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Therapeutic Effect of 4,4′-Diamino-Diphenyl-Sulfone, Corresponding Sulfide and Acetyl Derivatives in Streptococcic Infection.

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Although the therapeutic effect of sulfanilamide (para-amino-benzene-sulfonamide) in experimental beta-hemolytic streptococcus infection of mice has been definitely established, Buttle, Stephenson and others[1] have discovered that 4,4′-diamino-diphenyl sulfone,* (previously described in the chemical literature by Fromm and Wittman[2]) has still greater therapeutic effect upon the same infection. Buttle, *et al.*, stated that the sulfone compound is tolerated by mice in 5 mg doses given by mouth. Two mg can be given daily to normal mice. Although the product is more toxic for mice than sulfanilamide, the authors mentioned that it is not more toxic for normal rabbits and monkeys. They found that single doses of 2 g per kilo of body weight are tolerated by rabbits, and maintain that doses of 0.4 mg of diamino-diphenyl-sulfone are as effective as 40 mg of sulfanilamide.

Fourneau, Trefouël, etc.[3] found that sulfanilamide is tolerated by mouth in a dose of 2.5 g per kilo of body weight (or 50 mg per 20 g of mouse). The maximum tolerated doses per kilo of body weight for other compounds tested are as follows: diamino-diphenyl-sulfide, 0.5 g; diamino-diphenyl-sulfone, 0.1 g; diacetyl-amino-diphenyl-sulfide, more than 10 g; and diacetyl-amino-diphenyl-sulfone, 10 g. The authors found the minimum therapeutic dose in streptococcic infection of mice to be 0.001 g (per 20 g of mouse) for diamino-diphenyl-sulfide; 0.00005 g diamino-diphenyl-sulfone; 0.001 g, diacetyl-amino-diphenyl-sulfide; 0.00005 g, diacetyl-amino-diphenyl-sulfone; and 0.001 g for sulfanilamide. They state, however, that it is difficult to determine the minimum therapeutic dose,

* All of these compounds are 4,4′- but throughout the paper we will refer to them as diamino-diphenyl-sulfone, diamino-diphenyl-sulfide, etc.

1 Buttle, G. A. H., Stephenson, D., Smith, S., Dewing, T., and Foster, G. E., *Lancet*, June 5, 1937, p. 1331.

2 Fromm, E., and Wittmann, J., *Ber. dtsch. chem. Ges.*, 1908, **41**, 2269.

3 Fourneau, E., Trefouël, J., and Mme. J., Nitti, F., and Bovet, D., *Bull. l'Acad. Med.*, 1937, **118**, 210.

and, in general, they consider as the "active dose" the one which gives the same results as obtained under the same conditions with 0.0025 g of sulfanilamide given 2 days in succession by mouth.

Bauer and Rosenthal[4] found diamino-diphenyl-sulfone to be 30 times more active than sulfanilamide. They also found the maximum tolerated dose of diamino-diphenyl-sulfone to be 0.25 g per kilo of body weight given orally to mice.

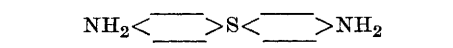
Cooper, Gross, and Lewis[5] found that diacetyl-amino-diphenyl-sulfone is more efficacious than sulfanilamide in the treatment of mice infected with beta-hemolytic streptococcus strain C 203.

Feinstone, Ott, Bliss and Long[6] found diamino-diphenyl-sulfone to be more active in experimental streptococcal infections than sulfanilamide and highly toxic for mice.

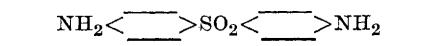
In view of the reported high therapeutic efficiency of diamino-diphenyl-sulfone, we found it of interest to determine whether this product is superior in its therapeutic effect to sulfanilamide. We were particularly interested in comparing the two products and ascertaining how much more effective the sulfone is as compared with sulfanilamide.

At the same time the diamino-diphenyl-sulfide and the acetyl derivatives were drawn into our study. The toxicity of these compounds also was studied.

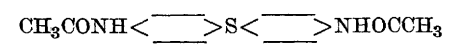
Chemistry. The structural formulæ of these products are presented as follows:



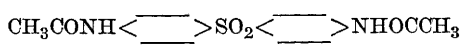
4,4'-diamino-diphenyl-sulfide



4,4'-diamino-diphenyl-sulfone



4,4'-diacetylamino-diphenyl-sulfide



4,4'-diacetylamino-diphenyl-sulfone

The above products are crystalline solid compounds which are only sparingly soluble in water at room temperature and slightly

[4] Bauer, H., and Rosenthal, S. M., *Public Health Rep.*, 1938, **53**, 40.

