A PHARMACOLOGICAL STUDY OF THREE SULPHONES.

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PART II.

HYDROLYSIS AND THE SPECIFIC TOXIC PHENOMENA.

BLOOD LEVELS AND THERAPY.

No information has been presented which indicates that high blood levels of any of the sulphones promotes greater rapidity of clinical response. The present dosage regimens are based upon empirical assumptions and no leprologist can say with certainty that it is necessary to maintain a definite prestated level of a sulphone in the blood (Ref. 1). It has yet to be shewn that a blood level of 6mg% of sulphetrone is any more effective than a blood

level of 2mg%; since the former blood level requires 8-10g. daily of sulphetrone and the latter 2g. daily, the matter is obviously of some importance both from a medical and economic point of view.

Recent work (Ref. 2) has shown that clinical response is obtained with extremely small doses of diaminodiphenylsulphone. Doses of 0.05. every other day are reported to give good results (Ref. 3); blood levels on this dosage are $<$ 0.1mg% therefore it would seem that the attempt to achieve and maintain bloodlevels of the order of 1-2mg% with diaminodiphenylsulphone is unnecessary.

This attempt was made because of the assumption that if a blood level of 5mg% of sulphetrone and 2mg% of diasone produced clinical response then the molecularly equivalent blood level of diaminodiphenylsulphone would do so too. (It was assumed, ad hoc, that 100% hydrolysis of the proprietary sulphone occurred though in fact it is improbable that this degree of breakdown does occur.) Fig 1 presents the constructed table shewing the chemical method of computing the molecular ratio of the sulphones.

FIG. 1.

Col. 1. Sulphone.	Col. 2. Molecular Wt.	Col. 3. Ratio of Col. 2 to DADPS*	Col. 4. Molecular Ratio.
DADPS*	248	248/248	1
Diasone	448	248/448	0.56
Sulphetrone ...	892	248/892	0.28

* Diaminodiphenylsulphone.

Therefore the "therapeutic" blood level of diaminodiphenylsulphone may be calculated empirically, as follows, using the blood levels of the proprietary sulphones that are maintained on the usual dosage regimens.

Diasone — 0.56 x 1.8mg% = 1.0mg%
Sulphetrone — 0.28 x 5.0mg% = 1.4mg%

It was found that a blood level of 1mg% of diaminodiphenylsulphone could be established and maintained by a daily oral dose of 0.2-0.3g. (v. Part I).

The ad hoc assumption that 100% *in vivo* hydrolysis occurs is obviously not correct since concentrations of sulphetrone are found in the urine of the order of 1000mg *in solution* (v. Part I). The solubility of diaminodiphenylsulphone being only 30mg% in urine, the sulphetrone must be present largely as the complete molecule. The degree of breakdown that does, if at all, occur with the proprietary sulphones is obviously a point of fundamental importance, and the following work was undertaken to establish

the evidence of, and degree of, *in vivo* hydrolysis of the proprietary sulphones. Brownlee states (Ref. 3) that breakdown of sulphetrone to diaminodiphenylsulphone does not occur. Similar statements are not made concerning other proprietary sulphones.

IN VIVO HYDROLYSIS.

a. *Therapeutic evidence.*

In Part I theoretical evidence was presented to shew that the proprietary sulphones probably owe their activity to their content of diaminodiphenylsulphone. For the purposes of this discussion if 100% hydrolysis of the proprietary sulphones occurs we may construct, as in Fig 2, a table to demonstrate how much diaminodiphenylsulphone would be available to the body from the usual dosage regimens of the proprietary sulphones.

FIG. 2.

Col. 1. Sulphone.	Col. 2 Daily oral dose.	Col. 3. %age Absorbed from gut.	Col. 4 Amount metabolised. (Col. 2 x Col.3)	Col. 5. Am'nt DADPS* liberated (Col. 4 x Molecular ratio)
DADPS*	... 0.3g.	85	0.26g.	0.26g.
Diasone	... 1.5g.	46	0.69g.	0.38g.
Sulphetrone	... 5.0g.	15	0.75g.	0.21g.

* Diaminodiphenylsulphone.

