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EXPERIMENTAL TUBERCULOSIS AND ITS CHEMOTHERAPY

BY

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In the past few years about one thousand compounds, falling into eleven series, were examined by us for tuberculostatic activity. Twenty-four of them were tested *in vivo* in the mouse and guinea-pig, but none affected the course of the disease. Compounds of known activity were included in the tests and the effects of combinations of these drugs were also investigated.

The present paper reports the *in vivo* results on the following members of the series examined:

Group A. p-*Alkoxy*-N-*arylbenzamidines* (Partridge, 1950)
570 *p*-Hexyloxy-*N*-phenylbenzamidine
486 *p*-Octyloxy-*N*-phenylbenzamidine
532 *p*-Butoxy-*N*-*p'*-butoxyphenylbenzamidine

Group B. Di-(p-N-*arylamidinophenoxy*)*alkanes* (Partridge, 1949)
354 1 : 3-Di-(*p*-*N*-phenylamidinophenoxy)propane
512 1 : 5-Di-(*p*-*N*-phenylamidinophenoxy)pentane
462 1 : 3-Di-(*p*-*N*-4'-ethoxyphenylamidinophenoxy)propane

Group C. Aliphatic amidines
449 2-Nonyldihydroglyoxaline
594 1-*N*-phenylamidinononane

Group D. Phenylacetamidines and analogues
621 *N*-phenylphenylacetamidine
528 *N*-*p*-Tolylphenylacetamidine

Group E. Amidino-cyclo*hexenes and* -cyclo*hexanes*
529 1-*N*-*p*-butoxyphenylamidino*cyclo*hexene

Group F. Halogenated-ω-aryloxyalkylamines and analogues (Drain, Peak, and Whitmont, 1949; Peak and Watkins, 1950)
585 Diethyl-2-(*p*-chlorophenoxy)ethylamine
336 Diethyl-2-(2' : 4' : 6'-trichlorophenoxy)ethylamine (Burger, Wilson, Brindley, and Bernheim, 1945)
264 Diethyl-2-(2' : 4' : 6'-tri-iodophenoxy)ethylamine (Saz and Bernheim, 1942)
494 Diethyl-2-(2' : 3' : 5'-trichlorophenoxy)ethylamine
586 2 : 4 : 6-Trichloro-*N*-2'diethylaminoethylaniline

Group G. iso-*Thio-semicarbazones, -ureas, and analogues* (Brooks, Charlton, Macey, Peak, and Short, 1950)

387 Acetone 3-ethyl*iso*thiosemicarbazone
393 *p*-1′-Pyrrolidylbenzaldehyde 3-ethyl*iso*thiosemicarbazone
429 *o*-Nitrobenzaldehyde thiosemicarbazone (suspensions in water and alcohol)
539 Benzaldehyde-3-butyl*iso*thiosemicarbazone

Group H. Guanidine derivatives

391 *p*-1′-Pyrrolodylbenzaldehyde guanylhydrazone
384 *p*-Dimethylaminobenzaldehyde guanylhydrazone

Group J. Basic sulphides, sulphoxides, sulphones, and related compounds

597 2-Diethylaminoethyl butyl sulphide

Group K. Basic phenolic ethers and analogues

492 3 : 4-Bis(*p*-2′-diethylaminoethoxyphenyl)hexane
545 Hexylresorcinol bis-(2′-diethylaminoethyl) ether (Chapman, Hager, and Shay, 1947)
706 1 : 4-Bis (2′-diethylaminoethoxy)benzene
709 1-(2′-Diethylaminoethoxy)-4-hexylbenzene
630 *p*-(1′-Hydroxy-1′-ethylbutyl)phenyl 2-diethylaminoethyl ether

Group L. 2-Sulphanilamido-5-alkylthia- and -oxa-diazoles (Brooks, Charlton, Macey, Peak, and Short, 1950)

344 2-Sulphanilamido-5-pentyl-1 : 3 : 4-thiadiazole
T.R.C. 245 2-Sulphanilamido-5-methyl-1 : 3 : 4-oxadiazole
T.R.C. 43 2-Sulphanilamido-5-methyl-1 : 3 : 4-thiadiazole

Group M. Active drug controls

Streptomycin (Feldman and Hinshaw, 1944)
Licheniformin (Callow, Glover, D'Arcy Hart, and Hills, 1947)
Para-aminosalicylic acid (Lehman, 1946). Referred to as PAS
Sulphetrone (Brownlee and Kennedy, 1948a)
2 : 6-Diaminobenzthiazole (Freedlander and French, 1947)

Group M was included in order to provide known tuberculostatic drug controls, since one purpose of this work was to find a satisfactory " screening " test in experimental tuberculosis. Licheniformin was tested in guinea-pigs at the request of Dr. P. D'Arcy Hart, as it had been found to be active in mice (Callow *et al.*, 1947).

