Metabolism of the Sulfones[1]

S. R. M. Bushby[2]

Before discussing the metabolism of DDS, I would like to comment on the empiric nature of the treatment of leprosy with antibacterial drugs. This treatment is basically empiric because sensitivity testing plays—perhaps it would be better to say has played—no part in selecting the appropriate drug for the treatment of particular patients. The importance that is attached to sensitivity testing in the treatment of other infections is shown by the fact that these tests form a major occupation of hospital bacteriologic laboratories.

Although knowledge of the *in vitro* sensitivity of an organism to an exhibited drug removes much of the empiricism from the treatment, other information, equally important, is necessary before the treatment can be regarded as having a sound rational basis. This extra information consists of knowing the metabolic changes that the drug may undergo and the concentrations and duration of active forms of the drug at the infection site. This information regarding concentration at an infection site is not easily obtained; reliance is therefore usually placed on knowing the concentration of the drug in the blood, and, when this is done, data regarding binding to plasma proteins become important.

In practice, information regarding metabolic changes to the drug can be ignored, without loss of rationality, provided that the concentration of the drugs is determined by a process that measures antibacterial activity; with the sulfones this is rarely, if ever, done. The Bratton and Marshall method ([1]) is the standard procedure for estimating these drugs, and detection by it depends on at least one of the amino groups being free, or, if substituted, for the substitution to be sufficiently labile to break down under the acid conditions of the test. The significance of this will be appreciated in a few moments.

Lowe ([7]) was probably the first investigator to endeavor to determine the minimum effective dose of DDS, but he made the tacit assumption that all strains of *Mycobacterium leprae* are of equal sensitivity to this drug. With the development of the mouse foot pad technic of Shepard ([9]), we can now determine the minimum concentration of dapsone in animal tissues that inhibits multiplication of the leprosy bacillus. In our own studies in mice, made in collaboration with Rees ([3]), the minimum effective dose is a concentration that lies between 0.001 and 0.0001 per cent DDS in the diet, and this dose produces very low levels of drug in plasma, muscle, and other tissues. They are in fact less than 0.2 μgm./ml. In my tests, the concentration of DDS that affects the growth of tubercle bacilli in 33 per cent blood is 62 μgm./ml., and at this concentration the effect is only partial; 1,000 μgm./ml. is required for complete inhibition. In the Peizer and Schecter medium the level that gives complete inhibition is about 60 μgm./ml. These concentrations are on the order of those necessary for an effect with sulfonamides against nonmycobacterial species. The leprosy bacillus must therefore be phenomenally sensitive to DDS.

In extrapolating to man this information about sensitivity gained in mice, however, we make the following assumptions:

1. That man and mouse deal with DDS in similar fashions, and

2. That the *in vivo* activity of DDS is due solely to the parent drug.

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In connection with these assumptions it must be noted that DDS is almost insoluble in water. However, it has been known for many years that DDS is changed *in vivo* to substances that are more soluble in water and might have antileprotic activity. The therapeutic significance of this change was confirmed when Woiwod and I ([1, 5]) showed that the principal derivative still had one free amino group and would, therefore, still have antibacterial activity. This derivative was identified as a glucuronide. Because of its acidic nature and its lability in weak acids with the yield of unchanged DDS, we considered the union to be between the nitrogen of one of the amino groups of DDS and the aldehyde carbon of glucuronic acid (Fig. 1); alternatively, according to Teewyn Williams ([12]), it could be an N-glucuronide (Fig. 1) with the glucuronic acid in its pyranose form.

This type of conjugation was hitherto unrecognized.