From this table it is interesting to see that the amount of diaminodiphenylsulphone available to the body when the proprietary sulphones are used is of the same order as the dose of diaminodiphenylsulphone which we have established to be the maximum therapeutic dose capable of continuous administration without toxic sequelae. If only 20% hydrolysis of the proprietary sulphones occurred it is obvious that the amount of diaminodiphenylsulphone available would be c.0.05g. That this figure is capable of giving clinical response is shewn by the work referred to above (Ref. 2).

b. *Experimental evidence.*

When any of the proprietary sulphones are ingested, blood and urine levels may be ascertained by diazotisation and coupling of the circulating sulphone as in the Bratton & Marshall procedure. However, this procedure does not give any information as to whether the sulphone present is present as the proprietary substance, the monoamino compound, the doubly hydrolysed compound (i.e., 4,4′ diaminodiphenylsulphone) or a various mixture of all of these. A blood level of 5mg% obtained from a patient

receiving sulphetrone therapy may represent not 5mg of sulphetrone but only 2mg% of sulphetrone and the rest diaminodiphenylsulphone. In order to determine the degree of *in vivo* hydrolysis of the proprietary sulphones the following method was developed.

Method for Urine. A sample of urine from a patient undergoing therapy with a proprietary sulphone is analysed for its concentration of sulphone by the usual technique (see previous paper). In order to facilitate the procedure a urine of >100mg% sulphetrone is obtained and >50mg% diasone is obtained, and after analysis the urines are adjusted exactly to some convenient figure (e.g., 100mg% and 50mg%) by the addition of water. Control solutions of sulphetrone and diasone are then prepared by weighing the pure powders and dissolving in a fresh urine obtained from a person not on sulphone or sulphonamide therapy. 100ml. portions of both the control and the urine to be tested are then extracted with three aliquots representing a total volume of 100ml. of ethyl acetate. The extracts are then dried over anhydrous sodium sulphate and 1ml. of each is estimated for diaminodiphenylsulphone content as described previously (Part I), except that the trichloracetic acid is replaced with a 50% solution of ethyl alcohol. The concentration of diaminodiphenylsulphone found in the "control" tube (usually negligible with sulphetrone) is subtracted from that obtained in the urine. This figure represents the amount of diaminodiphenylsulphone in mgs%. This over the figure obtained from the first analysis, will give the degree of hydrolysis that has occurred *in vivo*. The following figures show the amount of *in vivo* hydrolysis occurring in patients undergoing sulphone therapy with this Unit.

Drug.	No Observations.	mg% total sulphone.	mg% as DADPS*	Partition.
Diasone ...	5	50	2-10	4-20%
Sulphetrone ...	8	100	3-6	3-6%

* Diaminodiphenylsulphone.

Method for Blood. c.20ml. of blood from a patient are withdrawn. 1ml. of this sample is estimated for the sulphone in question as described in Part I. 10ml. of the remaining blood are mixed with 50ml. satd. $MgSO_4$ and then centrifuged until separation is obtained. 10ml. of the supernatent fluid is then shaken with 10mls. of ethyl acetate. 1ml. of the ethyl acetate extract is then estimated as for the extract obtained from urine.

A "blank" estimation is performed upon blood extracted from a person not undergoing sulphone therapy and to which blood has been added a known equivalent quantity of the sulphone to be estimated.

Result. Using this technique no diaminodiphenylsulphone could be discovered in the blood of patients undergoing sulphetrone therapy. With diasone, equivocal results were obtained, due to some grossness of the technical procedure. Further studies are contemplated: the above results and methods are reported in order that the work may be repeated in other research laboratories.

DISCUSSION.

That diaminodiphenylsulphone may be extracted from the urine of patients undergoing therapy with proprietary sulphones is evidence from the partial hydrolysis of these compounds in the body. The grossness of the methods used do not allow of any conclusions regarding the partition of the sulphones in the blood.

The presence of diaminodiphenylsulphone in the urine however is strong evidence for the presence of diaminodiphenylsulphone in the blood. The author holds the view that in the light of the evidence presented, both experimental and theoretical, the *in vivo* partial hydrolysis of the proprietary sulphones must be accepted.

TOXIC EFFECTS OF THE PROPRIETARY SULPHONES.