Experimental Methods

Strains.—Two virulent human strains, H.418 and H.37Rv, were employed: these were maintained by surface and submerged culture. There was a loss of virulence after repeated subculture in Dubos medium (Dubos and Davis, 1946), so that for *in vivo* tests strain H.418 was maintained on Lowenstein slopes and strain H.37Rv on Proskeuer and Beck's medium as used by Feldman and Hinshaw (1945); inocula were prepared by growing in Dubos medium or by grinding.

Drugs.—Most drugs were tested in the form of salts, usually the lactate or hydrochloride. Solutions were sterilized by heat unless unstable, when they were filtered.

In vitro tests (Table I).—Tests were carried out by surface and submerged culture. The Douglas modification of Long's medium was used for the former, both with

TABLE I

CHEMOTHERAPY OF TUBERCULOSIS

T.B. index = Group average ÷ control average. *In vitro* activity = reciprocal of inhibiting dilution × 1,000. Figures in parentheses indicate partial inhibition. Brownlee test on guinea-pig blood = tuberculostatic action of the blood after intraperitoneal injection of compound. S.C. = subcutaneous.

In vitro activity					*In vivo* activity				
					MICE		GUINEA-PIGS		
Serial number of drug	Long's medium	Long's medium + 10% serum	Dubos medium	Brownlee test on guinea-pig blood	Daily oral dose mg./g.	T.B. index	Daily oral dose mg./g.	T.B. index	Remarks on guinea-pig test
A. p.-Alkoxy-N-*arylbenzamidines*									
570	5,000	500–1,000 (5,000)	10	—	0.1	0.7	0.04	0.94	Toxic
486	1,000	100	10	—	0.2	1.4	0.2	1.1	
532	1,000	100	5	—	0.1	0.6	0.15	1.0	
B. Di-(p-N-*arylamidinophenoxy*)*alkanes*									
354	100	50–100	—	—	—	—	0.07	—	Toxic. Disease progressed in 2 survivors
512	500	500	50–100	—	0.1	1.1	0.15	0.81	
462	500	100	50–100	—	0.1	1.2	—	—	
C. Aliphatic amidines									
449	100–500	100 (500)	10	—	0.2	1.1	0.12	1.0	
594	500	10	5–10	—	0.2	0.7	—	—	
D. Phenylacetamidines and analogues									
621	50	—	5	—	0.1	0.64	—	—	
528	100	10	1	—	—	—	0.2	0.91	Toxic
E. Amidino-cyclo*hexenes and* -cyclo*hexanes*									
529	500	100 (10,000)	5	—	0.1	0.68	0.12	—	Toxic. Disease progressed in 2 survivors
F. Halogenated ω-aryloxyalkylamines and analogues									
585	10–50	10	5	—	0.2	1.12	—	—	
336	100	10	1–5	0.55 g. inactive	—	—	—	—	
264	100	10	10	0.7 g. inactive	0.1	0.80	0.1	1.0	
494	100	50–100	10–50	—	0.1	1.0	0.1	0.91	
586	100–500	50	5	—	—	—	0.15	1.0	
G. iso-*Thio-semicarbazones, -ureas, and analogues*									
387	10	10	10	0.5 g. inactive	—	—	—	—	
393	(50)	—	10	0.5 g. inactive	—	—	—	—	
429	10	—	10	0.5 g inactive	—	—	—	—	
539	100 (500)	50	10	—	0.1	0.68	0.3	0.89	
H. Guanidine derivatives									
391	(50)	10	5–10	0.5 g. inactive	—	—	—	—	
384	10	10	5–10	—	0.07	0.9	0.07	0.96	

