There are grave deficiencies in the information available to most of us about how the sulfones are dealt with in the body, and even the mechanism and the significance of the test used to demonstrate them are not as well understood as they might be. Our curiosity actively aroused on a number of points, we undertook to learn more about them. Those to whom inquiries were addressed were most cooperative, and the replies of most of them appear as a symposium in the correspondence section of this issue.

By-passing the fundamental question of whether the sulfones produce their effects by direct action on the bacilli themselves or indirectly, those which were asked of our consultants were of two categories and of the general trend of the following:

1. *What happens to the sulfones in the body?* Granting that when the complex derivatives like promin and diasone are given by mouth there is more or less degradation of them in the gastrointestinal tract to the toxic mother substance (diaminodiphenyl sulfone, DDS), is the therapeutic effectiveness of those drugs dependent on that change or may they also be active in the unmodified form?

What is the case with sulphetrone, also a disubstituted product but one

said not to undergo degradation and certainly tolerated in relatively large doses; with the monosubstituted sulfones ("half sulfones"), which too are said not to undergo degradation; and with promacetin, a sulfone-sulfonamide combination with neither amino group substituted, of which the same thing is said?

What happens to diaminodiphenyl sulfone (DDS) itself when it is used? Is it absorbed, utilized and excreted as such, or does it undergo metabolic changes?

How is it that promin, excessively toxic by mouth, is well tolerated in large doses when given intravenously? In what form does it then exert its therapeutic effect?

2. *What happens in the Bratton-Marshall test?* Just what is the process of "diazotization and coupling" which results in the production of color, and what does it reveal (i.e., does it measure quantitatively the sulfone base, or the whole molecule of the derivative used)?

Since sulfones which are "blocked" by acetylation of the amino linkages do not undergo direct diazotization (i.e., do not react until subjected to acid hydrolysis at 100°C.), how does it happen that substances like promin and diasone, whose amino groups are likewise linked with substituents, do respond to the direct test?

Considering the apparent disparities of blood levels of, say, 1 mgm. per cent for a patient receiving DDS, and 3 mgm. per cent for one receiving promin, whereas the latter figure is practically identical with the former one as regards the DDS constituent, would it not be better to reduce all such data to the DDS equivalent?

Regarding certain questions of the first category, William Feldman,[1] who with Corwin Hinshaw and their associates has done so much in experimental tuberculosis, says that they have always been plagued by them but that the chemists have been unable to answer them; and Sister Hilary Ross[2] and John Lowe[3] both comment on the lack of essential information of the pharmacology of these drugs. Titus and Bernstein,[4] realizing that the general conviction that both the toxicity and the therapeutic activity of the sulfones result from their degradation in the body was an assumption, undertook to elucidate the matter experimentally. The contributions to our symposium of these and other investigators have not a few points of real interest.

The replies to the questions of the second category deal with them thoroughly and clearly from the theoretical and practical points of view, showing what is supposed to happen in the test and wherein the process is not as positive and precise as may be thought. We are given an improvement of the Bratton-Marshall

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[1] FELDMAN, W. H. Personal communication.

[2] ROSS, SISTER HILARY. Contribution to the symposium (p. 251).

[3] LOWE, J. Contribution to the symposium (p. 249).

[4] TITUS, E. & BERNSTEIN, J. The pharmacology of the sulfones. Ann. New York Acad. Sci. **52** (1949) 719-728.

procedure,[6] and also a method for separating DDS from the water-soluble derivatives,[5] a matter recently dealt with elsewhere.[7]

SULFONES IN THE BODY

The prevalence of the view that the sulfone derivatives must be broken down in the body to be effective evidently stems from the earlier trials of the sulfa drug, when diaminodiphenyl sulfone was found to be the most active of all of them in experimental infections. Having also been found too toxic—in the doses used, based on those of the sulfonamides—for use in man,[8, 9, 17b] attention was concentrated on the production of derivatives which would be less toxic and more soluble than DDS, and more active than the diacetyl compound (rodilone) tested at the outset by Fourneau and his group.[10, 11] That was accomplished through elaborate and expensive processing to make derivative products bearing not-too-stable substituents in the amino groups.

The basis of sulfone activity; disubstituted products.—A widely-accepted doctrine of sulfone activity [2, 12] demands, besides the *para* relationship of the essential constituent groups, that one or both amino groups be free or potentially free. Theoretically, because in promin, diasone and sulphetrone both of the amino groups are occupied by substituents, they should be "blocked" and hence inactive, and in the test they should have no

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[5] TITUS, E. Contribution to the symposium (p. 256).

[6] BRATTON, A. C. Contribution to the symposium (p. 257).

