### A PHARMACOLOGICAL STUDY OF THREE SULPHONES Part III—THE SPECIFIC TOXIC PHENOMENA

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#### BLOOD DYSCRASIAS (Contcl.)

In the previous article the haemolytic effects of the sulphones was considered. The attempt was made to show that the mechanism of sulphone anaemia is by no means one of simple haemolysis. It was shewn that parenteral administration of the amount of sulphone calculated (from the absorption experiments detailed in Part I) to be available to the body from oral administration, does not produce any significant degree of anaemia.

The direct assessment of haemopoietic factors responsible for sulphone anaemia is beyond the scope of this article, but the endeavour has been made to discover how far a direct toxic haemolysis is responsible for the anaemia.

Haemolysis occurring in significant amounts is usually evidenced by (i) haemoglobinaemia (ii) methaemalbuminaemia (iii) increased faecal urobilinogenuria. As well, though not only with haemolysis, and not pathognomic of it, bilirubinaemia, urobilinuria, and an increased fragility of the red cells occur.

Table I shews that summarised results of repeated tests upon the 300 patients undergoing sulphone therapy at this Unit.

	TABLE I			
Examination	Diamino- diphenyl- sulphone	Diasone	Sulphetrone	
Haemoglobinaemia	O	0	0	
Methaemalbumin	$\phi +$	0	0	
Faecal Urobilin	+ +-	$+\phi$	0	
Bilirubinaemia	$\phi +$	$\phi +$	0	
Red Cell Fragility	0	0	0	
Legena	! + + +	Marked		
	+ +	Moderate		
	+	Slight		
	0	Not Significant		
	ф	Occasional		

The evidence for a haemolytic process is only clear cut in the case of diaminodiphenylsulphone. Sulphetrone cannot be called

a haemolytic drug in the dosage employed here (5-6g daily orally for a 65-70kg adult). Diasone becomes more clearly a haemolytic agent when the dosage is raised above 2.4g daily, but this dose has never been recommended in leprosy therapy.

A typical haemolytic process is described in the case history reported below.

#### Case History.

An adult male 35-40 years, in excellent general health, Ll case of leprosy, no trophic deformities. After having been established on 0.3 daily of diaminodiphenylsulphone for several months, the following laboratory results were obtained, R.B.C. 4.4 million, Hb 80% Sahli, Plasma bilirubin <0.2mg%, urine, urobilin occasionally moderately positive, all other routine laboratory tests N.A.D. Dose of diaminodiphenylsulphone increased by 0.1g daily every seven days. Two days after commencing 0.5g daily the morning urine was strongly reddish brown and urobilin could be detected by direct spectroscopic examination of a 3" layer. Plasma bilirubin was 1.5 mg%, methaemalbumin could be identified in the serum, the factor of faecal urobilinogen was ten times the normal, Fouchets test for bile in urine was a weak positive, Schlesingers test was very strongly positive, the urine could be diluted x 40 with alcohol before the F band of urobilinzinc disappeared. The urine contained 150mg% free sulphone, the blood level was nearly 3mg%. Methaemoglobin was never detectable in the blood. Three days later a faint icteric tint was visible in the conjunctivae which persisted for another 3 days. A reticulocytis count at this stage was 27%. Constitutional symptoms were not apparent, the only complaint being of a headache, severe during the evening. Owing to an oversight, values of red blood count and haemoglobin were not obtained. Porphyrins were not detectable in the urine and no detectable decrease of liver function was found by application of the alcoholic turbidity test (v.inf.).

#### Reticulocyte Count.

Owing to a technical difficulty, reticulocyte counts were found impracticable during the initial stages of the work. A series of 3 counts was made on successive days, using Osgoods technique, upon 6 patients who had been standardised for over six months upon the dosage level in question of sulphetrone and diaminodiphenylsulphone, and gave the following figures:

TAB	LE	2.
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				Day 1.	Day 2.	Day 3.
Diaminodiphe	enylsu	lphone	 	7	I 2	9
Sulphetrone			 	2	0	4

#### The White Blood count.

No significant changes in the white blood count or in the white blood picture have been observed in any of the 300 patients treated with any of the sulphones administered here.