TABLE I—*continued*

Serial number of drug	*In vitro* activity: Long's medium	Long's medium + 10% serum	Dubos medium	Brownlee test on guinea-pig blood	*In vivo* activity: MICE Daily oral dose mg./g.	MICE T.B. index	GUINEA-PIGS Daily oral dose mg./g.	GUINEA-PIGS T.B. index	Remarks on guinea-pig test
J. Basic sulphides, sulphoxides, sulphones, and related compounds									
597	500–1000	100–500	50	—	0.1	0.8	0.3	0.90	
K. Basic phenolic ethers and analogues									
492	1,000 (10,000)	100 (500)	10–50	—	0.1	0.7	0.1	—	Toxic. Disease progressed in 2 survivors
545	50–100	50–100	50	—	—	—	0.2	0.87	Toxic
706	100–500	100	1	—	—	—	0.15	0.84	
709	100	100	10	—	—	—	0.3	0.87	
630	50	50	10	—	—	—	0.1	0.88	
L. 2-Sulphanilamido-5-alkylthia- and -oxa-diazoles									
T.R.C. 43	10	—	—	1.0 g. active	—	—	—	—	
T.R.C. 245	10–50	10	—	1.0 g. active	—	—	0.5 0.3 S.C.	0.9 1.33	
344	50	—	—	0.5 g. inactive	—	—	—	—	
M. Active drug controls									
2:6 di-amino benzthiazole	10 (500)	5–10	5	—	—	—	0.27	0.8	
PAS	10	—	—	0.5 g. active	See Table IV				
Sulphe-trone	10	—	—	Active (Brownlee, 1948)					

and without the addition of 10 per cent ox serum, and tests were read four weeks after the inoculation of a small piece of floating pellicle. For submerged culture, Dubos's medium (Dubos and Davis, 1946) as modified by Forrest, D'Arcy Hart, and Walker (1947) was used. The inoculum was 0.03 ml. of a 7–10 day culture per 9 ml. test medium and tests were read after two weeks. All inhibition tests were carried out in duplicate in serial tenfold dilution of the drugs. Inhibition titres as great as 1 in 10^6 were obtained in Long's medium; titres were considerably lower in the Dubos medium.

Toxicity.—Dr. M. R. Gurd (Pharmacology Division) estimated the "acute" LD50 values in mice, for oral and subcutaneous administration of all the drugs, to lie between 0.1 and 1.0 mg./g., except T.R.C. 245, which had a value of 10 mg./g. for the oral route. In addition, he carried out chronic toxicity tests; mice were given 1/4 and 1/10th the LD50 subcutaneously for 14 days. If the drugs caused tissue damage they were not given subcutaneously to guinea-pigs. However, all drugs actually tested subcutaneously in guinea-pigs, except T.R.C. 245, were found to be so irritant that the *in vivo* tests had to be terminated and are not reported.

Brownlee test (Table I).—Brownlee, Green, and Woodbine (1948) suggested a method whereby the blood of a guinea-pig was tested for tuberculostatic action 2 hours after the intraperitoneal injection of 2 g. drug (unless the animal died before this time). In our tests 0.5–1.0 g. amounts of drug were used ; the blood was mixed with an equal volume of Long's agar and inoculated with strain H.418.

RESULTS

In vivo tests (Table I)

Mouse.—The albino strain of mice available to us in adequate numbers was relatively resistant to tuberculosis. Both intravenous and intraperitoneal inoculation of undiluted Dubos medium cultures of strains H.418 and H.37Rv were tested. H.418 was less infective to mice than H.37Rv, which was used throughout the tests. Groups of twenty animals were treated from the date of infection, the drug being mixed with the food. Streptomycin, 1,000 or 2,000 units daily, was given subcutaneously in two divided doses. Mice were killed after four to six weeks and all lungs examined for tuberculosis, the extent of the disease being recorded on an arbitrary scale of 0–6. As there were so few deaths in our experiments, drug action was assessed solely on lung lesions, which varied widely in their severity—i.e., from 0–6 in every group. Intravenous inoculation gave no better results than the intraperitoneal method, as shown in Table II. The addition of 50 per cent egg yolk to the intraperitoneal inoculum, as recommended by Pierce, Dubos, and Middlebrooke (1947), had no enhancing effect on virulence.