Tsutsumi ([10]) has since shown the presence of even another monoconjugate. This is the N-sulfamate (Fig. 2); it too would have antibacterial activity, but it is present in very low concentrations. A fourth metabolite, detected by Woiwod and me ([1, 5]), was the disubstituted N-glucuronide, and this does not have antibacterial activity. The monosubstituted metabolites will be estimated by the Bratton and Marshall method, irrespective of whether they revert to DDS or not during the estimation, but their concentration will be underestimated unless reversion occurs; this is because the monosubstituted sulfones give less intense color in the diazo reaction. Stable disubstituted forms of DDS will not be estimated, but these are of course inactive.
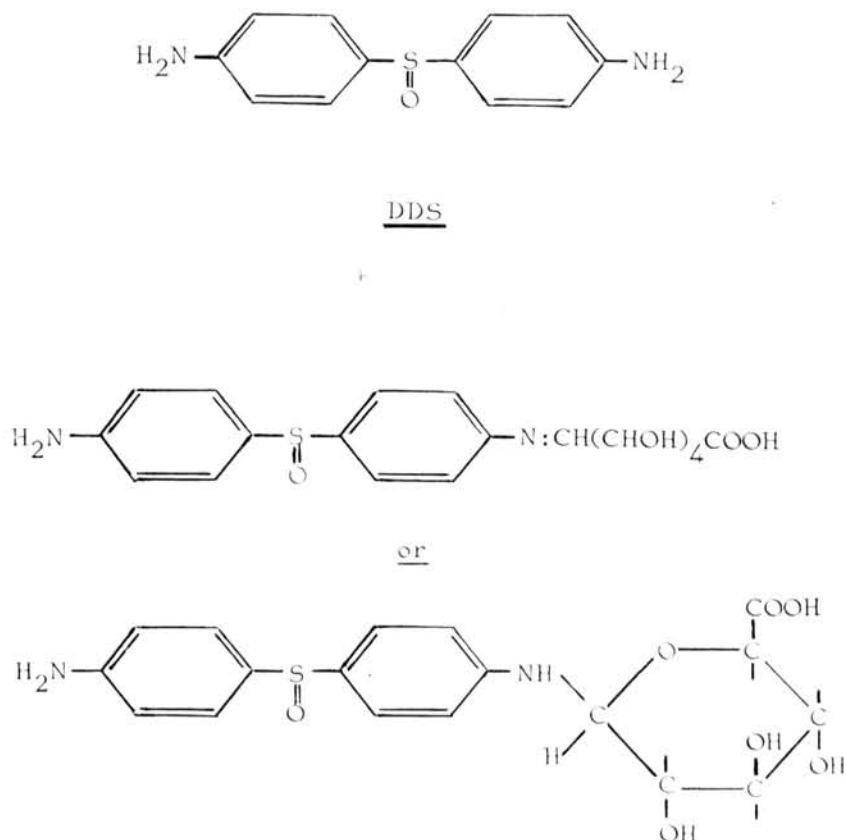

DDS

+

or

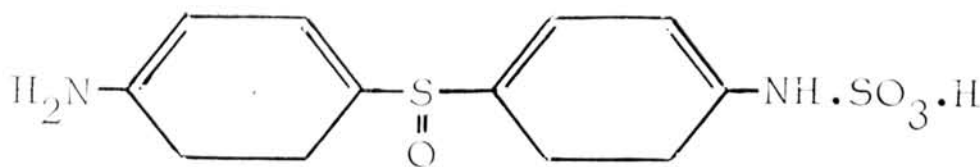FIG. 1. Glucuronide metabolite of DDS.

Fig. 2.   Sulfamate metabolite of DDS.

More recently Ellard ([6]) has examined the urine of both rabbit and man, and concluded from his results that the activity of DDS is due to the presence of unaltered drug. This conclusion was based on his finding that 41 per cent of the drug excreted was present in the unsubstituted form. Woiwod and I ([4, 5]) made most of our studies in rabbits, and we found that the proportion of the dose excreted as the mono-N-glucuronide depended on the pH of the urine; the conjugating bond of the derivative in this animal breaks readily under mild acid conditions to release the parent drug. With alkalinization of the urine, accomplished by administration of sodium citrate, the quantity of unsubstituted DDS in the urine became undetectable; similar studies in man showed that the proportion of free dapsone excreted under these conditions was less than 5 per cent. Incidentally, we had evidence that the glucuronide in man was more stable than that formed in the rabbit. We suggested that one of the amino groups of DDS may have been oxidized to a hydroxyl or carboxyl group and the resulting substance excreted as a glucuronide; we were, however, unable to recover any changed DDS, either free or from a conjugate.

