THE ACTIVITY *IN VITRO* OF SOME ANTILEPROTIC AND ANTITUBERCULOUS COMPOUNDS AGAINST A RANGE OF ACID-FAST MICROORGANISMS[1]

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"A chemotherapeutic agent of greater efficacy than the sulfones is urgently needed in leprosy." So stated Doull ([3]). In the search for such a compound it would be useful to have some kind of simple initial screening test to indicate the possible value of a drug before more costly and time-consuming studies are made in animals or man. In an attempt to evolve an *in vitro* test for screening new compounds with potential activity against *Mycobacterium leprae* a series of standard antituberculous and antileprotic drugs were investigated for their inhibitory effect on a range of mycobacterial species. It was hoped that some form of sensitivity pattern might emerge that would indicate the possible use of one or more of the organisms in a primary screening test.

MATERIALS AND METHODS

The strains of mycobacteria used were:

M. smegmatis	(NCTC[2] 333)	*M. marinum*	(NCTC 2275)
M. smegmatis	(NCTC 7017)	*M. rhodochrous*	(NCTC 8139)
M. phlei	(NCTC 8151)	*M. balnei*	10010
M. fortuitum	(NCTC 8573)	*M. balnei*	10012
M. ulcerans	(NCTC 7816)	*M. marianum*	

M. (unclassified) Binford strains Q and N

The chemical compounds and solvents used were:

Streptomycin (SM)	aqueous ethanol
Isoniazid (INH)	aqueous ethanol
Ethionamide (1314 TH)	dimethyl formamide
Dapsone (DDS)	ethanol
Thiacetazone (TBI)	dimethyl formamide
Thiambutazine (Ciba 1906)	dimethyl formamide

The compounds were dissolved in the solvents indicated at a concentration of 10,000 μg/ml. and diluted in distilled water to 1,000 μg/ml. or 100 μg/ml. as necessary. Thereafter two-fold dilutions were made in the medium of Dubos and Davis ([4]), or a modification of this medium containing 0.125% agar. To each tube was added 0.1 ml. of Dubos' medium albumin (Difco) and 0.1 ml. of culture of mycobacteria. The tubes for *M. ulcerans* and *M. balnei* were inoculated from 7 day old cultures and incubated at 32°C for 9 days. The inocula for the Binford strains were from 7 day old cultures, and tubes were incubated for 7 days at 37°C. Other mycobacteria were incubated at 37°C for 48 hours. The lowest concentration in which no growth was visible to the naked eye was designated the minimal inhibitory concentration (M.I.C.).

[1]Received for publication November 23, 1964.

[2]National Culture Type Collection, London.

RESULTS

Table 1 shows the M.I.C. of the compounds used against the various species of mycobacteria. Streptomycin proved by far the most active compound against the majority of the strains tested. Isoniazid and ethionamide showed slight activity against some species.

Of the remaining compounds, dapsone showed no activity against any of the organisms examined, thiambutazine was slightly active against the types labeled *M. marinum* and *M. balnei*, and the latter organism was inhibited to some extent by thiacetazone.

TABLE 1.—*Minimal inhibitory concentration (M.I.C.) of some antituberculous and antileprotic compounds against 12 strains of mycobacteria*

Strain	Medium	M.I.C. (μg/ml.)					
		Ethion-amide	Isoniazid	Thiace-tazone	Dapsone	Thiambu-tazine	Strepto-mycin
M. smegmatis (NCTC 333)	semi-solid	500	32.0	1000	>250	>250	<1.0
M. smegmatis (NCTC 7017)	semi-solid	63.0	1.6	500	500	>250	0.4
	liquid	500	3.2	1000	1000	>250	0.4
M. phlei (NCTC 8151)	semi-solid	500	500	1000	500	>125	32.0
M. fortuitum (NCTC 7816)	semi-solid	250	500	1000	500	>125	125
M. ulcerans (NCTC 7816)	semi-solid	1000	500	125	>125	>250	0.32
	liquid	1000	500	500	250	>250	0.32
M. marinum (NCTC 2275)	semi-solid	6.3	25.0	250	500	16.0	12.5
	liquid	6.3	25.0	250	500	16.0	12.5
M. rhodochrous (NCTC 8139)	semi-solid	1000	>100	1000	>1000	>250	6.3
	liquid	1000	>100	1000	>1000	>125	3.2
M. balnei (10010)	semi-solid	4.0	16.0	32.0	125	8.0	4.0
	liquid	4.0	32.0	32.0	125	8.0	2.0
M. balnei (10012)	semi-solid	2.0	32.0	63.0	250	8.0	2.0
	liquid	4.0	16.0	125.0	250	8.0	4.0
M. marianum	semi-solid	500	500	500	100	>125	6.3
	liquid	500	500	500	100	>125	3.2
M. (Binford Q)	semi-solid	32.0	16.0	125	250	32.0	<1.0
M. (Binford N)	semi-solid	16.0	16.0	125	250	63.0	<1.0

DISCUSSION

Doull ([3]) made a plea for more systematic screening of drugs and antibiotics to be used in leprosy by testing against certain mycobacteria and mycobacterial infections in the laboratory. He suggested

that, among several acid-fast organisms, *M. ulcerans* and *M. balnei* should receive attention.