TABLE II

TUBERCULOSIS IN MICE

Strain H.37Rv in undiluted Dubos culture

Route	Mice	Lung assessments. (Max. = 6; bold figures indicate death)	Average lung assessment
Intraperitoneal 0.5 ml. ..	White	**4**, 6, 5, 4, 3, 2, 2, 1, 0, 2, 1	2.7
	Dark	**4**, 5, 3, 3, 0, 2, 1, 1, 1, 1, 2	2.1
Intravenous 0.1 ml.	White	6, 5, 4, 3, 3, 2, 2, 2	3.4
	Dark	6, 2, 2, 4, 2, 2, 1, 2, 3, 2, 2, 0, 1	2.2

Guinea-pig.—An injection of 0.0001–0.005 mg. of a three-week culture of strain H.418 was used in most experiments ; this was later changed to 0.5 ml. of a 1 in 100 dilution of a 14 day Dubos culture. The laboratory bred animals in groups of at least six and weighing about 500 g. were infected intramuscularly. All received an ample diet and were weighed at weekly intervals. Treatment commenced three to four weeks after infection, when the animals were tuberculin positive, and continued for ten weeks. The drugs were administered with the food, the drug solutions being mixed with the dry diet. Streptomycin, which was used as an active drug control, licheniformin, and some other drugs were tested subcutaneously. Drugs were assessed by their action in prolonging life and limiting the spread of the disease. For macroscopic estimation of tuberculosis the scoring method of Sher and Kloeck (1946) was followed, each organ being given a maximum value as follows: lymph nodes 8, spleen 24, liver 28, lungs 40. The assessments for all

the controls in these experiments are given in Table III. This test was found to give reproducible results providing that groups of at least six animals were employed. Smaller numbers give unreliable results. Occasionally, in order to confirm that the greatest tolerated dose was being given, groups of only three animals had higher doses than the main test group.

TABLE III

TUBERCULOSIS IN THE GUINEA-PIG

0.0001–0.005 mg. strain H.418 intramuscularly

Number of animals	Individual T.B. assessments of controls in 11 tests. (Max. 100; bold figs. indicate death)	Average	Streptomycin index for the test†
6	**44, 74, 91,** 68, 63, 78	69.6	0.29
6	54, 33, 31, 39, 23, 49	38.2	0.13
12*	22, 42, 27, 66, 49, 45, 61, 37, 47, 39, 58	45.0	0.16
6	**56, 66, 72,** 66.5, 53, 71.5	64.2	0.11
12	**95, 83, 92, 65.5, 74,** 55, 48, 55, 61.5, 41, 59.5, 55	65.4	0.25
9	12, 22, 55, 33, 39, 37, 45, 16, 38	33	0.06
6*	56, 46, 58, 58, 36	50.8	Not tested
6*	58, 59.5, 48, 61.5, 65	58.4	0.16
12*	22, 42, 27, 66, 49, 45, 61, 39, 37, 58, 47	45	Not tested
12*	**34,** 14, 63, 66, **56,** 36, 66, 70, 31, 83, 33	50.2	,, ,,
9	**95, 83, 88,** 44.5, 47, 50, 41, 51, 46	60.6	0.16

* One animal died a few days after injection, not from T.B.

†Average lung assessment of streptomycin treated animals / Average lung assessment of control animals

In both the mouse and the guinea-pig tests, group means of the estimates of " the T.B. assessments " were calculated and compared statistically with the " control " group mean. Estimates were considered to differ significantly at a value of " P " corresponding to a probability of 0.05. None of the 25 new drugs showed any activity. In Table I results are expressed as a T.B. index

$$\left(\text{i.e.,} \quad \frac{\text{Mean T.B. assessment of the group}}{\text{Mean T.B. assessment of the controls}} \right)$$

The wide scatter of the results in the mouse test was reflected in the low T.B. index, which was necessary before a result could be accepted as differing significantly from the controls. Table III shows that the variation was much less in the guinea-pig and that streptomycin had a fairly constant index even though the extent of the disease varied widely in the different experiments. For these reasons guinea-pigs were employed in all the experiments on the effects of combinations of drugs. Results of the latter investigations are given in Table IV. Spleens of the animals given zero assessments were found to be non-infectious when injected into healthy guinea-pigs; also, some animals which were left on test after the cessation of treatment did not develop the disease.

Developing chick embryo test.—Various workers in America have used this method (e.g., Emmart, 1945 ; Dubos and Davis, 1946 ; Lee and Stavitsky, 1947), employing different routes and with varying results, but the test is not yet on a firm basis. Our experiments were limited to inquiry for a more uniform infection than in the mouse test. Varying dosages of strains H.418 and H.37Rv were inocu-

TABLE IV

EFFECT OF COMBINATIONS OF DRUGS ON EXPERIMENTAL TUBERCULOSIS

u = units. S.C. = subcutaneous. * = result significant (P = 0.05 or less). Bold figures of T.B. assessment denote death of animal. T.B. index = group average ÷ control average

