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STUDIES IN THE CHEMOTHERAPY OF TUBERCULOSIS: PART I. SULPHONES

BY

E. HOGGARTH AND A. R. MARTIN

From Imperial Chemical Industries Limited, Hexagon House, Blackley, Manchester

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After the introduction of prontosil and sulphanilamide for the treatment of streptococcal infections, much interest was taken in the use of sulphonamides and 4 : 4′-diaminodiphenylsulphone for the treatment of tuberculosis in guinea-pigs. For example, Rich and Follis (1938) showed that sulphanilamide exerted a striking effect (as judged by such criteria as spleen size, and distribution and extent of lesions) on such an infection, but it was not until four years later that Feldman, Hinshaw, and Moses (1942) using the N : N′-bis-sodium dextrosebisulphite derivative of 4:4′-diaminodiphenylsulphone (promin) were able to demonstrate a definite difference in survival times between groups of treated and control animals. We had begun the work reported here on sulphones as possible antituberculous drugs in 1942, and when the paper by Feldman *et al.* appeared we were encouraged to proceed further along these lines. But it seemed to us unlikely that improved drugs would be found among solubilized forms of 4 : 4′-diaminodiphenylsulphone of the type represented by promin and diasone (which in all probability owe their activity to breakdown *in vivo* to the parent sulphone, with its known risk of serious toxicity), and it was our intention to examine sulphones of as widely differing chemical types as possible. No rapid and simple screening test for selecting compounds active *in vivo* was available when the work started, and we were forced to rely upon an *in vitro* method, although the limitations of this approach were fully realized at the time. To anticipate the main conclusion of the present report, it may be said at once that when a convenient *in vivo* method became available (Martin, 1946) the determination of *in vitro* activity was shown to be a completely unreliable guide for the discovery of compounds with activity *in vivo*. This conclusion is in accordance with current opinion among other workers (see, e.g., Feldman and Hinshaw, 1945).

Experimental Methods

In vitro.—Each compound was finely ground with a small quantity of " Dispersol OG " and diluted with water to give a final concentration of drug of 1:100. Further dilutions (1:300, 1:900, etc.) were prepared from this and 0.5 ml. of each dispersion added to 4.5 ml. amounts of Long's synthetic medium containing 1.5 per cent of agar, to give final concentrations of compound as follows:

Serial number of tube:	1,	2,	3, etc.
Final concentration of compound:	1:1,000,	1:3,000,	1:9,000.

The medium was allowed to solidify with the tubes slightly inclined, and each tube was sown with a small particle of a culture of the H37 strain of " human " tubercle bacilli on Lowenstein's medium. Care was taken that all particles were as nearly as possible of the same size. Tubes were incubated in a moist atmosphere for 14 days at 37° C. and the degree of growth assessed by comparison with that in control tubes. The *in vitro* activities of the compounds in all the following tables are quoted as " *in vitro* indices " which were arrived at as follows: The serial number of the last tube in which no growth took place is recorded as the first figure of the index; the second figure of the index is the serial number of the first tube in which full growth occurred. The index " 4/6 " would therefore indicate that the compound in question completely inhibited growth (under the conditions described) in the fourth tube (i.e., at a concentration of 1:27,000) and had no inhibitory action whatever in the sixth tube (i.e., at a concentration of 1:243,000). The majority of the compounds listed in the following tables were tested at least twice and the indices found to be reproducible. Similar limiting concentrations for complete inhibition of growth and complete absence of inhibition were found for a number of the more active compounds.

using Long's liquid synthetic medium in which the organism was allowed to grow as a pellicle.

