

THE *IN VITRO* ACTIVITY OF 4,4'-DIAMINODIPHENYL
SULFONE AGAINST VARIOUS ACID-FAST
MICROORGANISMS¹

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The antituberculosis property of 4,4'-diaminodiphenyl sulfone (DDS) in experimentally infected guinea-pigs (¹), and its beneficial effect on experimental infection due to *Mycobacterium ulcerans* in mice (²), suggested that this drug may have an inhibitory effect on other mycobacteria. In a preliminary report (⁴) it was recorded that infection was retarded by the administration of DDS in the food of mice experimentally infected with *M. avium*, *M. fortuitum*, *M. microti*, and a photochromogenic mycobacterium. It was also recorded that *in vitro* tests disclosed wide variations in sensitivity to DDS among 21 strains of *M. tuberculosis*, as well as among 34 strains of unidentified *Mycobacterium spp.*

The present report describes the procedure and the results of *in vitro* tests of the sensitivity to DDS of a variety of acid-fast microorganisms.

METHODS

The tests were performed on slants of egg-yolk agar containing DDS in concentrations of 3.1, 12.5, 50.0, and 100.0 megm. per milliliter.² The medium was prepared as follows:

The DDS was removed from its container with a sterile spatula, weighed in a sterile test tube, and dissolved in 50 per cent aqueous ethyl alcohol (volume/volume) which had been prepared in sterile glassware with sterile distilled water. That concentration of ethyl alcohol was needed to dissolve the water-insoluble DDS sufficiently. A stock solution of 20,000 megm. was prepared and maintained at 50°C, because at lower temperatures the solution was not clear due to the partial precipitation of DDS. Suitable dilutions of the stock solution were made with the 50 per cent alcohol maintained at 50°C in such a manner that the required amount of DDS for 100 cc. of medium would be contained in the 0.5 cc. solution. Previously it had been found that the addition of 0.5 cc. of 50 per cent aqueous ethyl alcohol to 100 cc. of egg-yolk agar would not cause inhibition of growth of *M. tuberculosis*, *M. bovis*, or *M. avium*.

The 0.5 cc. of DDS solution was added to 90 cc. of sterile melted nutrient agar³ which had been cooled to approximately 50°C. This was followed by the addition of the yolk from a fresh egg, the total volume of which is approximately 10 cc. The materials

¹This investigation was supported in part by Research Grant E 2357 from the National Institutes of Health, United States Public Health Service.

²The 4,4'-diaminodiphenyl sulfone was kindly supplied by Dr. A. C. Bratton, of Parke, Davis and Co., Detroit, Michigan.

³The medium was Difco nutrient agar or trypticase-soy agar, Digestive Ferments Co., Detroit, Michigan.

were mixed by rotating gently, to avoid foam, and then distributed into sterile screw-capped tubes (20 by 100 mm.) in approximately 4-cc. amounts and slanted. The tubes were ready for use when the agar became firm. A control tube of egg-yolk agar with no DDS, and another with agar to which had been added 0.5 cc. of 50 per cent ethyl alcohol per 100 cc., were used for each test.

The tubes were inoculated with 0.1 cc. of bacterial suspension containing approximately 0.1 mgm. of bacteria. The suspensions were prepared in sterile water by grinding with a glass rod a loopful of growth in the bottom of a test tube. For some of the microorganisms a suitable suspension could be made with the inoculating loop alone. The suspensions were compared to a density standard comparable to tube No. 1 of McFarland's nephelometer. It had been determined that the amount of inoculum could vary in fivefold dilutions without variation in the results of the sensitivity tests.

The tests were read after 14 days of incubation at 37°C except *M. bovis*, which was read after 28 days, and *M. ulcerans*, which was incubated at 31°C for 28 days. The sensitivity of each strain was recorded as the lowest concentration of DDS which inhibited growth.

RESULTS

The egg-yolk agar control, without DDS, supported good growths of all the microorganisms tested. Nor was there any inhibition of growth in any of the tests on the control medium containing 0.25 per cent ethyl alcohol. On the test media there was usually a sharp endpoint of growth; that is, there was seldom a gradual diminution of growth from the lower to the higher concentration of DDS. This was due, perhaps, to the fourfold difference in concentration of DDS from tube to tube. Some of the photochromogens failed to develop the characteristic yellow pigment when they grew in the tubes with the higher concentrations of DDS.

The results of the sensitivity tests are recorded in Table 1. The number of strains of *M. tuberculosis* resistant to isoniazid, streptomycin, or to *p*-aminosalicylic acid is too small for valid comparison, but there was no apparent cross resistance between these drugs and DDS. Wide variation of *in vitro* sensitivity to DDS within species of mycobacteria as well as between groups can be observed. Only the *Nocardia asteroides* seemed consistently to be relatively resistant.