Because of the very low proportion of unsubstituted DDS that we found in the urine, Woiwod and I ([4, 5]) suggested that the antileprotic activity of DDS is due mainly to the monosubstituted derivative. Ellard ([6]), on the other hand, finding much larger proportions (40 - 50%), considered that the activity was due to DDS itself. The difference in our findings, and therefore in our conclusions, may have occurred because the patients studied by Ellard were not given alkalies; the pH value of their urines ranged from 6 to 7, and alkalinization, similar to that accomplished by us, would have raised these

values to near or above pH 8. We considered that the free DDS present in urine was probably due to breakdown after passing through the kidneys, but Ellard suggested the very opposite. Bridges and Tecwyn Williams ([2]) have shown that N-glucuronides of sulfonamides can develop *in vivo* nonenzymatically, and Ellard ([6]), by implication, suggested that as the rates of the synthesis and decomposition of the glucuronides vary considerably with pH, the proportion of free DDS in the urine will vary with the pH of the urine. He is suggesting, therefore, that at least a part of the glucuronide forms in the urine. I agree with the conclusion regarding decomposition, but not with the suggestion that synthesis occurs after passage through the kidneys. We found that the proportion of the conjugate was maximum in alkaline urine, and Bridges and Tecwyn Williams found that nonenzymatic synthesis was optimal at pH 3 - 4, with little or no conjugate forming above pH 7; the synthesis of the DDS-N-glucuronide is therefore unlikely to occur after passage through the kidney. Moreover, Tsutsumi ([10]) claims to have identified the N-glucuronide of DDS actually in the plasma of rabbits.

Even if we agree that the question is still unsettled as to whether the DDS present in the blood is solely in the substituted form, the knowledge that the drug is present in the plasma in more than one active form, raises the question whether the various forms are equally capable of reaching sites of infections. Observations on binding to proteins, by workers at the Japanese Institute for Leprosy Research ([8]), are pertinent; they have shown that, whereas the parent drug is highly bound to plasma proteins, the more water-soluble derivates are at most only slightly bound.

We too have made some observations on this problem and the results confirm those

of the Japanese. In a modification of the Bratton and Marshall method which we use in the Technicon Autoanalyser, the serum is dialyzed against 0.5N hydrochloric acid, which lowers the pH of the blood to about 2.0; at this pH the DDS that is absorbed to protein passes into the acid. We varied the method by dialyzing into normal saline. Under these conditions only unbound drug passes freely into the dialysate and is subsequently estimated. We compared the effects of this modification on the estimation of DDS that is present in the blood of dogs after oral administration and after adding DDS directly to dog serum. The results are shown in Table 1; they show that approximately 52 per cent of the DDS dialyzes into saline when the drug is derived from that present in the alimentary tract, but only 34 per cent dialyzes when DDS is added directly to the serum.

TABLE 1. *Differences in the proportions of bound and free DDS in serum after oral administration and when adding to serum in vitro. The total drug dialyses into acid but only unbound drug dialyses into water.*

	DDS content of serum μgm./ml.	
	Adsorbed into blood from gut (10 mgm./kgm. orally)	Add to serum (5 μgm./ml.)
Total (dialyzable into acid)	4.93	4.8
Free (dialyzable into saline)	2.65	1.7
Bound (non-dialyzable into saline)	2.28	3.1

The significance of these observations is that the metabolites are likely to be present in higher concentrations at the infection site than the unchanged drug. This conclu-

sion, however, ignores the probability that the parent drug, being more lipid-soluble, will be more readily taken up by the mycobacteria than the hydrophilic metabolites.