In vivo.—Therapeutic tests were carried out on groups, each of 24 mice, infected intravenously with 1 mg. of "human" tubercle bacilli (strain 905). Details of the method have been published (Martin, 1946). The examination of each substance was preceded by a chronic toxicity test extending over a period of three weeks. The mice were selected and randomized as described for the therapeutic test. Each compound was given at a range of doses using 12 animals for each dose level, and each group was weighed at weekly intervals. The dose chosen for the therapeutic test was the highest which permitted normal growth, and on which the animals appeared to be in good condition. It was not always possible to choose this dose with full confidence, and in such cases one or more smaller doses were also given. Doses are quoted in the tables as mg. per 20 g. mouse, and were administered orally twice daily for five days and once on Saturdays. The first dose was given shortly before infection and dosing continued until the first specific death occurred in the control group—i.e., for about 14 days in most cases. In the tables of therapeutic results the column headed "Increased mean survival time" gives the difference between the mean survival times of treated mice and untreated control animals. The column headed "Increase required for significance" gives the time increase which would be necessary for statistical significance at the level $P=0.05$. If the figure in the first of these columns is positive and exceeds the value in the second column, the compound shows *in vivo* activity. Negative values equal to or greater than the "required increase" indicate that the toxicity of the drug has had a significantly adverse effect on treated mice. Values of magnitude approaching that required for significance (positive or negative) may be expected to arise through the operation of small uncontrolled variables once in twenty times, although the compound has no influence on the infection. That the test will detect activity *in vivo* is shown by the results for 4:4′-diaminodiphenylsulphone (No. 371) and 2:4′-diamino-5-thiazylphenylsulphone (No. 4879) (see Table XXIII).

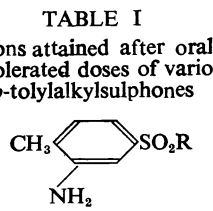
Blood level concentrations.—Only those compounds carrying aromatic amino groups were estimated. Details of the method used in these laboratories, for the determination of blood concentrations after the oral administration of such compounds, have been described previously (Martin, Rose, and Bevan, 1943). The method is based upon diazotization and coupling to form an azo colour.

Results

It had been observed that the activity *in vitro* of diphenylsulphone itself (index 1/5) was not greatly different from that of 4:4′-diaminodiphenylsulphone (index 2/5). It was therefore permissible for the purpose in hand to disregard the substituent amino groups of diaminodiphenylsulphone and attempt to find some combination of hydrocarbon residues which, united by the sulphone linkage, would possess higher intrinsic activity. It was, of course, realized that such a compound would be unlikely as such to be effective *in vivo* and the next step envisaged was the introduction of groups such as amino, methoxy, etc., which it was thought might confer appropriate pharmacological properties on the new parent sulphone structure. In Table II below are listed sulphones representing the various possible combinations of the groups phenyl, *p*-tolyl, 4-diphenylyl, α- and β-naphthyl, *cyclo*-hexyl, and *cyclo*-pentyl. Highest activity seems here to be associated with hydrogenated cyclical nuclei, and further sulphones containing *cyclo*-alkyl and alkyl residues were tested. These are listed in Tables III, IV, V, and VI. The high indices shown by phenyl-, *p*-tolyl-, and *p*-*n*-butylphenyl-alkylsulphones (containing alkyl residues with 5 to 8 carbon atoms) seemed sufficiently marked to warrant the undertaking of the second part of the investigation, namely the introduction of further substituents into selected parent sulphones. From the Tables VII, VIII, X, XI, and XII it will be seen that the introduction of a single amino, alkoxy, or hydroxy group into the aromatic ring of such sulphones does not alter the *in vitro* index greatly. The β-diethylaminoethylamino group, however, reduces *in vitro* activity (Table IX), as does the presence of two methoxy groups (Table XIII). The presence of amino groups in some of these compounds made it possible to estimate them readily and the concentrations which were attained in the blood of mice after oral administration of

TABLE I

Blood concentrations attained after oral administration of maximum tolerated doses of various *m*-amino-*p*-tolylalkylsulphones