Comparison of the results for the *Mycobacterium spp.* disclosed no real differences in sensitivity between the photochromogens and the skotochromogens. However, as a group, the nonchromogens appear to be relatively resistant to DDS *in vitro*; of the 71 nonchromogens only 15 (21.1%) were inhibited by 12.5 mcgm. or less, whereas 22 (75.8%) of the 29 skotochromogens were inhibited by 12.5 mcgm. or less. Also only three (10.4%) of the 29 skotochromogens were able to grow in the presence of 100 mcgm., whereas 22 (31.0%) of the nonchromogens were resistant to 100 mcgm.

Inspection of the data for *M. avium* shows that these microorganisms are relatively sensitive to DDS when compared to the nonchromogens; 39 of 55 strains (71.0%) were inhibited by 12.5 mcgm. or less, against only 15 of 71 strains (21.1%). This is of particular interest

TABLE 1.—Sensitivity of acid-fast microorganisms to 4,4'-diaminodiphenyl sulfone (DDS) in egg-yolk agar.

Type of microorganism	No. of strains	No. of strains inhibited by respective concentration of DDS in micrograms ^a				Not inhibited by 100
		3.1	12.5	50	100	
<i>Mycobacterium tuberculosis</i>	201	12 ^b	35 ^c	102 ^d	41	11
<i>bovis</i>	19	2	5	6	3	3
<i>avium</i>	55	31	8	7	3	6
<i>microti</i>	1					1
<i>balnei</i>	1					1
<i>ulcerans</i>	3	2	1			
<i>fortuitum</i>	1					1
<i>smegmatis</i>	1		1			
<i>Mycobacterium</i> spp.						
photochromogen ^e	20	9	7	2	1	1
skotochromogen ^e	29	14	8	4		3
nonchromogen ^e	71	4	11	13	21	22
<i>Nocardia asteroides</i>	15				2	13

^aLowest concentration in micrograms per cubic centimeter which inhibited growth.

^bIncludes 3 resistant to isoniazid (INH).

^cIncludes 2 resistant to INH, 1 to *p*-aminosalicylic acid (PAS), 2 to INH and PAS, and 3 to INH, PAS and streptomycin.

^dIncludes 3 resistant to INH, 2 to PAS and 1 to INH, PAS and streptomycin.

^eThese terms refer to the groups proposed by Runyon (5).

because these nonchromogens were indistinguishable from *M. avium* on the basis of cultural characteristics.

DISCUSSION

These findings are not directly comparable to those reported by Gupta (3), who used Lowenstein-Jensen medium which was inspissated at 90° C for 1 hour after addition of the DDS. Also, Gupta's data were recorded as ratios of the minimal inhibitory concentrations of test strains as compared to a standard, without record of the actual minimal inhibitory concentration. Nevertheless, there are certain similarities between the results obtained by him and these reported here. This study confirmed that there is no cross resistance between DDS, isoniazid, *p*-aminosalicylic acid and streptomycin. In both studies, the *Nocardia asteroides* appeared to be relatively resistant to DDS. Concerning *M. tuberculosis*, approximately 6.0 per cent of 68 strains examined by Gupta appeared to be resistant to DDS, which is similar to the data presented in this report; that is, 11 of 201 strains (5.5%) were not inhibited by 100 mcgm. of DDS per cubic centimeter of medium. A comparison of data for the other microorganisms cannot be made, but it appears that Gupta also found wide variation in the *in vitro* activity of DDS against acid-fast microorganisms.

SUMMARY

Tests for *in vitro* inhibitory activity of 4,4'-diaminodiphenyl sulfone (DDS) against acid-fast microorganisms were made on egg-yolk agar medium containing DDS in concentrations of 3.1, 12.5, 50.0, and 100.0 mcg./cc. Each egg-yolk agar slant was seeded with 0.01 mgm. of bacteria in 0.1 cc. of sterile water. All tests were read after 14 days of incubation at 37°C except those for *M. bovis*, which were incubated for 28 days, and those for *M. ulcerans*, which were incubated at 31°C for 28 days. The sensitivity of each strain was recorded as the lowest concentration of DDS which inhibited growth.

As a group, *Nocardia asteroides* appeared to be relatively insensitive to DDS: 13 of 15 strains were not inhibited by 100 mcgm. Among the mycobacteria, however, there was a wide variation in sensitivity. For example, of 201 strains of *M. tuberculosis*, 12 were inhibited by 3.1 mcgm., 35 by 12.5 mcgm., 102 by 50 mcgm., 41 by 100 mcgm., while 11 were not inhibited even by the last concentration. There appeared to be no cross-resistance between DDS and streptomycin, isoniazid, or *p*-aminosalicylic acid.