#### Methaemoglobinaemia.

The spectrum of methaemoglobin has never been encountered in any of the patients undergoing sulphone treatment in this Unit. 50 examinations made at intervals have never been positive, even in diaminodiphenylsulphone cases where frank haemolysis was occurring due to high dosage experiments.

The blood of patients receiving up to 18g of sulphetrone daily does not appear to contain methaemoglobin.

#### Blood Dyscrasias Conclusion.

The evidence presented in the latter part of Part II and in this part of this article may be interpreted to show that the anaemia of the sulphones is not a process of simple haemolysis. Definite evidence for a haemolytic process is available in the case of diaminodiphenylsulphone, when doses greater than 300mg daily are used. Sulphetrone however has been administered in doses up to 8.0g daily without direct evidence of haemolysis occurring. It is suggested, therefore, that the anaemia produced by sulphetrone is probably mainly dyshaemopoietic in nature. Limited evidence available for the mechanism of the anaemia produced by diasone therapy indicates that in the usual dosage range employed in leprosy, haemolysis occurs to a very slight extent.

#### Effects of the Sulphones upon liver function.

The liver is reported to be the organ responsible for the detoxication of the sulphonamides, the major function involved is the acetylation of the  $N_4$  amino group. Liver damage has been reported upon sulphonamide therapy (Ref. 1) and since diaminodiphenylsulphone has two amino groups presumably capable of acetylation, it was felt that the study of the action of the sulphones upon liver function was of importance.

This study was complicated by the fact that some degree of liver dysfunction is common among the Ibos of Southern Nigeria. Only those showing no detectable degree of liver dysfunction were made the subject of this study. Liver function tests upon those already having some degree of dysfunction will be reported at the end of this section.

#### Procedures.

Method No. 5. The urinary urobilin excretion was measured on three successive days by a modification of the method of Wilbur and Addis (Ref. 2). To 5ml of the acid urine sample (preserved with toluene); three drops of iodine mitis are added. After two or three minutes, 5ml of a saturated alcoholic solution of zinc acetate are added. Filter, stand the filtrate in the dark for 15 mins. Examine with a direct vision spectroscope for the band of urobilin. If present, dilute the sample with 60% alcohol until the band can no longer be seen. Record the dilution as a factor. This estimation is conveniently carried by using standard Pyrex test tubes self graduated at 5 ml and 10ml in a series of two test tube racks.

Method No. 2. The amount of circulating gamma globulin was measured in arbitrary units by the alcohol turbidity test (Ref. 3). This test has been shown to produce results comparable with those of the thymol turbidity test. In practise

it is much more convenient for a routine series of examinations than Maclagans test.

*Test No. 3.* Plasma bilirubin was estimated by the van den Bergh test using cobalt sulphate as a standard.

*Test No. 4.* The detoxicating power of the liver was measured by the intravenous hippuric acid test of Quick.

*Test No. 1.* Palpation for enlargement of the liver was a routine examination.

#### Results.

These are shown in tabular form in Table 3.

			0			
TEST (see preceding	Diamir Number Prior to Therapy	odiphenylsulphon Number after 6—10 months continuous Therapy 0.3g daily		Number Prior to Therapy	Sulphetrone Number after 610 months continuous Therapy with 5-6g	
descriptions)		Test Normal	Test Abnormal		Test Normal	Test Abuormal
No. I	22	22	0	37	37	0
(Palpation)						
No. 2	22	20	2	29	29	0
(Turbidity)						
No. 3	8	8	0	8	8	0
(Bilirubin)						
No. 4	8	8	0	8	8	0
(Hippuric acid)						
No. 5	22	6	16	37	35	2
(Urobilin)						
Analysis,						

TABLE 3.

None of the 59 patients upon diaminodiphenylsulphone or sulphetrone therapy had any entargement of the liver during the period studied.

Two of the 22 patients upon diaminodiphenylsulphone had a significant increase (6 units, 10 units) in the amount of circulating gamma globulin. Neither of these two patients had clinically palpable livers, the excretion of hippuric acid was within normal limits, the albumin/globulin ratio was within normal limits, and the plasma bilirubin was normal.

No increase of plasma bilirubin was found in the 15 patients studied upon either drug.

The detoxicating power of the liver was not affected by either drug in any of the 16 patients studied.