Drug	MICE		GUINEA-PIGS			Remarks
	Dose in mg./g.	T.B. index	Dose in mg./g.	T.B. assessments (max. = 100)	T.B. index	
Streptomycin	1,000 u/mouse S.C.	0.36*	10,000 u/pig, S.C. in 2 doses	4, 2, 8, 2, 8, 19	0.16*	Disease progresses on cessation of treatment
	500 u/mouse S.C.	0.68				
Sulphetrone	4.0 oral	0.71	1.45 oral	29, 26, 10, 22, 49, 16, 14, 20	0.52*	,, ,,
Licheniformin	Active	(Callow *et al.*, 1947)	0.01 S.C. in 2 doses	**18,** 39, 12, 0, 10, 16, 21, 18, 39	0.43*	Toxic. Kidneys enlarged. Low weight gain
Licheniformin + sulphetrone	Synergic effect	(Callow *et al.*, 1947)	As above	2, 2, 2, 2, 0, 2, 13, 2, 4	0.07*	1 out of 3 pigs did not develop disease when treatment stopped. 2 out of 3 pigs did
Streptomycin + sulphetrone	—	—	As above	0, 0, 0, 2, 0, 4, 12, 8, 2	0.07*	not develop disease when treatment stopped
Controls	—	—	Nil	22, 42, 27, 66, 49, 45, 61, 39, 37, 58, 47	1.0	
PAS ..	5.0 oral	0.21*	1.5 increased to 2.3 oral	**48, 75,** 15.5, 2, 35, 18	0.53*	
			0.7 S.C.	38, 10, 16, 8, 11.5, 19.5	0.28*	5% Na salt caused tissue damage
Streptomycin	—	—	10,000 u/pig as above	8, 11.5, 13, 14, 5, 5	0.16*	
PAS + streptomycin	Youmans *et al.* (1947)		PAS orally and streptomycin as above	8, 5, 5, 2, 2	0.07*	
Controls	—	—	Nil	**95, 83, 88,** 44.5, 47, 50, 41, 51, 46	1.0	

lated on to the chorioallantoic membrane and into the yolk sac. The rate of infection was assessed by the macroscopic appearance of lesions, together with an examination of smears. In contrast to the reliability of the technique in virus work, uniform infection was never obtained either with inocula of ground suspensions or Dubos medium cultures made directly on to the chorioallantoic membrane of 9–10 day embryos, or into the yolk sac at 7 and 10 days. Streptomycin (1,000 units/egg by the allantoic sac) had no consistent effect on the large inoculum of tubercle bacilli found necessary to secure reliable infection and would probably have been missed, if sought for, by this method. Work on this test was therefore discontinued.

Discussion

The testing of potential chemotherapeutic agents for the treatment of tuberculosis admits of so many variables that an extensive literature has developed. While there is a general agreement on the reliability of the guinea-pig test, as used by Feldman and Hinshaw (1945) and Brownlee and Kennedy (1948a and b), the large amount of drug necessary for the test and the time taken are marked disadvantages. Apart from the egg test, which has not found favour with most workers, and the infection of the cornea of rabbits (Gardiner, Rees, and Robson, 1949), some form of mouse test has been advocated by various workers. Callow *et al.* (1947) infected mice by an aerosol but considered "it a matter of speculation which animal may yield results more indicative of therapeutic value in man"; this statement is still true. Youmans and McCarter (1945) used the intravenous route and paid marked attention to the pathology of the disease, but Martin (1946) assessed drug action solely on the basis of survival times. The method of treatment of the experimental animal also varies considerably so that it is difficult to compare the results of different workers. When reporting their results, workers on guinea-pigs usually give details, pictorially or numerically, of the extent of the disease in every animal. For example, in our own experiments, the variation shown in Table III was typical and the disease was progressive in every case. With mice, however, the lung lesions varied from 0–6 in each test (cf. Table II). Martin (1946) did not report the lung assessments, but Youmans and McCarter (1945) found that the percentage of lung disease in their control animals varied from 10–60 per cent and, in one test, from 0–75 per cent. Pierce *et al.* (1947) found that mouse strains varied in their resistance to infection and it may well be that there is less variation when the mice of a susceptible strain die in 4–6 weeks. However, in our experiments, we invariably found that a test on six guinea-pigs was more convincing than one on twenty mice. Whichever animal eventually finds favour, there is no doubt that some active drugs may be missed, as already definite specific differences in response by the mouse and guinea-pig have arisen. We confirmed the activity of sulphetrone in guinea-pigs (Brownlee and Kennedy, 1948a) and its inactivity in mice (Callow *et al.*, 1947). Our tests also confirmed the activity in both animals of PAS (Feldman, Karlson, and Hinshaw, 1947; Youmans, Raleigh, and Youmans, 1947). Given subcutaneously as a 5 per cent (w/v) solution this drug caused tissue damage, but it was more effective by this route than when given orally. Feldman, Karlson, Carr, and Hinshaw (1949) recently reported similar findings. Streptomycin was not so strikingly active in the mouse as in the guinea-pig (in one test it would have been discarded), and the claim by Freedlander and French (1947) that 2 : 6-diaminobenzthiazole was active in guinea-pigs was not confirmed under our conditions. Where supplies were sufficient, compounds of Groups A to K were tested in mice and guinea-pigs, but all were inactive in both tests, so that further light was not thrown on this problem.