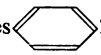


Number	R	Dose mg./20 g. mouse	Blood concentrations (mg./100 ml.) at time after dosing		
			1 hr.	2 hr.	4 hr.
3631	methyl ..	5	8.0	8.2	4.0
3630	*n*-propyl	5	5.5	3.2	1.6
3622	*n*-amyl ..	5	2.1	1.6	1.0
2972	*n*-heptyl..	7.5	0	—	—

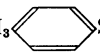
maximum tolerated doses were measured. Those compounds which have small alkyl groups were well absorbed, though somewhat rapidly excreted. With increasing size of alkyl group, the maximum concentration reached in the blood decreased rapidly. This is illustrated for the *m*-amino-*p*-tolylalkylsulphones (Table I).

While the work described above was in progress, attempts were also being made to obtain compounds more effective than 4:4′-diaminodiphenylsulphone by replacing the amino groups by other substituents. Attention was concentrated on these compounds (Tables XIV and XV) rather than on phenylalkylsulphones when it was realized that members of the aminophenylalkylsulphone series having an alkyl group large enough to confer high activity *in vitro* were so poorly absorbed that high activity *in vivo* was most unlikely. A further development of this aspect was the preparation of heteroarylphenylsulphones (Table XVI), interest in which was first aroused by reports by Feldman, Hinshaw, and Mann (1944) on the activity of promizole (2:4′-diamono-5-thiazylphenylsulphone, corresponding to our compound No. 4879) against a tuberculous infection in guinea-pigs. As a final variation the effect of replacing the sulphone linkage itself by other related linkages was examined. The linkages chosen were the sulphonic ester (-SO_2O-) and sulphonamide (-SO_2NH-) groupings and compounds corresponding to both phenylalkyl- and diphenyl- sulphones were examined (Tables XVII to XXI). Several of the phenylalkane and phenylbenzene sulphonates have very high *in vitro* indices, while those of the N-alkyl- and N-phenyl- benzenesulphonamides are about the same as those of the corresponding sulphones.

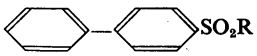
TABLE II

(*a*) Phenylsulphones 

Number	R	*In vitro* index
1645	phenyl	1/5
2651	*p*-tolyl	<1/6
2653	4-diphenylyl	1/3
2692	α-naphthyl	<1/6
2693	β-naphthyl	<1/4
2763	*cyclo*pentyl	2/4
2671	*cyclo*hexyl	3/6

(*b*) *p*-Tolylsulphones 

2599	*p*-tolyl	<1/4
2601	4-diphenylyl	<1/1
2624	α-naphthyl	<1/6
2625	β-naphthyl	<1/1
2603	*cyclo*pentyl	2/5
2602	*cyclo*hexyl	3/6
3113	4-methyl*cyclo*hexyl	3/6

(*c*) 4-Diphenylylsulphones 

2600	4-diphenylyl	<1/4
2626	α-naphthyl	<1/5
2620	β-naphthyl	<1/1
2627	*cyclo*pentyl	1/6
2617	*cyclo*hexyl	<1/5

(*d*) α- and β-Naphthylsulphones 

2628	(α) *cyclo*pentyl	2/>6
2618	(α) *cyclo*hexyl	3/>6
2621	(β) *cyclo*pentyl	3/8
2619	(β) *cyclo*hexyl	3/>6

(*e*) Di-*cyclo*alkylsulphones 

2747	*cyclo*pentyl	2/5
2723	*cyclo*hexyl	2/6

TABLE III

Phenylalkylsulphones 

Number	R	*In vitro* index
2892	methyl	<1/3
2891	ethyl	<1/4
2760	*n*-propyl	1/4
2758	*iso*propyl	1/4
2845	*n*-butyl	3/>6
2846	*iso*butyl	2/5
2842	1-methylpropyl	2/6
2849	*n*-amyl	4/7
2952	*iso*amyl	3/6
2839	2-methylbutyl	3/6
2843	*n*-hexyl	5/9
2860	4-methylamyl	5/>8
2672	*n*-heptyl	4/9
2844	*n*-octyl	5/8
2967	*n*-dodecyl	<1/1