[7] SMITH, M. I., JACKSON, E. L., CHANG, Y. T. & LONGENECKER, W. H. Metabolic fate of 4,4′-diaminodiphenylsulfone (DDS) in the rabbit and its isolation from urine. Proc. Soc. Exper. Biol. & Med. **71** (1949) 23-25.

[8] LONG, P. H. Contribution to the symposium (p. 247).

[9] BROWNLEE, G. Contribution to the symposium (p. 247).

[10] (*a*) FOURNEAU, E. J., TREFOUEL, J., TREFOUEL, MME. J., NITTI, F. & BOUET, D. Chimiotherapie de l'infection pneumococcique par la di-(p-acétylaminophenyl)-sulfone (1399F). Compt. rend. Acad. Sci. **204** (1937) 299. (*b*) Action antistreptococcique des derives sulfures organiques. Ibid. 1763. (Cited.)

[11] These workers, according to Titus & Bernstein who used this substance recently, found it to be one-fortieth as toxic as the parent sulfone but much less active. How, being conjugated and presumably thoroughly "blocked," it can have any activity at all is a question which arose too late to be put to our consultants.

[12] SWEET, L. A. & PAYNE, E. H. Contribution to the symposium (p. 254).

"diazotization value" without preliminary acid hydrolysis.[6, 13, 14] That, however, is not the case. Because of their instability in acid different proportions of the linkages (varying from 50 to 65 per cent [6]) are dissociated and diazotized in the direct test, with the consequent production of color of corresponding intensities. Since compounds are much more stable at pH 7.0 or above, there would seem to be good reason for the opinion [4, 15] that the degradation of sulfones given by mouth occur chiefly in the stomach.

Sulphetrone.—Although sulphetrone on direct test also gives readings of more than 50 per cent of the theoretically possible, it is nevertheless held to resist degradation in the body and to act as the whole molecule.[16] Its lack of toxicity is only relative, however, and the Nigeria workers [17, 18] are convinced that some of what is absorbed is degraded.

At this point note must be made of an apparent conflict. M. I. Smith and his associates [13, 14] believe that the usual sulfones are *wholly* metabolized in the body to DDS, because urines give the same values on direct diazotization and after acid hydrolysis. Michael Smith [18b] at first assumed that they were completely hydrolyzed, but when he extracted the DDS from the urine and blood he found it to constitute only a small part of the total drug present; and so Lowe [3, 17b] marvels at the very low blood levels of DDS that will produce clinical effects. How these conflicting findings are to be reconciled is not apparent.

Promacetin.—An entirely different situation would seem to exist with promacetin, which is a peculiar *mestizo* (sulfonamide-sulfone) product said [12] not to be made as a derivative of DDS although chemically it is a sulfone. It is said definitely that it does not undergo degradation in the body, which is only logical since its two amino groups are free; but it seems odd that only

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[13] SMITH, M. I., JACKSON, E. L., JUNGE, J. M. & BHATTACHARYA, B. K. The pharmacologic and chemotherapeutic action of some new sulfones and streptomycin in experimental tuberculosis. American Rev. Tuberc. **60** (1949) 62-77; *reprinted,* I. J. L. **17** (1949) 425-441.

[14] SMITH, M. I. Contribution to the symposium (p. 252).

[15] JOHANSEN, F. A. Personal communication.

[16] BROWNLEE, G. Sulphetrone: therapeutics and toxicology. Lancet **2** (1948) 131-134; *reprinted,* I. J. L. **17** (1949) 73-85.

[17] (*a*) LOWE, J. & SMITH, M. The chemotherapy of leprosy in Nigeria. I. J. L. **17** (1949) 181-195. (*b*) LOWE, J. Treatment of leprosy with diamino-diphenyl sulphone by mouth. Lancet **1** (1950) 145.

[18] SMITH, M. (*a*) A pharmacological study of three sulphones. Part I. Absorption, distribution & excretion. Lep. Rev. **20** (1949) 78-88. (*b*) Idem. Part II. Hydrolysis and the specific toxic phenomena. Ibid. pp. 128-134.

about 87 per cent of them react on direct test. In the one trial in leprosy as yet reported [19] it has proved therapeutically active.

Monosubstituted compounds.—Other derivatives of DDS comprise those with only one amino group substituted, the "half sulphones" and about which Brownlee [20] said rather plaintively that there was a tendency "to look back over our shoulder." M. I. Smith's group [13, 14] speak especially of the alkyl and hydroxyalkyl derivatives of this class, which appear not to undergo degradation. Presumably, having one amino group free, they do not have to be modified to exhibit activity. One of them,the hydroxyethyl product (HES), one of low solubility, is now undergoing preliminary trials in leprosy.