The complete inactivity of the twenty-four new drugs tested by us, in spite of their high *in vitro* activity even in the presence of serum, was discouraging, since the maximum amount of each compound was always given. The inactivity of R.D. 539, benzaldehyde-3-butyl*iso*thiosemicarbazone, is interesting in the light of the recent confirmation of the findings of Domagk (1948) by Hoggarth, Martin,

Storey, and Young (1949), who found that the thiosemicarbazones of substituted benzaldehydes showed marked activity in mice ; their most active compound did not completely inhibit growth *in vitro* in 1 in 10^3 although partial inhibition occurred at 1 in 10^5 to 1 in 10^6, but it attained a high blood concentration (Spinks, 1949). T.R.C. 245, with an activity in the presence of serum of 1 in 10^4, was relatively non-toxic and significant blood levels, as estimated by the Marshall test, were maintained throughout the twenty-four hours. The Brownlee test indicated that the blood was tuberculostatic after two hours and Dr. G. Brownlee confirmed this. It was only after the complete failure of large scale tests (only one small test is reported in Table I) that further investigations, using U.V. absorption, revealed that the drug was present for only two hours after injection and had disappeared after four hours although some product in the blood still gave a positive Marshall reaction. When later repeated, the Brownlee test gave a negative value at four hours. This test, however, picked out streptomycin, sulphetrone, and PAS, and further work on other active compounds should confirm its usefulness.

Only preliminary, and in view of recent publications confirmatory, work on drug combinations is reported here. The experiments in which streptomycin and sulphetrone were combined, and licheniformin and sulphetrone were combined, were carried out at the request of Dr. P. D'Arcy Hart, who had noticed the synergistic effect of sulphetrone and licheniformin in mice. This effect, and that between sulphetrone and streptomycin, as reported by Brownlee and Kennedy (1948b), were marked. As seen in Table IV several animals showed no evidence of the disease and the spleens of the animals with zero assessments were not infective to normal guinea-pigs. Two out of three animals given streptomycin plus sulphetrone, and 1 of 3 given licheniformin plus sulphetrone, did not develop the disease when treatment was stopped after ten weeks. Of many hundreds of infected animals, these were the only ones which were apparently cured of the disease. A combination of PAS (oral) and streptomycin also gave better results than either drug alone, although no animal showed zero assessments ; this confirms the findings reported for mice by Youmans *et al.* (1947) and, for guinea-pigs, by Block, Vennesland, Ebert, and Gomori (1949) and Karlson and Feldman (1949). The effect of both drugs when given subcutaneously would be interesting.

Summary

1. About one thousand compounds of eleven different series were tested *in vitro* against *M. tuberculosis* (human).

2. Twenty-four compounds, with high *in vitro* activity, were tested in mice or guinea-pigs, or both. None was active.

3. The small-scale guinea-pig test was found to give more reliable and more convincing results than the mouse test.

4. The following combinations of drugs were more effective than either drug alone ; licheniformin and sulphetrone, streptomycin and sulphetrone, PAS and streptomycin.

5. PAS was more active subcutaneously than orally in guinea-pigs.

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Chemical Research Division, for the supply of compounds. They are also indebted to Dr. M. R. Gurd, of the Pharmacology Research Division, for toxicity determinations and Mr. D. E. Williamson for technical assistance. Dr. R. K. Callow supplied the licheniformin and Dr. C. H. Kellaway provided the sulphetrone. The work on T.R.C. 43 and T.R.C. 245 was, in part, carried out on behalf of the Therapeutic Research Corporation.

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