TABLE IV

(*a*) *p*-Tolylalkylsulphones CH_3–C$_6$H$_4$–SO_2R

Number	R	*In vitro* index
2988	methyl	1/5
2759	*n*-propyl	2/5
2764	*iso*propyl	1/4
3001	*n*-amyl	5/8
2650	*n*-heptyl	5/>6
2673	*n*-dodecyl	<1/3

(*b*) *p*-*n*-Butylphenylalkylsulphones n-C_4H_9–C$_6$H$_4$–SO_2R

3403	methyl	2/8
3404	*n*-propyl	5/>8
3405	*n*-amyl	6/>8
3817	*iso*amyl	5/>8
3406	*n*-heptyl	2/8

TABLE V

α- and β-Naphthylalkylsulphones C$_{10}$H$_6$–SO_2R

Number	R	*In vitro* index
2840	(α) *n*-propyl ..	3/>6
2654	(α) *n*-heptyl ..	2/>6
2652	(β) *n*-heptyl ..	1/6

TABLE VI

Dialkylsulphones R-SO_2-R′

Number	R	R′	*In vitro* index
2858	*cyclo*pentyl	*n*-heptyl	4/>8
2762	*cyclo*hexyl	*n*-propyl	2/5
2722	*cyclo*hexyl	*n*-heptyl	4/>6
2761	*cyclo*hexyl	*n*-dodecyl	<1/1
2942	*n*-heptyl	*n*-heptyl	<1/>6

TABLE VII

p-Aminophenylalkylsulphones NH_2–C$_6$H$_4$–SO_2R

Number	R	*In vitro* index
3134	methyl	<1/3
3373	*n*-propyl	1/5
5505	*iso*propyl	1/4
1080	*n*-amyl	2/6
3808	*iso*amyl	1/4
3914	2-methylbutyl ..	2/5
3910	*cyclo*pentyl ..	1/4
3082	*n*-heptyl	4/6

TABLE VIII

p-Alkyl-*m*-aminophenylalkylsulphones R–C$_6$H$_3$(NH$_2$)–SO_2R'

Number	R	R′	*In vitro* index
3631	methyl	methyl	<1/5
3630	methyl	*n*-propyl	2/5
3622	methyl	*n*-amyl	5/8
2972	methyl	*n*-heptyl	4/7
3558	*n*-butyl	*n*-propyl	4/>8
3664	*n*-butyl	*n*-amyl	4/8
3889	*n*-butyl	*iso*amyl	5/8

TABLE IX

p-β-Diethylaminoethylaminophenylalkylsulphones

$(C_2H_5)_2N(CH_2)_2NH$–C$_6$H$_4$–SO_2R

Number	R	*In vitro* index
3160	methyl	<1/3
3114	*n*-amyl	<1/3
3110	*n*-heptyl	2/5

TABLE X

Anisylalkylsulphones CH_3O–C$_6$H$_3$–SO_2R

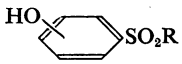
Number	Position of methoxyl group	R	*In vitro* index
3717	*o*	methyl	1/4
3740	*m*	methyl	<1/5
3458	*p*	methyl	<1/4
3718	*o*	*n*-propyl	1/4
3739	*m*	*n*-propyl	2/5
3459	*p*	*n*-propyl	3/7
3721	*o*	*n*-amyl	3/6
3804	*m*	*n*-amyl	4/8
3479	*p*	*n*-amyl	5/>8
3938	*p*	*iso*amyl	4/8
3722	*o*	*n*-heptyl	4/7
3209	*p*	*n*-heptyl	4/8

TABLE XI

p-Alkoxyphenylalkylsulphones RO–C$_6$H$_4$–SO_2R'