[5] Cooper, F. B., Gross, P., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 375.

[6] Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 565.

more soluble in hot water. They are more soluble in alcohol or acetone.

The diamino-diphenyl sulfone was made from pure diamino-diphenyl-sulfide by oxidation and all products were recrystallized several times in order to obtain them in a condition of the highest purity.

The toxicity was studied on rabbits by oral administration. For this purpose a small rubber tube was introduced as far as possible into the oesophagus of the rabbit, and by means of a syringe the drug, suspended in aqueous solution was forced through the tube into the stomach of the animal. The toxicity of sulfanilamide was reported by us previously.[7, 8]

TABLE I.
Toxicity Tests in Rabbits *Per Os.*

Drug	Dose per kilo of body wt.	
	Tolerated g	Lethal g
No. 2341	0.3	
4,4'-diamino-diphenyl-sulfide		0.5
		0.5
		0.75
No. 2344	0.3	
4,4'-diamino-diphenyl-sulfone	0.3	
	0.5	
	0.5	
		0.75
No. 2345	2.0	
4,4'-diacetyl-amino-diphenyl-sulfide	3.0	
	5.0	
		6.0
		8.0
No. 2356	10.0	
4,4'-diacetyl-amino-diphenyl-sulfone	15.0	
	15.0	
	20.0	
Sulfanilamide	1.5	2.0

From the data presented in Table I one can see that diamino-diphenyl-sulfone has been tolerated in a dose of 500 mg per kilo of body weight, while the tolerated dose of sulfanilamide is 1.5 g. Diamino-diphenyl-sulfone is, therefore, about 3 times more toxic for the rabbit. The diamino-diphenyl-sulfide is 5 times more toxic

[7] Raiziss, G. W., Severac, M., and Moetsch, J. C., *J. Chemoth.*, 1937, **14**, 1.

[8] Raiziss, G. W., Severac, M., Moetsch, J. C., and Clemence, L. W., *J. Chemoth.*, 1938, **14**, 91.

than sulfanilamide. On the other hand, the corresponding diacetyl derivative of the sulfone is tolerated in very high doses, up to 20 g per kilo of body weight. This is undoubtedly due to the fact that the absorption of the product by the intestinal route is very slow and incomplete. In fact, when very large doses of this drug were given to animals, we observed a more frequent excretion of feces which contained considerable quantities of the drug. The diacetylamino-diphenyl-sulfone could easily be isolated from the excreta by extracting the drug with acetone. Upon recrystallizing, a pure product was isolated. Our conclusion, however, is that the diacetyl compounds are considerably less toxic than the diamino products.

Therapeutic Effect in Experimental Streptococcic Infection of Mice. In this study mice were infected with a virulent culture of beta-hemolytic streptococci of the strain C 203, 0.5 cc of which in these experiments killed mice in a dilution of 10^{-6} (1,000,000) to 10^{-7} (10,000,000). Mice were infected with about 1000 minimum lethal doses of the culture by intraperitoneal injection. An hour and a half later, treatment was given by mouth. These treatments were continued daily for 4 days more. The mice were kept under observation for 28 days.

In Tables II, III, IV, and V, a summary of various individual experiments is presented. Table II presents results of treatment by ascending doses of diamino-diphenyl-sulfone, 0.0001 g causing only a 40% survival of mice, an indication that the dose was not effective; 0.0005 g showed a good therapeutic result, particularly in

TABLE II.

Percentage of Mice Surviving Effects of Infection Following Administration of 4,4'-diamino-diphenyl-sulfone.

Daily dose, g	No. of mice used	7 days %	14 days %	21 days %	28 days %
0.0001	10	60	50	40	40
0.0005	30	90	80	70	66⅔
0.001	15	80	66⅔	66⅔	66⅔
0.002	5	100	80	80	80

TABLE III.

Percentage of Mice Surviving Effects of Infection Following Administration of 4,4'-diamino-diphenyl-sulfide.

Daily dose, g	No. of mice used	7 days %	14 days %	21 days %	28 days %
0.0005	10	40	30	30	30
0.001	15	53	33⅓	33⅓	33⅓
0.002	5	20	20	20	20
0.003	10	50	50	40	40
0.005	5	20	20	20	20

TABLE IV.
Percentage of Mice Surviving Effects of Infection Following Administration of 4,4'-diacetyl-amino-diphenyl-sulfone.