Because of the low concentrations of drug in the tissues, and because of the instability of the mono-N-glucuronide in acid, the question whether the antileprotic activity of DDS is due to the unmodified drug or to a monosubstituted derivative may never be answered directly. However, information that may have some bearing on this question could be obtained from studies with the monosubstituted forms of DDS that are available for clinical use. For example, Woiwod and I (⁴, ⁵) found that the monosubstituted glycine derivative, sulfone-cilag (Fig. 3), was excreted unchanged after either oral or parenteral administration; its activity can therefore not be due to free DDS. So it would be pertinent to know whether the minimum concentration that inhibits the multiplication of the leprosy bacillus, as measured by the foot pad technic, is of the same order as that of DDS. We should note, however, that these monosubstituted forms of DDS are less active than the parent drug against the pyogenic cocci, when compared both *in vitro* and *in vivo*, and so, if their minimum inhibitory concentration for the leprosy bacillus should prove to be higher than that of DDS, it would be reasonable to assume that the activity of DDS was due to DDS *per se* and not to a monosubstituted metabolite. I hope someone will make these experiments and thus attempt to settle this question.

So far, I have confined my discussion to DDS and to one of its monosubstituted forms. To recapitulate my contentions, the antileprotic activity of DDS appears to be due, at least partly, to its monosubstituted metabolite, whereas the activities of the monosubstituted analog seem to be due to the drug *per se*. The monosubstituted forms of DDS are, however, seldom used, but their disubstituted analogs, e.g., solapsone, Promin and the diacetyl derivative are still not uncommonly used. These drugs have no free amino groups, and, as I have already indicated, there is convincing evidence to show that antibacterial activity of

H₂N—⬡—SO₂—⬡—NH.CH.CH₂.CH—⬡

SO₃Na   SO₃Na

Semi-sulphetrone

H₂N—⬡—SO₂—⬡—NH.CH₂.COONa
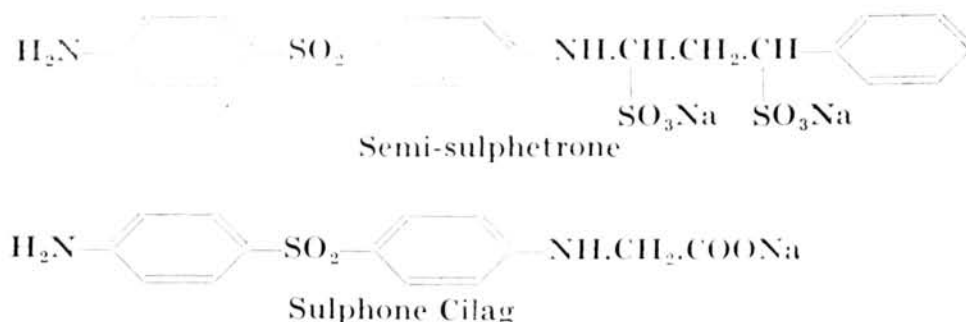
Sulphone Cilag

Fig. 3. Monosubstituted derivatives of DDS.

the sulfones depends on at least one of the amino groups being free; the disubstituted forms of DDS must therefore be inactive until one, or both, of the substituted amino groups becomes free.

The oral and parenteral activities of these disubstituted forms of DDS were accounted for some years ago. A small amount of DDS is present as an impurity in most of these preparations and the quantity is further increased after oral administration, because a variable portion of the drug reverts to DDS during passage through the alimentary tract. After parenteral administration little of the drug reverts to DDS, and the activity then appears to be due to the contaminating DDS and also to the corresponding monosubstituted form. This monosubstituted form is present in the drug as an impurity, but it is also formed nonenzymatically *in vivo* from the disubstituted form; in water these disubstituted analogs of DDS form equilibrium mixtures consisting of both the disubstituted and the monosubstituted forms. According to Tsutsumi ([11]), however, Promin, after intravenous injection, is desulfonated to form the N-glucosides of DDS, and therefore closely resembles the N-glucuronide of DDS. I am not aware of any studies with the diacetyl derivative when it is used as a repository; its low solubility will make these difficult.