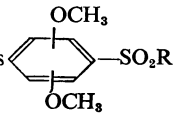
Number	R	R′	*In vitro* index
3465	*n*-propyl	methyl	3/7
3486	*n*-propyl	*n*-propyl	4/>8
3520	*n*-propyl	*n*-amyl	4/>8
3522	*n*-amyl	*n*-amyl	4/>8

TABLE XII

Hydroxyphenylalkylsulphones 

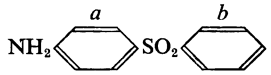
Number	Position of hydroxyl group	R	*In vitro* index
3460	*p*	methyl	1/4
3478	*p*	*n*-propyl	1/5
3745	*o*	*n*-amyl	4/6
3805	*m*	*n*-amyl	4/8
3494	*p*	*n*-amyl	4/7

TABLE XIII

Dimethoxyphenylalkylsulphones 

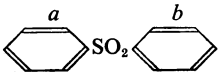
Number	Positions of methoxyl groups	R	*In vitro* index
4031	2:4	methyl	<1/3
4032	2:5	methyl	<1/3
4092	2:4	*n*-propyl	1/3
4093	2:5	*n*-propyl	1/5
3896	2:4	*n*-amyl	2/6
3895	2:5	*n*-amyl	1/5

TABLE XIV

4-Aminodiphenylsulphones 

Number	Substituent(s) in ring *b*	*In vitro* index
2269	none	2/5
2630	*p*′-methyl	1/5
3385	*p*′-methoxy	<1/4
4577	*p*′-chloro	<1/7
3521	*p*′-hydroxy	<1/6
5405	*p*′-β-diethylamino-ethylamino	<1/4
3239	2:4-dimethoxy	<1/6
3624	3:5-dimethoxy	<1/5
4286	2:4-dihydroxy	4/7
2790	2:5-dihydroxy	2/4
3686	3:5-dihydroxy	2/6
371	*p*′-amino	2/5

TABLE XV

Miscellaneous Diphenylsulphones 

Number	Substituent(s) in ring *a*	Substituent in ring *b*	*In vitro* index
3384	*o*-amino	*p*′-methoxy	<1/4
3535	*o*-amino	*p*′-hydroxy	1/6
4326	3:4-dimethoxy	none	1/3
3336	*p*-methoxy	*p*′-methoxy	<1/4
3236	2:4-dimethoxy	none	<1/5
3491	2:4-dimethoxy	*p*′-methoxy	<1/5
3335	*p*-hydroxy	*p*′-hydroxy	3/6
3402	2:4-dihydroxy	none	3/6
3537	2:4-dihydroxy	*p*′-hydroxy	3/5
3441	2:4-dimethoxy	*p*′-chloro	<1/4
3551	2:4-dihydroxy	*p*′-chloro	3/8
3433	*p*-chloro	*p*′-chloro	<1/5

TABLE XVI

Heteroarylphenylsulphones R-SO_2R′

Number	R	R′	*In vitro* index
2719	8-quinolyl	phenyl	<1/1
2745	6-quinolyl	phenyl	3/6
2695	8-quinolyl	*p*-tolyl	<1/4
2694	6-quinolyl	*p*-tolyl	<1/6
4879	5-(2-aminothiazyl)	*p*-aminophenyl	1/2
5163	5-(2-aminothiazyl)	*p*-tolyl	<1/2
5106	5-(2-amino-4-methylthiazyl)	*p*-aminophenyl	2/5
5445	5-(2-amino-4-methylthiazyl)	*p*-chlorophenyl	3/6

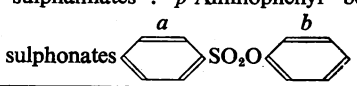
TABLE XVII

Phenylalkanesulphonates R-SO_2OR′

Number	R	R′	*In vitro* index
4033	methyl	*p*-anisyl	1/6
4161	methyl	*p*-hydroxyphenyl	1/5
3723	*n*-amyl	*m*-anisyl	4/>8
3744	*n*-amyl	*p*-anisyl	5/>8
3737	*n*-amyl	*m*-hydroxyphenyl	5/>8
3783	*n*-amyl	*p*-hydroxyphenyl	5/>8
3983	*iso*amyl	*p*-hydroxyphenyl	4/>8
4088	*iso*amyl	*p*-aminophenyl	4/8