Since the sulphones are in some measure degraded to diaminodiphenylsulphone the toxic effects of any proprietary drug may be due to a combination of two factors:

a. The toxic action of diaminodiphenylsulphone.

b. The toxic effect of the whole molecule of the sulphone or portion of the molecule remaining after liberation of diaminodiphenylsulphone.

In this article the toxic phenomena of three sulphones will be studied in an attempt to discover whether diaminodiphenylsulphone possesses intrinsic toxic qualities which renders its use in leprosy therapy inadvisable.

BLOOD DYSCRASIAS.

Anaemia.

That the administration of the sulphones whether orally or parentally results in an anaemia (Ref. 4), is well known. That the anaemia produced may differ quantitavely with (a) the type of sulphone and (b) the mode of administration is not generally realised.

Sulphetrone. The anaemia produced is in the majority of cases not severe on a dosage of not more than 5.0g. daily *when the exhibition of the drug is gradual.* A fall of $>$ 3g. Hb% in the first six weeks is usual, and if iron is given concurrently with the sulphetrone the body stabilises itself within a matter of two or three months. The anaemia is hypochromic in type, no evidence for a marked haemolytic process has been found from studies of plasma bilirubin, red cell fragility, urobilinogen excretion or the presence of methaemalbuminaemia.

If the exhibition of the drug is rapid there occurs in Southern Nigeria a syndrome of glandular enlargement, splenomegaly, pyrexia and disturbance of liver function exhibition the blood picture similar to that of infectious mononucleosis. This effect is seen with all the sulphones when the exhibition of the drug is rapid. So far as is known it occurs only in this area—no such similar syndrome has been reported elsewhere. This particular action of the sulphones will be made the subject of a separate

report. No patient exhibiting this phenomenon is included in the group for purposes of the present report.

Brownlee (Ref. 3) reports that sulphetrone combines with alimentary iron and also depresses the bacterial flora of the gut. Both these factors may be responsible for the anaemia producing effect of sulphetrone administered orally.

In an effort to study the degree of responsibility of both the diaminodiphenylsulphone part of the sulphetrone molecule and the remaining cinnamyl-like portion for the anaemia produced by sulphetrone the following experiment was performed. In Fig. 2, Col. 4 it is shewn that the amount of sulphetrone available to the body on a daily oral dose of 5g. is 0.75g. Sulphetrone was given intramuscularly in a dosage of 0.75g. daily to a group of five patients. The resultant anaemia was not of significance. In the five cases under test no loss of Hb greater than 0.5g. was recorded.

It is realised that this study is by no means as satisfactory as desired.

Diasone. When diasone is given orally in doses of 1.8g. daily an anaemia rather greater than that produced by 5g. of sulphetrone daily is produced. When the dose is raised above 2.0g. daily then a definite haemolytic anaemia is detectable by means of raised urobilinogen, excretion, raised icterus index, and the occasional presence of methaemalbumen in the blood plasma.

The fall in the red cell count with oral diasone is of a much greater degree than with sulphetrone. Red cell count decreases of the order of 1.5 to 2.0 million are not uncommon when the daily dose is 1.8g.

Fig. 2, Col. 4 shews that the amount of diaminodiphenylsulphone available to the body is c.0.7g. when the daily oral dose is 1.5g. When diasone is given parenterally in a dosage approximating to 0.7g. daily (5ml. of a 40% solution thrice weekly) the anaemia produced is (as with parenteral sulphetrone) of nothing like the same degree. In 14 patients treated for two months with parenteral diasone as described above, the anaemia was not severe enough to warrant the administration of iron or the cessation of treatment.

Diaminodiphenylsulphone. In a dosage of 0.2-0.3g. daily the anaemia produced by this drug is of a mild self-correcting type occurring mainly in the first month of treatment. The anaemia is of a haemolytic nature, though perhaps other factors also enter into its production.

General Comment. The study of anaemia in Southern Nigeria

is made difficult by the many intercurrent haemolytic infections to which the patients are exposed. There is also the unknown effect of the various degrees of liver dysfunction present in a large percentage of primitive African people in this area. Thus the effect of any drug upon the body may not be a pure cause and effect relationship. The anaemia produced by the sulphones is always more severe and treatment more difficult in patients with liver dysfunction. Diaminodiphenylsulphone cannot be shewn however to be more anaemia-producing in these cases than diasone or sulphetrone.

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