Over 70% of the patients upon diaminodiphenylsulphone therapy have increased excretions of urobilin whereas but 5% of the patients receiving sulphetrone therapy have increased excretions. When the diaminodiphenylsulphone was withdrawn from five of these sixteen patients showing increased excretion, the excretion of urobilin returned to normal after 21 days. It is therefore suggested that the urinary excretion of urobilin is a manifestation of a haemolytic process in these cases, and not pathognomic of disturbed liver function.

#### Comment.

The results presented show no evidence that the sulphones studied have any effect upon the liver function. Histopathological studies of the liver were not possible because of the absence of post-mortem material. The paucity of results from 300 patients is due to

1. The number of cases already presenting (prior to treatment) evidence of liver dysfunction which excluded them from the study.

2. The syndrome (v. Part II) induced by rapid exhibition of the sulphones which causes a marked disturbance of liver function.

The cases already presenting evidence of liver dysfunction were not excluded from treatment. A selected number of these cases on diaminodiphenylsulphone are reported herewith.

#### Cases with palpable liver but no laboratory evidence of dysfunction.

Of 8 cases exhibiting a palpable liver prior to treatment 6 showed no laboratory evidence of dysfunction after 10 months treatment. Two showed an increase of gamma globulin but no other evidence (Tests 3 and 4) of dysfunction.

Cases with palpable liver and laboratory evidence of dysfunction. —Eight cases exhibiting a palpable liver and alcohol turbidity values >4 Units, plus urobilin excretion factors averaging 15 showed no increase in turbidity after 7-9 months treatment. In fact a definite decrease of about 2 turbidity units was observed in all cases.

## Cases without a palpable liver but with laboratory evidence of dysfunction.

Six cases exhibiting a turbidity of >5 units, and an increased urobilin excretion averaging 25 showed two increases of turbidity 8—10:6—10) two decreases of turbidity (10—3:7—5), and two remaining stationary.

#### Comment.

No evidence is presented to show that patients with liver dysfunction prior to therapy cannot be treated with the sulphones. Treatment of patients with marked liver dysfunction is difficult because of the **severe anaemia** produced when the usual doses of the sulphones are given. These patients should not be given more than roomg of diaminodyphenylsulphone daily and a special check should be kept upon their haemoglobin and red cell count.

### Liver function tests after $2\frac{1}{2}$ years sulphetrone therapy.

Forty patients were placed upon sulphetrone therapy in early 1947. Twenty-six of these examined after  $2\frac{1}{2}$  years continuous treatment showed no evidence of disturbed liver function (Tests 1, 2, 5). The remainder were not examined.

#### Urinary Manifestitations of Toxicity.

Although, as far as is known, the kidney is not primarily concerned with detoxicating the sulphonamides, yet it is this organ which eliminates the drug from the blood stream. Because of tubular re-absorption, precipitation of the sulphonamides in the tubules sometimes occurs causing crystalluria, haematuria and anuria. Whether or not this tubular reabsorption occurs with the sulphones is unknown. Determined experimentally here was the solubility of diaminodiphenylsulphone, diasone and sulphetrone in both acid and alkaline urine. Sulphetrone is freely soluble, as is diasone, and precipitation of these drugs could not occur physiologically in urine. Diaminodiphenylsulphone however is relatively insoluble and the determination of its urine solubility is therefore of importance. To this end a sufficient quantity of urine was obtained, divided into two parts, one part was adjusted to pH6 with dilute acetic acid, the other to pH8.0 with dilute caustic. To about 250ml of each in a conical flask was added 1g of diaminodiphenylsulphone and both flasks were shaken for 15-20 mins.

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at room temperature. The solutions were then filtered twice through a fine Whatman (Grade 42) paper, and 10ml of each solution diluted to 100ml with water. 1ml of the final solution was estimated for sulphone content as described in Part I.

#### **Result:**

Solubility of diaminodiphenyl sulphone in urine at pH6 =  $30mg^{0}_{0}$ Solubility of diaminodiphenyl sulphone in urine at pH8 =  $30mg^{0}_{0}$ 

Under the conditions of the analytical method, no difference in the solubility of diaminodiphenylsulphone could be detected in either acid or alkaline urine.

