

Comparative Effects on Experimental Tuberculosis of 2,4'-Dichlorobenzophenone and 4,4'-Diaminodiphenylsulfone.

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The bacteriostatic action *in vitro* of benzophenone and of many of its derivatives on cultures of tubercle bacilli was tested by Freedlander.¹ The most pronounced inhibitory action was exerted by 2,4'-dichlorobenzophenone. In addition to being markedly bacteriostatic for a culture of a rapidly growing avirulent strain of human tubercle bacilli, this compound was also highly effective in inhibiting the growth of cultures of a recently isolated slow growing virulent strain of the organism. The highest dilution in which bacteriostatic action was effective for the avirulent strain was 1:100,000 while for the virulent strain inhibition was recorded in a dilution of 1:60,000, the highest dilution tested. Sodium p,p'-diaminodiphenylsulfone-N,N'-didextrose sulfonate (promin) was also tested and the results were quite inferior to those obtained from most of the chlorine derivatives of benzophenone.

The high bacteriostatic capacity of 2,4'-dichlorobenzophenone against tubercle bacilli *in vitro* suggested the desirability of an experiment to determine what effect, if any, this compound might have against virulent tubercle bacilli *in vivo*.

Previous observations had indicated that one of the most effective compounds in deterring experimental tuberculosis in guinea pigs is 4,4'-diaminodiphenylsulfone.^{2,3} Consequently an experiment was planned so that (1) the results obtained from 2,4'-dichlorobenzophenone could be compared with the results obtained from a drug of established

tuberculotherapeutic efficacy and (2) the results following the administration of the drugs could be compared with the results in animals that were not treated.

Methods. Four groups, each consisting of 10 adult male guinea pigs, were used. The respective groups were identified as follows: Group 1, infected but not treated (controls); Group 2, infected and treated with 0.33% of 4,4'-diaminodiphenylsulfone in the feed; Group 3, infected and treated with 0.5% of 2,4'-dichlorobenzophenone in the feed, and Group 4, infected and treated with 1.0% of 2,4'-dichlorobenzophenone in the feed.* The administration of the drugs was started 2 days before the animals were inoculated with tubercle bacilli. All animals received subcutaneously in the sternal region 0.1 mg of a 20-day-old culture of the virulent variant of H37 (H37RV).† The guinea pigs were caged in pairs and the experiment was terminated 60 days after the animals had been inoculated with tubercle bacilli. At the time of necropsy tissues from the liver, spleen, lungs, tracheobronchial lymph nodes, the site of inoculation and the contiguous lymph nodes were preserved for subsequent histologic examination. In addition, approximately half of each spleen of the guinea pigs

* Preliminary trials had indicated that guinea pigs weighing from 400 to 500 g would consume an average of 40 g of feed daily, containing 1% by weight of 2,4'-dichlorobenzophenone. When the amount of the drug was increased to 2% and to 4% of the daily food intake inappetence developed and most of the animals receiving feed containing 4% of the drug died within 3 weeks. Both compounds used in this study were supplied through the kindness of Dr. L. A. Sweet, Parke, Davis & Co., Detroit, Michigan.

† The origin and characteristics of H37RV have been described previously.²

¹ Freedlander, B. C., PROC. SOC. EXP. BIOL. AND MED., 1942, **52**, 153.

² Feldman, W. H., Hinshaw, H. C., and Moses, H. E., *Am. Rev. Tuberc.*, 1942, **45**, 303.

³ Feldman, W. H., Hinshaw, H. C., and Moses, H. E., unpublished data.

TABLE I.

Summary of Comparative Effects of 4,4'-diaminodiphenylsulfone and of 2,4'-dichlorobenzophenone on Experimental Tuberculosis of Guinea Pigs. All Animals Received Subcutaneously 0.1 mg of Human Tubercle Bacilli (Strain H37RV). Drugs Were Supplied Daily in the Feed. Duration of Experiment, 62 Days.

Group	Numerical index of infection determined microscopically				
	Spleen (Max. 35)	Lungs* (Max. 30)	Liver (Max. 25)	Site of inoculation* (Max. 10)	Avg index (Max. 100)
1. Controls	26.1	19	16.3	10	71.4
2. Treated†	2.9	3.8	1.2	6.4	14.3
3. Treated‡	25	18.5	21.5	10	75.0
4. Treated§	27.8	16	19	10	72.8

* Includes contiguous lymph nodes.

† 4,4'-diaminodiphenylsulfone, 0.33% of the feed.

‡ 2,4'-dichlorobenzophenone, 0.5% " " "

§ 2,4'-dichlorobenzophenone, 1.0% " " "

in Groups 1, 2 and 4 was removed aseptically and utilized for attempts to culture tubercle bacilli.

Results. Animals surviving. Of the 40 animals inoculated with tubercle bacilli when the experiment began, 34 were living 60 days later. All of the control or untreated group survived; of those that received 4,4'-diaminodiphenylsulfone seven were living; of those that received in the feed 0.5% of 2,4'-dichlorobenzophenone, 9 were living; while of the 10 that received in the feed 1% of the benzophenone derivative, 8 were living. The cause of death of the 3 animals in the group that received 4,4'-diaminodiphenylsulfone was not evident at necropsy. Each had residual tuberculous changes at the site where tubercle bacilli had been injected and in one animal there was a slight progressive tuberculous involvement of the spleen. In none of the 3 was there sufficient tuberculosis to account for death. The opposite was true of the 3 animals which died while receiving 2,4'-dichlorobenzophenone. In each of these there was present severe tuberculosis that appeared sufficient to have been lethal.