The mother substance, DDS.—No question in this field seems yet to have arisen from the clinical side regarding diaminodiphenyl sulfone itself, which for some years now has been used in veterinary medicine in England and is put out by the Imperial Chemical Pharmaceuticals as "evlosulphone," and which has recently been applied in leprosy by Cochrane, Lowe and others.[21, 17, 22, 23] There may be such a question, however. M. I. Smith holds that DDS does not undergo acetylation in the body,[7] and that in the rabbit it is definitely excreted unchanged.[24] On the other hand Titus and Bernstein found that dogs excrete it partly as an unidentified phenolic conjugated, and Titus now writes that it is "excreted largely as a water-soluble degradation product or products." Bratton thinks that in patients it may be found unchanged and as monoacetyl or diacetyl derivatives, and perhaps also as other degradation products. (He says that it is possible that promin may exist in seven or eight different forms.)

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[19] JOHANSEN, F. A. *et al.* Promacetin in treatment of leprosy; progress report. Publ. Hlth. Rep. **65** (1950) 195-207; *reprinted,* I. J. L., elsewhere in this issue (p. 221).

[20] BROWNLEE, G. Remarks on sulfone treatment. I. J. L. **17** (1949) 111-112 (correspondence).

[21] COCHRANE, R. G. (*a*) A comparison of sulphone and hydnocarpus therapy of leprosy. I. J. L. **16** (1948) 139-144 (Havana Congress paper). (*b*) New developments in the therapy of leprosy. Proc. Fourth Internat. Cong. Trop. Med. & Malaria, Washington, D. C., 1948, vol. 1, pp. 364-373; *reprinted,* I. J. L. **17** (1949) 283-293. (*c*) Sulphone treatment of leprosy. I. J. L. **17** (1949) 299-304 (editorial). (*d*) The use of diaminodiphenyl sulfone. I. J. L. **18** (1950) (correspondence).

[22] MOLESWORTH, B. D., NARAYANASWAMI, P. S. & SIMPSON, I. A. The treatment of lepromatous leprosy with 4:4′-diaminodiphenyl sulfone in oil. I. J. L. **17** (1949) 197-210.

[23] FLOCH, H. & DESTOMBES, P. Traitement de la lèpre par la "sulfone-mère" (diaminodiphenyl-sulfone). I. J. L. **17** (1949) 367-377.

[24] SMITH, M. I. Personal communication.

Sulfones administered parenterally.—As for the problem of what happens to promin when it is injected parenterally, it was shown long ago [25, 26] that several times as much can be given that way as by mouth. Hinshaw and Feldman [26] found that the intravenous route was the least disturbing, and that route was adopted by Faget *et al.*[27] in treating leprosy. Most of what is injected is rapidly—and wastefully—poured off in the urine, from which Johnson [25] could recover almost all unchanged, whereas only 30 per cent of what is given by mouth appeared in the urine and one-fifth of that was in the conjugated form.

Johansen [15] believes that the injected drug does not break down and is active either as such or in the form of some degradation product other than diaminodiphenyl sulfone. Sweet [12] thinks that it is probably "only partially broken down," with an alternative possibility that only one of the substituted groups is split off to form an intermediate (i.e., monosubstituted) product. Lowe [3] holds it evident that "only a small proportion. . . is broken down," but enough to produce the very low DDS concentrations needed for therapeutic activity. The same considerations would presumably apply to the parenteral use of sulphetrone [21d, 28] although Cochrane is inclined to accept the view that it is not degraded at all. However, no DDS determinations of bloods or urines of actual patients treated with such drugs parenterally have been seen. M. I. Smith [13] says that sulphetrone given to the rabbit intravenously is largely excreted unchanged, while Titus [5] finds that with dogs 2 or 3 per cent of the dose of promin injected intravenously appears in the urine as DDS.

Stability in the body.—The apparent fact that relatively little if any of such drugs administered parenterally is degraded may perhaps be related to the fact that they are relatively stable at the pH of the blood, although that condition may not apply

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[25] JOHNSON, R. M. Absence of toxic manifestations following the parenteral administration of promin. J. American Med. Assoc. **114** (1940) 520 (abstract only).

[26] HINSHAW, H. C. & FELDMAN, W. H. Treatment of experimental tuberculosis; use of sodium p,p′-diaminodiphenylsulfone-N,N′-didextrose sulfonate ("promin") with notes on some toxic effects observed in man. J. American Med. Assoc. **117** (1941) 1066-1068.

[27] FAGET, G. H., POGGE, R. C., JOHANSEN, F. A., DINAN, J. F., PREJEAN, B. M. & ECCLES, C. G. The promin treatment of leprosy; a progress report. Publ. Hlth. Rep. **58** (1943) 1729-1741.