This solubility is of the same order as that of sulphamerazine or sulphapyridine at an acid pH. Unlike these two sulphonamides however the increase of pH does not, apparently, increase the solubility. (A change of pH from 5.5 to 8.0 increases the solubility of sulphamerazine from  $30 \rightarrow 210 \text{mg}_0^{\circ}$  in urine.

There would seem no point therefore in the administration of alkalies to patients on diaminodiphenylsulphone therapy from the point of view of preventing deposition of the drug in the kidney. No work was attempted to discover whether the administration of alkalies increases the rate of excretion of the sulphones.

#### Crystalluria.

During treatment with diaminodiphenylsulphone concentrations of the drug in the urine >100mg% in solution have been found. Concentrations of >30mg% are common. However, in spite of the fact that the experimental evidence shews that diaminodiphenyl-sulphone is only soluble to 30mg% in the urine no case of crystal-luria has been observed among the 70 patients treated for periods up to 10 months with this drug.

Urines containing approximately 100mg% were hydrolysed with boiling dilute HC1 to discover if any of the diaminodiphenylsulphone was present in the form of a more soluble metabolic product. No evidence of this was found, neither was any evidence of acetylation obtained.

The urine did not give a positive test when tested for glycuronic acids; conjugation of this nature, therefore, would not appear to be the solution to the apparently increased solubility of diaminodiphenylsulphone.

With both diasone and sulphetrone no evidence of crystalluria has been forthcoming after nearly four years' experience of these drugs.

#### Hæmaturia.

Has never been encountered during therapy with any of the sulphones.

#### Urobilinuria.

The urine of patients on diaminodiphenylsulphone is frequently a dark yellow brown. Diasone and sulphetrone do not, in moderate doses, have this effect upon the urine. This colour of the urine is due to the presence of urobilin. This substance is presumably present because of the hæmolysis that occurs on diaminodiphenylsulphone therapy. When doses of >0.3gdiaminodiphenylsulphone are given the colour of the urine changes, after a lag period of nearly a week, to a reddish brown and this change is indicative of the excessive hæmolysis occurring in vivo. This colour change is detectable by the patient and may serve as a useful warning if correctly interpreted.

Routine examination of the urine for urobilin by Schlesingers tests is a valuable routine procedure when diaminodiphenylsulphone therapy is instituted. A moderate degree of urobilinuria is not an unusual phenomenon, but when a strongly positive Schlesingers test is obtained at >1-10 dilutions of urine, and a concurrent fall in hæmoglobin is encountered, a hæmolysis of some degree of severity may be occurring. Caution must be exercised in the interpretation of strongly positive Schlesinger tests: **they are not pathognomic of hæmolysis in the presence of liver dysfunction.** 

Although a positive Schlesinger test may be read with the eye alone, confirmatory evidence with a direct vision spectroscope is advisable.

Ehrlichs test for the chromogen, whilst of the same significance as Schlesingers test, **cannot be applied to the urines of patients receiving sulphones.** An orange dye precipitates when Ehrlichs reagent is added to urines containing the sulphones (if the urine sulphone concentration is low only a deep yellow colour may be obtained) because of the formation of a sulphone-benzaldehyde compound. This compound obscures the cherry red colour of the positive Erhlich test. Patients on sulphetrone therapy occasionally excrete a brown urine **not** containing urobilin. The significance of this is not known.

#### Porphyrins.

Examination of numerous urines has never revealed a positive test for coproporphyrins. The urine has always been examined by the following techniques.

- 1. Direct spectroscope examination of  $\frac{1}{2}$ " layer fresh urine.
- Direct spectroscope examination of 1" layer urine + HCI standing for 20 mins.
- 3. Spectroscope examination of acetic acid/ether extract.
- 4. Urochlor reaction.
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It was felt that with the evidence already available (that diaminodiphenylsulphone is responsible for a hæmolytic process) there ought to be produced an increased excretion of coproporphyrin I. In spite of very careful examination none of the tests above have ever shewn a positive result.

#### **Renal Function.**

Six patients on diaminodiphenylsulphone therapy were submitted to the water retention and excretion test of Volhard (Synopsis of Medicine—Letherby Tidy) prior to and four months after the commencement of therapy.

No significant difference between the two series was demonstrable.

No histological reports are available.

No case of anuria has ever been encountered with any of the sulphones used in this Unit.