The inhibitory effect on *M. bovis* and on *M. avium* was similarly variable. More *M. avium* strains were sensitive to the lowest concentration of DDS than was either *M. tuberculosis* or *M. bovis*. Two of three strains of *M. ulcerans* were inhibited by 3.1 and one was inhibited by 12.5 mcgm. of DDS. There was no real difference in sensitivity between the photochromogens and skotochromogens, and variations within both groups were evident. The nonchromogens appeared to be the most insensitive of the *Mycobacterium spp.* although they, too, were variable in sensitivity.

RESUMEN

Pruebas relativas a la actividad inhibidora *in vitro* de la 4,4'-diaminodifenil-sulfona (DDS) contra microbios ácidosresistentes se hicieron en un medio de agar-yema de huevo que contenía la DDS a concentraciones de 3.1, 12.5, 50.0 y 100.0 mcgms./cc. Cada película inclinada de agar-yema de huevo se sembró con 0.01 mgm. de bacterias en 0.1 cc. de agua estéril. Todas las pruebas fueron leídas al cabo de 14 días de incubación, excepto por las del *M. bovis*, que fueron incubadas durante 28 días, y las del *M. ulcerans*, que se incubaron a 31° C. por 28 días.

La sensibilidad de cada cepa registrada como la concentración más baja de DDS que inhibía la proliferación.

Como grupo, la *Nocardia asteroides* pareció ser relativamente insensible a la DDS: 13 de 15 cepas no fueron inhibidas por 100 mcgms. Sin embargo, entre las micobacterias, hubo amplia variación en la sensibilidad. Por ejemplo, de 201 cepas del *M. tuberculosis*, 12 fueron inhibidas por 3.1 mcgms, 35 por 12.5 mcgms, 102 por 50 mcgms, 41 por 100 mcgms, mientras que 11 no fueron inhibidas ni aun por la última concentración. No pareció que hubiere resistencia cruzada en la DDS y la estreptomicina, la isoniazida o el ácido *p*-aminosalicílico.

El efecto inhibidor sobre el *M. bovis* y el *M. avium* varió en forma semejante. Más cepas del *M. avium* fueron sensibles a la mínima concentración de DDS que ya del *M. tuberculosis* o del *M. bovis*. Dos de tres cepas del *M. ulcerans* fueron inhibidas por

3.1 y una por 12.5 megms. de DDS. No hubo diferencia real en sensibilidad entre los fotocromógenos y los escotoeromógenos y las variaciones dentro de ambos grupos eran evidente. Los no cromógenos parecían ser los más insensibles de la *spp. Mycobacterium*, aunque ellos también eran variables en sensibilidad.

RESUMÉ

Des essais portant sur le pouvoir d'inhibition in vitro de la 4-4'-diaminodiphényl sulfone (DDS) sur les micro-organismes acido-résistants ont été menés sur un milieu agar-jaune d'oeuf contenant de la DDS à des concentrations de 3.1, 12.5, 50.0, et 100.0 megm./cc. Chaque disque d'agar—jaune d'oeuf a été ensemencé avec 0.01 mgm. de bactéries dans 0.1 cc. d'eau stérile. Tous les résultats furent relevés après 14 jours d'incubation à 37°C., sauf en ce qui concerne *M. bovis* qui fut incubé pendant 28 jours, et *M. ulcerans*, qui fut incubé pendant 28 jours à 31°C. La sensibilité de chaque souche fut consignée comme étant la plus faible concentration inhibant la croissance.

Comme groupe, *Nocardia asteroides* se révéla relativement insensible à la DDS : 13 des 15 souches ne furent pas inhibées par 100 megm. Pour les mycobactéries, cependant, on enregistra une grande variation de la sensibilité. Parmi 201 souches de *M. tuberculosis*, par exemple, 12 furent inhibées par 3.1 megm., 35 par 12.5 megm., 102 par 50 megm., et 41 par 100 megm., tandis que 11 n'étaient pas inhibées même à cette dernière concentration. Il n'apparaît pas y avoir de résistance croisée entre la DDS et la streptomycine, l'isoniazide ou l'acide p-aminosalicylique.

De même, le pouvoir inhibiteur sur *M. bovis* et *M. avium* a été variable. Plus de souches de *M. avium* ont été sensibles aux concentrations les plus faibles de DDS que cela n'a été noté avec *M. tuberculosis* ou *M. bovis*. Deux des trois souches de *M. ulcerans* ont été inhibées par 3.1 megm. et une par 12.5 megm. de DDS. Il n'y a pas eu de réelles différences de sensibilité entre les photochromogènes et les skotochromogènes, mais les variations dans chacun de ces groupes ont été notables. Les non-chromogènes se sont révélés les plus tolérants de l'espèce *Mycobacterium*, bien que eux aussi aient présenté des variations de sensibilité.

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