[28] DHARMENDRA, DE. N. C., BOSE, R. & KAPUR, P. L. Sulphetrone given intramuscularly in the treatment of leprosy. I. J. L. **18** (1950) in press.

to diasone since Muir [29] was apparently unable to give larger doses of it intravenously than by mouth. If promin is broken down at all after intravenous injection the question arises why increase of the usual 5 cc. maximum dose to 15 cc. does not result in corresponding increase of toxicity—or, for that matter, clinical benefit.[30] It may be that the drug exercises its effects within the tissue cells concerned, and that only a limited amount of it can be taken up by those cells. Beyond that point we have Brownlee's suggestion,[17b] based on the antibacterial activity of sulphetrone *in vitro*, that there may be degradation to the active form inside the bodies of the bacteria themselves, although for the situation *in vivo* one would not venture beyond implicating the tissue cells concerned.

THE BRATTON-MARSHALL PROCEDURE

With regard to what occurs in the Bratton-Marshall test, the contributions to the symposium contain much that is elementary, and also much that to many will not be familiar. Of interest is the fact that plasma or serum is now called for instead of whole blood, because 10 per cent or more of the "blood level" is carried out with the erythrocyte proteins.[2] But, contrariwise, it is also said [24] that some of the drug is bound to the erythrocytes themselves and so is lost with them. Another new point is that a special solvent should be added because the dyes which the chromogen forms with the sulfones are less soluble than these formed with the sulfonamides.[6, 14]

Uncertainty as to just what forms of the drug are present in any biological material from cases treated with a derivative becomes evident. As has been seen there may be the unmodified drug, the mother substance, and the acetylated form, and perhaps other forms as well, and consequently there must be a measure of empiricism in the concentration figures derived from colorimeter readings. The direct test gives an expression of the amount of "free" drug present, and hydrolyzing by boiling with acid releases any conjugated drug and permits determination of the "total" amount present. The difference represents what is called, somewhat vaguely, "conjugated material."

To go into this matter a little: The two amino groups of the DDS molecule being free for direct diazotization and coupling with the chromogen reagent, a solution of DDS containing x number of molecules will

[29] MUIR, E. Preliminary report on diasone in the treatment of leprosy. I. J. L. **12** (1944) 1-6.

[30] JOHANSEN, F. A. & ERICKSON, P. T. Studies on the therapy of leprosy. Proc. Fourth Internat. Cong. Trop. Med. & Malaria, Washington, D. C., 1948, vol. 1, pp. 364-373; *reprinted*, I. J. L. **17** (1949) 273-282.

consequently give $2x$ units, so to speak, of color. The same number of molecules of promin will give $1.3x$ units of color, according to Bratton's figures—65% of the possible linkages, and 65% of the color value of the DDS actually present.

If in, say, the blood of a promin-treated patient all of the molecules of the drug present were unmodified, then obviously the concentrations would be determined quite accurately by comparison with the standards prepared with that drug. But if the specimen should contain some of the original promin, and because of degradation also some DDS, the color would be more intense and the reading fallaciously high. For example, a 50:50 mixture would give rise to a color value of $\frac{2x + 1.3x}{2} = 1.65x$, and (from the difference between 1.3 and 1.65) a reading 27% too high. Michael Smith had the same thing in mind when he pointed out that an (apparent) blood level of 5 mgm. per cent for a patient on sulphetrone might actually represent only 2 mgm. per cent of that substance and the rest DDS. And Floch and Destombes remarked that with a derivative it cannot be told from the test how much is the "active DDS" and how much the whole substance.

To indulge in a little more of this academic meandering, this time in connection with the apparent difference between the "free" drug revealed by the direct test and the "total" drug found after acid hydrolysis: The chemists seem concerned only with the fraction blocked by acetylation, but it may be asked if all of the difference is necessarily ascribable to that factor. With promin, if the unmodified molecules present give a 1.3 color factor on direct test, and if on acid hydrolysis they are reduced to DDS molecules with their 2.0 color factor, it would follow that materially higher readings will then occur even in the absence of conjugated material.

The thought recurs that it might be most instructive, and least confusing in comparing the findings in patients treated with different drugs, if the values of all determinations were to be recorded in terms of the DDS base, regardless of the drug actually used and of the question of therapeutic availability of all of the base. Sister Hilary suggests that if the blood levels of the different drugs as they are used in practice were expressed in terms based on the molecular weight of DDS they would be very close, and the data of Michael Smith are in line with that idea.

But after all, Sister Hilary points out, the clinician is interested only in how much of the particular drug given is available for therapeutic effect, and in practice the total determination is never made because the conjugated drug is held to be inactive. Bratton, on the other hand, while of course appreciating the practical value of the colorimetric determinations as practiced, believes that clinicians should bear in mind the limitations of interpretation of the readings, and that the terminology used should be such as will avoid implanting the impression that we know precisely what substances are present in the materials tested.

H. W. WADE.