TABLE XVIII

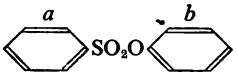
Phenyl sulphanilates : *p*-Aminophenyl benzenesulphonates



Number	Substituent in ring *a*	Substituent in ring *b*	*In vitro* index
3898	*p*-amino	none	2/5
3997	*p*-amino	*o'*-amino	2/5
4089	*p*-amino	*m'*-amino	<1/4
3998	*p*-amino	*p'*-amino	<1/5
3616	*p*-amino	*p'*-methoxyl	<1/3
4409	*p*-amino	*m'*-hydroxy	4/6
4590	*p*-amino	*p'*-hydroxy	3/5
4587	*p*-amino	*p'*-chloro	4/6
4090	*m*-amino	*p'*-amino	<1/5
4315	*o*-amino	*p'*-amino	3/5
4144	*p*-methoxy	*p'*-amino	<1/4
4197	*p*-chloro	*p'*-amino	6/8

TABLE XIX

Miscellaneous Phenyl benzenesulphonates



Number	Substituent in ring *a*	Substituent in ring *b*	*In vitro* index
4211	none	*m'*-methoxy	4/7
3233	none	*o'*-hydroxy	4/6
4091	*m*-amino	*o'*-amino	<1/5
4314	*o*-amino	*o'*-amino	3/7
3617	*m*-amino	*o'*-methoxy	1/6
3899	*m*-amino	*m'*-methoxy	3/7
4099	*m*-amino	*p'*-methoxy	3/6
4142	*p*-methoxy	*o'*-amino	<1/7
4143	*p*-methoxy	*m'*-amino	3/5
3900	*p*-methoxy	*m'*-methoxy	4/6
3901	*p*-chloro	*m'*-methoxy	5/6

TABLE XX

N-Alkylbenzenesulphonamides R$C_6H_4SO_2N(R')(R'')$

Number	R	R'	R''	*In vitro* index
2831	methyl	*n*-butyl	H	3/6
2985	methyl	*n*-amyl	H	4/7
2984	methyl	*n*-heptyl	H	5/6
2829	methyl	ethyl	ethyl	3/6
2827	methyl	pentamethylene		2/>6
2990	methyl	*n*-amyl	methyl	4/7
2836	methyl	*n*-butyl	*n*-propyl	4/7
2992	methyl	*n*-heptyl	methyl	4/6
4682	methyl	diethylaminoethyl	ethyl	<1/2
4735	amino	ethyl	ethyl	1/4
3241	amino	amyl	H	2/6
4730	amino	diethylaminoethyl	ethyl	<1/1

TABLE XXI

Phenyl benzenesulphonamides R$C_6H_4SO_2NHC_6H_4$R'

Number	R	R'	*In vitro* index
2480	amino	H	< 1/4
6419	H	H	3/6
6418	methyl	H	3/7
6420	H	methyl	< 1/7
6417	methyl	methoxy	1/7
6416	methyl	chloro	4/8

Turning to the results of therapeutic tests, 10 new compounds were selected on the basis of high *in vitro* activity. 4 : 4'-Diaminodiphenylsulphone and No. 4879 (which corresponds to promizole) were also included. Before carrying out these tests, six of the compounds whose constitutions lent themselves to the method of analysis were given orally to mice at maximal doses and the blood concentrations determined (Table XXII).