#### Comment.

The study of the toxic effects of the sulphones commenced in Part II has been continued. The amount of information presented on diasone is unfortunately meagre. The reason for this is, as stated in Part I of this series. Having dealt with the three sulphones, special reference is now made to diaminodiphenylsulphone.

# DIAMINODIPHENYLSULPHONE—A PHARMACOLOGICAL REPORT.

#### Introduction.

Thirty years elapsed between the synthesis of diaminodiphenylsulphone and the demonstration of it antibacterial effects. A further ten years passed without diaminodiphenylsulphone finding a place in the treatment of human bacterial diseases. This long neglect has been due to the marked toxic effects which occur when diaminodiphenylsulphone is used in a dosage adequate for the control of an acute bacterial infection. These effects, seen in man, are severe hæmolytic anæmia, cyanosis and renal damage.

When reports of the value of diasone and promin in leprosy were published an obvious question was whether the sulphones were active per se, or whether they were degraded to diaminodiphenylsulphone in vivo. Two considerations strengthened the belief that they were not active per se:

1. That both were estimated by diazotisation of a free amino group by Bratton and Marshall's method.

**2**. That given orally they were far more toxic than when given intravenously.

As demonstrated (in Part II) the amount of diaminodiphenylsulphone available from the daily dose of diasone was calculated and it was realised that this amount was less than one tenth of the daily dose used for the treatment of acute infections.

Early in 1947 Dr. Ernest Muir and the author had a discussion with Dr. Wevill of the Imperial Chemical Industries (Pharmaceuticals Division) that resulted in the supply of 5kg of pure diaminodiphenylsulphone for experimental trial in leprosy. Owing to factors outside the author's control, the first pharmacological experiments were delayed until October 1948. These experiments were designed to determine.

1. The maximum dose of diaminodiphenylsulphone capable of continuous daily administration.

2. The relationship between dosage, blood and urine levels.

3. The nature of the toxic effects encountered.

and are reported in detail below. There is now evidence from this and other leprosy centres (Cochrane 1949, Leprosy Review XX, I, 4, Molesworth Ibid 1949, XX, I, 3) that diaminodiphenylsulphone may be used in doses >0.3g daily orally without toxic effects greater than those seen with the proprietary sulphones, and with clinical response at least equal to that produced by them.

#### The maximum oral dosage for continuous daily administration.

A short preliminary experiment had shewn that diaminodiphenylsulphone required 6-8 days continuous administration to achieve a plateau blood level. In view of this, in the following experiment the daily dose was increased at 14 day intervals.

Six patients of normal body weight and in good general health were placed on the following course of diaminodiphenylsulphone.

Period.	Daily Dose in grammes.	Total dose in grammes at end of Period.
1st and 2nd week	0.I	I.4
3rd and 4th week	0.2	4.2
5th and 6th week	0.3	8.4
7th and 8th week	0.4	14.0
9th and 10th week	0.5	21.0

After eight weeks, when a total of 14.4g of diaminodiphenylsulphone had been administered the presence of very large amounts of urobilin in the urine together with the presence of methæmalbumen in the blood necessitated the discontinuance of the experiment. None of the patients complained of any constitutional symptoms, their treatment was discontinued on laboratory signs of toxicity only. The following Fig. gives the laboratory data in tabular form:

Patient No.	Con DAI Blood mg%	cen. DPS X Urine mg%	Methæm-l Albumin,	Bilirubin- æmia.	Urobin- uria.	Porphyrins.
1.	2.0	80 <b>*</b>	+	1.5	+ + +	0
2.	1.8	80*	0	0.9	+ + +	0
3.	2.8	70 <sup>%</sup>	+	2.0	++ + +	- 0
4.	2.8	110 <sup>*</sup>	0	2.0	+ + +	0
5.	3.0	130*	+	2.0	+ + +	0
6.	3.6	90*	+	2.0	+ + +	0
	Lu	egend: -	+++ Strong ++ Positi + Weakl o Not d * Single	gly Positiv ve. y Positive etectable: sample.	′е.	

Fouchets test for bilirubinuria was not positive in any of the urines examined.

No methæmoglobin was detectable by direct spectroscope examination of fresh blood.

The van den Bergh reaction of all sera was an indirect positive.

The urines were all highly coloured—a deep red-brown—but no red cells were observed upon microscopic examination of the deposit and the benzidine reaction was negative in all cases.