So far, these comments regarding the metabolism of DDS have been directed mainly toward use of the drug in the treatment of leprosy, but perhaps I may conclude with some remarks regarding the use of DDS in malaria. In this infection advantage can be taken of the synergic action of

DDS and other antimalarials, and the duration of action becomes important if the doses of the drugs are to be properly matched. We have recently determined the half-life of DDS in the serum of five human volunteers. They took 100 mgm. DDS and the half-life averaged 28 hours, but ranged from 17 to 32.5 hours. Peak concentrations of 1.5 to 2.0 μgm. ml. occurred in three to six hours, but concentrations of about 0.2 μgm. ml. were still present at three days and the drug was still detectable at four days; no drug, i.e., <0.05 μgm./ml., was detectable in the serum at seven days, although traces, i.e., 0.2 μgm./ml., were still being excreted in the urine after 12 days or more. These results agree with those of earlier workers. Although complete elimination of the drug takes almost two weeks, in the control of malaria, a disease in which we are presumably mainly interested in the concentrations of the drug in the blood, it is the plasma half-life of about 28 hours that is important in the endeavor to match the dose of DDS with that of a second drug.

I commenced this discussion by referring to the empiric nature of sulfone therapy; I will end it by suggesting that when all the implications of drug metabolism and distribution are considered, the basis of the chemotherapy of leprosy seems unlikely to differ significantly from that of other infections. In practice, successful therapy is based on the empiric observation that infections caused by organisms that have some degree of sensitivity to certain drugs usually respond to treatment with these drugs.

SUMMARY

Although the sulfones have been the standard treatment of leprosy for 20 years, their administration still remains essentially empiric. The development of a rational approach has been hindered by lack of information regarding sensitivity of the leprosy bacillus to these drugs and their metabolites.

Now that the multiplication of the leprosy bacillus in animals is known to be prevented by administration of sulfones, the minimum effective concentration is determinable. In order to place treatment with the sulfones on a less empiric basis, it would seem necessary only to determine the optimum regimen for maintaining this concentration of drug in the tissues of man. In practice, however, the problem is complicated by: (1) The knowledge that DDS is changed *in vivo* and that at least one of the metabolites is probably active. (2) The metabolism of the sulfones in the mouse may differ significantly from that in man, and the minimum effective concentration of diazotizable drug in the tissues of man may therefore differ from that of mouse. (3) The difficulties of making direct observations in man of the concentrations in tissues may make reliance on plasma concentrations appear necessary. (4) The adsorption of DDS and of its metabolites to plasma proteins differs, so that the concentrations in plasma may not reflect those tissues. (5) The hydrophilic properties of the drug and its metabolites differ greatly; this may affect their distribution within tissues. Because of these complications the problem may have to be solved indirectly by comparing in mice the effects of DDS and a stable monosubstituted derivative, e.g., the glycine derivative.

The half-life of DDS in man is about 28 hours, but this knowledge has not been important for effective treatment of leprosy, for low but probably adequate concentrations are still present in the blood and tissues for several days. However, as DDS is now being recommended for the treatment of malaria in conjunction with the antifolates, this knowledge is necessary in order to arrange a suitable dose regimen for the two drugs.

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DISCUSSION

Dr. Peters. Dr. Bushby referred to a paper by Ellard, who measured not only directly diazotizable materials, but also those released after mild and strong hydrolysis. It would help to know if the N-glucuronide is completely hydrolyzed by direct diazotization or possibly contributes to the other fractions. Furthermore, what are the materials contributing to the diazotizable substances released by the mild and strong acid treatments?

Dr. Bushby. The N-glucuronide detected, which is a major metabolite, is broken down under acid conditions without heat. I do not know the nature of other metabolites contributing to the slight increase occurring during acid hydrolysis with heat. It ususally is attributed to the acetylated form, but we were unable to detect any acetylated form in our studies.