TABLE XXII

Blood concentrations attained after oral administration of maximum tolerated doses to mice

Number	See Table	Dose mg./20 g. mouse	Blood concentrations (mg./100 ml.) at time after dosing		
			1 hr.	2 hr.	4 hr.
371	XIV	3	3.2	3.1	2.8
4286	XIV	5	12.2	4.5	0.9
4879	XVI	10	10.0	9.1	6.9
5106	XVI	5	9.1	10.0	7.5
4197	XVIII	8	2.0	1.5	1.5
4409	XVIII	9	6.9	3.6	1.4

We could not be sure that some of the remaining compounds were absorbed to any appreciable extent because no method of analysis was available for them and mice tolerated, without gross toxic effects, relatively large doses. Certain others were definitely absorbed because small doses were toxic to mice, but we have no knowledge of the blood levels corresponding to these doses. The results of therapeutic tests are listed in Table XXIII. As would be expected, activity was found with 4 : 4'-diaminodiphenylsulphone (No. 371) and with 2 : 4'-diamino-5-thiazylphenylsulphone (No. 4879). No activity was observed with any of the others.

TABLE XXIII

THERAPEUTIC TESTS IN MICE. 24 mice in each group, infected intravenously and treated orally.

Number	See Table	Dose mg./20 g.	Increased mean survival time	Increase required for significance
3938	X	4.0	−1.8	1.9
3983	XVII	3.0	−0.7	1.9
2985	XX	1.0	−0.7	1.15
		2.5	+0.3	
		5.0	−0.6	
371	XIV	1.0	+1.1	1.8
		2.0	+2.2	
		3.0	+1.9	
		4.0	+2.5	1.7
4286	XIV	5.0	0	2.0
5405	XIV	0.1	+0.3	1.6
		0.25	−0.3	
4879	XVI	2.5	+0.8	1.8
		5.0	+2.2	
		5.0	+1.7	1.4
5106	XVI	1.0	+0.1	1.8
		2.5	+0.2	
5445	XVI	10.0	+0.6	1.8
4197	XVIII	8.0	−2.3	1.5
3901	XIX	10.0	−0.5	1.5
4409	XVIII	8.0	+0.9	1.5
6416	XXI	1.0	+0.1	1.15
		2.5	−0.3	
		5.0	−0.8	

SUMMARY

1. The testing of a large number of sulphones and related sulphonates and sulphonamides against *Mycobacterium tuberculosis in vitro* is recorded.

2. Therapeutic tests in mice have shown that high *in vitro* activity does not necessarily lead to activity *in vivo*.

Chemical papers referring to the methods of preparation of the compounds mentioned here (where these are not already well known) have appeared (Burton and Hoggarth, 1945; Hoggarth, 1947). Some of the compounds listed were originally submitted by Dr. Burton, of the University of Leeds, for general antibacterial examination. Our thanks are due to him and also to Miss M. Scott of these laboratories for help with the preparation of some of the larger samples required for the therapeutic tests. The statistical assessment of the therapeutic experiments was carried out by Dr. O. L. Davies, of our Statistical Department.

REFERENCES

Burton, H., and Hoggarth, E. (1945). *J. chem. Soc.*, (a) 14; (b) 486.

Feldman, W. H., and Hinshaw, H. C. (1945). *Amer. Rev. Tuberc.*, **51**, 582.

Feldman, W. H., Hinshaw, H. C., and Mann, F. C. (1944). *Amer. Rev. Tuberc.*, **50**, 418.

Feldman, W. H., Hinshaw, H. C., and Moses, H. E. (1942). *Amer. Rev. Tuberc.*, **45**, 303.

Hoggarth, E. (1947). *J. chem. Soc.*, (a) 110; (b) 114.

Martin, A. R. (1946). *J. Path. Bact.*, **58**, 580.

Martin, A. R., Rose, F. L., and Bevan, H. G. L. (1943). *J. Pharmacol.*, **77**, 127.

Rich, A. R., and Follis, R. H. (1938). *Johns Hopk. Hosp. Bull.*, **62**, 77.