiring at most only 4 to 5 gm. a week as against the 36 gm. per week recommended for the oral route, and it is likely to be the more effective. In leprosy institutions where the giving of injections does not entail any extra cost, parenteral administration of sulphetrone will be much less expensive than its oral use.

REFERENCES

1. BBOWNLEE, G. Sulphetrone: Therapeutics and toxicology. Lancet **2** (1948) 131-134.
2. BROWNLEE, G. Personal communication.
3. BROWNLEE, G., GREEN, A. F. and WOODBINE, M. Sulphetrone: A chemotherapeutic agent for tuberculosis: Pharmacology and chemotherapy. British J. Pharm. & Chemother. **3** (1948) 15-28.
4. COCHRANE, R. G. A comparison of sulphone and hydnocarpus therapy of leprosy. Internat. J. Leprosy **16** (1948) 139-144.
5. COCHRANE, R. G., RAMANUJAM, K., PAUL, H. and RUSSELL, D. Two-and-a-half years' experimental work on the sulphone group of drugs. Lep. Rev. **20** (1949) 4-64.
6. DHARMENDRA. Treatment of leprosy with sulphone drugs. Mem. V Congr. Internac. Lepra, Havana, 1948; Havana, 1949, pp. 826-834.
7. SMITH, M. A pharmacological study of three sulphones. Part I. Absorption, distribution and excretion. Lep. Rev. **20** (1949) 78-88.
8. WHARTON, L. H. Preliminary report on a new sulphone drug "sulphetrone." Internat. J. Leprosy **15** (1947) 231-235.