These results were interpreted as the commencement of a marked hæmolytic process, and it was felt that a daily oral dose of 0.5g represented the minimum toxic dose. 0.3g daily was adopted as the therapeutic maximum dose, and chronic toxicity studies reported in these articles have shewn that this dose can be continuously administered without ill effects.

Rapid exhibition of the drug is strongly contra indicated: higher blood levels than usual are produced and toxic manifestations including methæmalbuminæmia with frank jaundice are seen. When the drug is exhibited slowly these effects do not occur. A suggested administration is 0.1g daily rising by 0.1g every 14 days to a maximum of 0.3 daily for adults. Results obtained with this dosage regimen have already been reported as part of this study.

#### Conclusion.

Part I of this series was concerned with the aspects of absorption and persistence of the sulphones, and the results presented shew that from this aspect the non-proprietary diaminodiphenylsulphone possessed advantages over the proprietary sulphones.

Part II described work performed to estimate the degree of breakdown of diasone and sulphetrone and shewed that diaminodiphenylsulphone may be recovered from the urine of patients on diasone and sulphetrone therapy.

Studies of the anæmia producing mechanism of the sulphones shewed that the anæmia was the result of both dyshæmopoiesis and hæmolysis when the proprietary sulphones were administered and that hæmolysis was the minor cause. The anæmia caused by diaminodiphenylsulphone being no more severe than with the proprietary sulphones the attempt was made to discover whether diaminodiphenylsulphone had any specific toxic action that prohibited its use for continuous therapy.

Studies of blood dyscrasias, liver function, renal function and urinary abnormalities shewed that diaminodiphenylsulphone possessed no intrinsic chronic toxic potentialities when a dose of 0.3g daily was not exceeded.

The author is unable to ascertain exactly what is a therapeutic blood level of the sulphones, but refers to work which, if substantiated, will prove of great importance, namely that clinical improvement follows doses of diaminodiphenylsulphone of such an order that blood levels are not detectable.

Further research into the therapeutically optimum dose of diaminodiphenylsulphone is obviously necessary. The author suggests that the approach to this question must not be the same as that of the chemotherapy of an **acute** bacterial disease. In this latter case, it has been shewn that the maximum amount of the chemotherapeutic agent must be administered as quickly as possible (i.e., high initial blood levels must be obtained) for effective treatment. Molesworth (*Lep. Rev.* 1949 XX, 1, 3) adopts this approach in the treatment of leprosy.

Finally, a brief pharmacological report is given of diaminodiphenylsulphone shewing that the major toxic effect is that of hæmolysis. This hæmolysis was to some extent anticipated by the presence of increasing amounts of urobilin in the urine.

#### Summary.

Diaminodiphenylsulphone (in oral doses not exceeding 0.3g daily) is suggested as a therapeutic agent for the treatment of leprosy for the following reasons:

- i. it is extremely well absorbed.
- ii. it is slowly excreted.

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iii. in the dosage suggested it is no more toxic than the proprietary sulphones.

iv. the cost of therapy with diaminodiphenylsulphone is less than 1/10th of that with the proprietary sulphones.

In cases of known kidney dysfunction the oral dose of diaminodiphenylsulphone should not exceed 0.1g daily.

The oral administration of sulphetrone should be replaced by parenteral administration, owing to the poor absorption of this drug. A suggested dosage (v. Part 11) is 5.0g weekly given in two roml injections of a 25% aqueous solution.

Sulphetrone would seem to be a completely non-toxic substance, if its anæmia producing effect is excepted, in the dosage recommended for leprosy therapy.

The anæmia produced when the proprietary sulphones are administered parenterally is much less than that produced when oral administration is used.

The sulphones cannot be shown to have any effect upon the liver function.

The sulphones cannot be shown to have any effect upon the renal function.

No effect upon the white blood count or picture can be demonstrated.

It is suggested that the estimation of blood levels of the sulphones as a means of adjusting dosage is an unnecessary procedure.

Further clinical study of the effect of various dosage regimens of the sulphones is strongly advocated.

#### Acknowledgments.

The acknowledgments were, by an oversight, placed after Part I of this series. They should be read here. As well I should like to acknowledge the very real help afforded by Dr. T. F. Davey.

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