Mayer ([7]), in his comprehensive review, considered that, in testing for any antibacterial activity, *in vitro* screening was a useful preliminary in chemotherapeutic exploration. He introduced the interesting idea that differences in enzymatic make-up of organisms grown *in vitro* and *in vivo* might be accompanied by differences in response to drugs. He suggested that investigation of this might be pursued by comparing the response to drugs of such cultivable mycobacteria as *M. ulcerans, M. balnei* and *M. johnei,* which had been isolated from tissues and grown *in vitro.*

Leach and Fenner ([6]) had examined strains of *M. ulcerans* and *M. balnei* and the effect upon them of streptomycin, thiosemicarbazone, and isonicotinic acid hydrazide. They found that *M. ulcerans* was inhibited by 1 μg/ml. of streptomycin and that *M. balnei* was unaffected by this concentration but was completely suppressed by 10 μg/ml. We found that *M. ulcerans* was inhibited by 0.32 μg/ml. of streptomycin and *M. balnei* by 2.0 μg/ml. *M. marinum,* however, which is said to be the same organism as *M. balnei,* was found to be suppressed by 12.5 μg/ml. of streptomycin. Like Leach and Fenner, we found that *M. ulcerans* and *M. balnei* were unaffected by thiosemicarbazone and isoniazid.

Wolinsky *et al.* ([8]) studied the drug susceptibility of "atypical" mycobacteria, human and bovine strains of *M. tuberculosis,* a small number of saprophytes, and three miscellaneous organisms, one of which was *M. ulcerans.* They examined antituberculosis drugs mainly, and only one compound that can be regarded as active against leprosy (thiambutazine). Their results were not given as M.I.C. values but roughly as "susceptible," "moderately resistant," and "resistant." With respect to the organisms and drugs common to both investigations, there is a similarity in the findings. *M. ulcerans* was shown "resistant" by Wolinsky *et al.,* and we found an M.I.C. of 500 μg/ml. of thiambutazine against that organism. *M. phlei* and *M. fortuitum* were "resistant" to isoniazid and thiambutazine in their studies, and our strains of these organisms required concentration of 500 μg/ml. and 125 μg/ml. respectively of these drugs to cause inhibition.

Karlson ([5]), using slants of egg-yolk-agar containing dapsone in various concentrations, tested the sensitivity of a number of types of mycobacteria, including *M. ulcerans, M. fortuitum, M. balnei* and *M. smegmatis.* He showed that within species as well as among groups of mycobacteria there was a fairly wide range of sensitivity to dapsone, with a fair proportion showing no inhibition by 100 μg/ml. Of his three strains of *M. ulcerans,* however, two were sensitive to 3.1 μg/ml. and the remaining one to 12.5 μg/ml., a concentration considerably lower than the figure we could demonstrate. In fact, with

the strain we used we could not obtain a figure of sensitivity to dapsone less than 100 μg/ml.

The strains of mycobacteria designated "Binford Q and N" were obtained from Dr. C. H. Binford. They had been derived from isolations from granulomatous lesions produced in golden hamsters inoculated with material from lepromatous patients in his experiments designated Q and N ([1, 2]).

There appears to be agreement that *M. balnei* and *M. marinum* are the same organisms in spite of different reported origins of each. Cultures representative of these respective strains were examined and found to exhibit somewhat similar behavior in their relative susceptibility to thiambutazine. Sensitivities to thiacetazone, however, differed widely.

None of the mycobacterial strains examined presented a pattern of sensitivity against the compounds employed that would indicate its usefulness in a screening procedure to test new compounds that might be active against *M. leprae.*

SUMMARY

The minimal inhibitory concentrations of three antileprotic and three antituberculous drugs were determined against 12 strains of mycobacteria. It was hoped that some pattern of sensitivity might emerge that could provide a simple *in vitro* screening test for compounds with potential activity against *M. leprae.* No satisfactory pattern was found for the mycobacteria and chemical compounds tested.

RESUMEN

Las concentraciones inhibitorias mínimas de tres drogas antilepróticas y antituberculosas fueron determinadas contra 12 razas de micobacterias. Se esperaba que algún modelo de sensibilidad pudiera emerger, que pudiera proveer un simple test *in vitro* para compuestos con actividad potencial contra el *M. leprae.* No fué encontrado un modelo satisfactorio para las micobacterias y los compuestos químicos ensayados.

RÉSUMÉ

Les concentrations inhibitoires minimals de trois drogues antileprotiques et antituberculeuses ont été determinées contre 12 varietés des mycobacteries. On a eu l'espoire de qu'un certain type de sensibilité pouvait emerger de ceci qui pouvait donner d'un test simple *in vitro* pour le componant avec une activité potential contre le *M. leprae.* Encore n'était pas trouvé une modèle satisfactoire pour las micobactéries et les componants chimiques eprouvés.

Acknowledgments.—We wish to thank Dr. R. Chaussinand for the provision of the strain *M. marianum,* Dr. C. H. Binford for the strains Binford Q and N, and Mr. C. H. Collins for the cultures of *M. balnei.*

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