Dr. Powell. In urine normally there are small amounts of diazotizable materials. These appear to vary from day to day. It seems difficult, therefore, to know precisely when values reach undeterminable levels after cessation of administration of DDS. I would like to ask Dr. Bushby if he corrected for the small amounts of material normally present, and at what point he decided the values of density readings were too low to be significant. Our own values have approximated nondetectable, or at least nonsignificant, values, usually three to five days after cessation of the administration of DDS, whether daily, weekly, or in single doses.

Dr. Bushby. Dr. Powell: were you extracting from the urine or making direct estimations on the urine?

Dr. Powell. We were using the Bratton-Marshall procedure, as modified by Molesworth,[1] with standards prepared by the addition of the parent DDS to aliquots of urine.

Dr. Bushby. Estimations were made on extractions into a nonpolar solvent after acid hydrolysis. This reduces the blank figure almost down to undetectable levels.

Dr. Powell. How much does this increase accuracy, sensitivity, and reliability of the procedure?

Dr. Bushby. It eliminates the problems arising from variable blanks.

Dr. Levy. I would like to ask Dr. Bushby if the mono-N-glucuronide would be extractable into chloroform or ethyl acetate from an alkalinized urine or blood.

Dr. Bushby. It would not.

Dr. Levy. Is this the material accounting for some or much of the circulating dapsone that can be measured in the blood, and, if so, how can this be reconciled with the rather common finding that most of the dapsone in the blood is readily extractable?

Dr. Bushby. That depends on the pH. If it is made acid and then extracted, it will come out.

Dr. Levy. In the method described in Simpson's paper[1] the extraction was made from material made slightly alkaline with dibasic sodium phosphate.

Dr. Bushby. I cannot answer the question directly. But if, when they make their estimation, they first acidify in order to break labile bonds and then take the pH back to the alkaline side in order to get the free DDS to pass into the solvent, then this glucuronide would be estimated.

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Dr. Levy. Possibly the N-glucuronide might not be so readily extractable as the Bratton-Marshall-reacting material.

Dr. Bushby. This is certainly our experience.

Dr. Shepard. I would like to ask Dr. Bushby in what species sulfone-cilag was excreted in unchanged form.

Dr. Bushby. It was in the rabbit.

Dr. Peters. In view of the reports of hemolytic anemia following high doses of DDS in very sensitive individuals, one might suspect that hydroxylamine or nitroso derivatives could be formed. These would not be diazotizable. I wonder if there is any evidence or indication that these compounds have been isolated or indicated as metabolites of DDS.

Dr. Bushby. Not as far as I know. If they are not diazotizable or do not give a positive Ehrlich reaction, I would not think they would have been detected by other methods.

Dr. Chang. Dr. M. I. Smith and his associates at the National Institutes of Health, Bethesda, Maryland, studied more than 20 mono-derivatives of DDS before 1949, and tested them in experimental tuberculosis in guinea-pigs. One derivative, 4-amino-4'-β-hydroxyethylaminodiphenyl sulfone (HES), was found to be the least toxic drug in various species of animals.[2] It is 10 times less toxic and only 2 times less active than DDS.[3] Payne and his associates[4] treated 57 pulmonary tuberculosis patients with HES partly combined with streptomycin. The drug was well tolerated in a dose of 1.5 to 3 gm. daily for 6 months. Its antituberculosis activity was apparent. Floch[5] treated 10 cases of lepromatous leprosy with HES, 1 gm. daily, for 2 to 7 months. The drug was well tolerated, and its antileprosy activity was the same as that observed with other sulfones. However, Cochrane[6] claimed that HES was toxic, as observed in his trial of only 7 cases of leprosy. The most interesting point with reference to HES is that it is not converted into DDS *in vivo*. It is excreted as 4-amino-4'carboxymethylaminodiphenyl sulfone (the sulfone-cilag) in the urine of the cat[7] and in man (Chang, Y. T., unpublished). One would like to see a thorough clinical investigation of HES made before such a valuable drug is abandoned from the leprosy field.

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