Relative amounts of tuberculosis. For the sake of brevity the comparative amounts of tuberculosis in the organs of predilection and at the site of inoculation of the four groups of guinea pigs have been summarized in

‡ The scheme followed in recording numerically the amount of tuberculosis in experimentally infected guinea pigs takes into account the degree of involvement and the progressive or nonpro-

Table I.‡

The results show that there was no significant difference in the severity of the tuberculosis in the control group and in the 2 groups of guinea pigs that received 2,4'-dichlorobenzophenone. In contradistinction to the results obtained from the chlorine derivative of benzophenone were the effects exerted by 4,4'-diaminodiphenylsulfone. Whereas the average numerical index of infection (on the basis of 100) was 71.4 for the untreated animals the average index of infection of those animals treated with the sulfone compound was only 14.3. Not only was there markedly less tuberculosis in the latter group than was true of the untreated controls and the animals that received 2,4'-dichlorobenzophenone, but of much more importance was the fact that the histologic character of the tuberculous processes in the group that received the sulfone compound was with few exceptions that of a nonprogressive or arrested process.

The results indicated definitely that under the conditions imposed, 2,4'-dichlorobenzophenone did not influence the expected course of the disease favorably. This inability on the part of 2,4'-dichlorobenzophenone to exert a deterrent effect becomes especially im-

pressive character of the tuberculous changes. This scheme and the importance of the microscopic examination of tissues in tuberculochemo-therapeutic studies have been described previously.⁴

⁴ Feldman, W. H., *Am. Rev. Tuberc.*, 1943, **48**, 248.

pressive when compared with the results obtained under the same conditions with 4,4'-diaminodiphenylsulfone.

Effects on the blood. The only observations made on the changes in the blood of the 4 groups of animals were limited to determinations of hemoglobin.[§] When the animals were killed 60 days after infection specimens of blood were obtained by cardiac puncture from 3 of the groups. The results expressed in grams of hemoglobin per 100 cc of blood are as follows: Group 1, untreated controls, 13.3; Group 2, treated with 4,4'-diaminodiphenylsulfone, 10.2; Group 3, treated with 1% of 2,4'-dichlorobenzophenone by weight in the feed, 11.2. If one assumes that the average concentration of hemoglobin for normal guinea pigs is 14 to 15 g per 100 cc of blood it is evident that neither drug reduced the amount of hemoglobin to critical levels.

Blood concentrations of the drugs. When the experiment was terminated the concentration of 4,4'-diaminodiphenylsulfone in the animals in Group 2 ranged from 1.8 to 5.7 mg per 100 cc of blood, the average being 4.1 mg. No determinations were made concerning the concentration in the blood of 2,4'-dichlorobenzophenone. We are not aware of practical methods whereby this can be

[§] The hemoglobin values were determined by the Cenco-Sheard-Sanford photometer.

accomplished.

Results of splenic cultures. Among the 10 spleens cultured for tubercle bacilli from the group of untreated controls acid-fast bacilli were obtained from 9. Seven splenic cultures were made from the group that received 4,4'-diaminodiphenylsulfone; the results were negative for 5 and positive for 2. Eight spleens were cultured from the animals in Group 4 (1% of 2,4'-dichlorobenzophenone by weight of the feed) and acid-fast bacilli were obtained from each.

Summary and conclusions. The effect of 2,4'-dichlorobenzophenone on experimental tuberculosis of guinea pigs was studied. Although 4,4'-diaminodiphenylsulfone was capable of exerting an impressive inhibitory effect, the chlorine derivative of benzophenone did not retard or influence favorably the course or character of the disease as exemplified among the animals in the untreated or control group. The following conclusions are drawn: 1. Favorable bacteriostatic results *in vitro* of chemical compounds against tubercle bacilli are not necessarily indicative of favorable results in experiments *in vivo*. 2. 4,4'-diaminodiphenylsulfone under the conditions imposed proved capable of combating a tuberculous infection rather satisfactorily. 3. The one chlorine derivative of benzophenone studied proved ineffective in altering the expected course of the disease.

14304

Effect of Some Chemicals, Which Affect Smooth Muscle Contraction, on Ciliary Activity in *Paramecium*.

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Mast and Nadler,¹ Merton,² Oliphant³ and others have shown that monovalent cation salts and hydrates induce reversal in the direction of the effective beat of the cilia in protozoa while, with few exceptions, bi-

and tri-valent cation salts and hydrates do not. Similar observations have been made regarding ciliary reversal in the metazoa, and the reproductive cells of plants and animals have been observed to swim backward.

¹ Mast, S. O., and Nadler, J. E., *J. Morph. and Physiol.*, 1926, **43**, 105.

² Merton, H., *Biol. Zentralbl.*, 1935, **55**, 268.

³ Oliphant, J. F., *Physiol. Zool.*, 1942, **15**, 443.