Daily dose, g	No. of mice used	7 days %	14 days %	21 days %	28 days %
0.0001	10	70	40	30	20
0.0005	10	60	20	20	20
0.001	20	85	75	70	55
0.002	30	77	60	60	60
0.005	5	80	80	80	80

TABLE V.
Percentage of Mice Surviving Effects of Infection Following Administration of 4,4'-diacetyl-amino-diphenyl-sulfide.

Daily dose, g	No. of mice used	7 days %	14 days %	21 days %	28 days %
0.0005	5	20	20	20	20
0.001	5	0	0	0	0
0.002	25	84	68	56	56
0.005	15	73	60	53	53
0.010	10	80	80	50	50

the first 14 days. The administration of 0.001 g did not materially improve the results. 0.002 g, however, gave the best effect. Comparing the survival of mice following the sulfone with that following sulfanilamide, one may designate 0.0005 g to be the minimum therapeutic dose. Approximately the same result is obtained with

TABLE VI.
Percentage of Mice Surviving Effects of Infection Following the Administration of Sulfanilamide, 4,4'-diamino-diphenyl-sulfone and Allied Compounds.

Drug	Daily dose, g	No. of mice used	7 days %	14 days %	21 days %	28 days %
Sulfanilamide	0.002	85	67	52	51	37
	0.005	25	68	48	48	48
	0.010	40	75	68	65	60
No. 2341 4,4'-diamino-diphenyl-sulfide	0.003	10	50	50	40	40
No. 2344 4,4'-diamino-diphenyl-sulfone	0.0005	30	90	80	70	66⅔
	0.001	15	80	66⅔	66⅔	66⅔
	0.002	5	100	80	80	80
No. 2345 4,4'-diacetyl-amino-diphenyl-sulfide	0.002	25	84	68	56	56
	0.005	15	73	60	53	53
No. 2356 4,4'-diacetyl-amino-diphenyl-sulfone	0.002	30	77	60	60	60
	0.005	5	80	80	80	80

0.005 g of sulfanilamide. Contrasting the minimum therapeutic effect of diamino-diphenyl-sulfone with that of sulfanilamide we may conclude that the sulfone is about 10 times as therapeutic as sulfanilamide.

In Table VI we present a summary of results obtained with all 4 compounds used in these studies. The difficulties are obvious in selecting a dose which is considered to be the minimum therapeutic. It requires a careful study of individual experiments and of the averages. With certain reservations we consider the minimum therapeutic doses given daily for 5 consecutive days, according to the type of experiments performed, to be: for sulfanilamide, 0.005 g, for 4,4′-diamino-diphenyl-sulfone, 0.0005 g, for diamino-diphenyl-sulfide, 0.003 g, for di(acetyl-amino)-diphenyl-sulfone, 0.001 g, and for di(acetyl-amino)-diphenyl-sulfide, 0.002 g.

Conclusions. 1. 4,4′-diamino-diphenyl-sulfone is about 3 times as toxic for the rabbit as is sulfanilamide; 4,4′-diamino-diphenyl-sulfide is 5 times as toxic. 2. The corresponding diacetyl derivatives of sulfone and sulfide are less toxic than sulfanilamide. 3. The minimum therapeutic doses given daily for 5 consecutive days are as follows: for sulfanilamide, 0.005 g, for 4,4′-diamino-diphenyl-sulfone, 0.0005 g, for diamino-diphenyl-sulfide, 0.003 g, for di(acetyl-amino)-diphenyl-sulfone, 0.001 g, and for di(acetyl-amino)-diphenyl-sulfide, 0.002 g. 4. In mice infected with beta-*Streptococcus hemolyticus,* 4,4′-diamino-diphenyl-sulfone is therapeutically active in a dose 10 times smaller than that required by sulfanilamide. This indicates a considerably higher therapeutic efficacy of the sulfone over sulfanilamide.

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Occurrence of Positive Vaginal Smears in Spayed Mice.*

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We[1] reported that spayed mice painted with 1:2:5:6 dibenzanthracene had positive vaginal smears sporadically. In preparing to

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1 Perry, I. H., and Ginzton, L., *Am. J. Ca.*, 1937, **29**, 680.