Antituberculous Activity of Substituted Thioureas. (20241)

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Mycobacteria, actinomycetes and streptomycetes are members of the order Actinomycetales and are characterized as organisms forming elongated cells which have a definite tendency to branch. This morphological feature places the members of this order close to the fungi taxonomically. In an earlier study(1) one of us (R.L.M.) investigated the hypothesis that this morphologic relationship between mycobacteria and fungi may be paralleled by a biochemical relationship and thereby suggesting a basis for the search for new antituberculosis agents. It was indeed found that a number of recognized antifungal substances exerted strong and specific antimycobacterial *in vitro* activities while possessing only minor effectiveness against the common Gram negative and Gram positive bacteria. Particularly interesting among the sulfur-containing compounds exhibiting such properties were thiourea and its derivatives. The most active substance was sulfamidothiourea (2255 RP).

We have now extended these earlier studies and investigated more than 350 thiourea derivatives and related compounds for their antimycobacterial and antifungal activities. Many of the disubstituted thioureas, especially the thiocarbanilides demonstrated not only *in vitro* tuberculostatic and fungistatic activities but were also found to have excellent chemotherapeutic effects in mice and guinea pigs infected with the tubercle bacillus. From among the many thiocarbanilides studied, several have been selected for inclusion in this paper as representative of the various types of substituents located in the p,p'positions (Table II).

In vitro activities of disubstituted thioureas. Numerous derivatives demonstrated considerable activity against the human, bovine and avian types of the tubercle bacillus as well as the BCG (human) strain. However, none of these compounds showed appreciable activity against the saprophytic mycobacteria or any of the usual Gram negative or Gram positive bacteria. Furthermore, a number of compounds simultaneously exhibited excellent activities against the tubercle bacillus and a variety of fungi, as evidenced by the examples shown in Table I.

Chemotherapeutic activities in mice. Experiments with representative types of thioureas. CF1 mice, weighing between 15 and 20 g each, were infected intravenously with 0.5 ml of a 1:10 dilution of culture of $H_{37}Rv$ (or Ravenel) grown for 7 days in Kirchner's liquid medium containing 0.05% Triton A-20 and 0.5% bovine serum albumin. Immediately upon infection, groups of 10 mice each were fed for 21 days with a diet consisting of ground food mixed with finely pulverized (200 mesh) test compounds. Normal diet was restored for an additional fifteen days and all surviving animals then sacrificed. All active thiourea derivatives were tested at varying concentrations and the percentages indicated in Table II represent the lowest dose levels given to the infected groups of animals. *T50* represents the calculated survival time (days) of 50% of the mice and is obtained by plotting the cumulative percentage dead on a probability scale against time on an arithmetic scale(3). Since the average T50 for the infected, untreated groups was 20 days in this experiment, values greater than 23 days are indicative of chemotherapeutic activity.

The results obtained in the diet experiments with 11 selected substituted thioureas bearing a variety of substitutions in the p,p'-positions are shown in Table II. From these results it is readily apparent that the presence of certain substituents, such as alkoxy groups, in p,p'positions either with or without nitrogen, or alkyl groups, has transformed the inactive unsubstituted thiocarbanilide (Su-1495/E) into substances with considerable antitubercular activity in mice.

Additional experiments with Su 1795. The extent of activity of the disubstituted thiou-

TABLE I. *In Vitro* Activities of Selected Thioureas.

	Substance		Su 1814	Su 1380	Su 1515	Su 1795
Structure:		R	$CH_3CH_2CH_2CH_2-$	CH_3CH_2O-	$CH_3CH_2CH_2CH_2O-$	CH_3CH_2O-
	$R-\langle\rangle-NHCSNH-\langle\rangle-R_1$	R_1	$-CH_2CH_2CH_2CH_3$	$-OCH_2CH_3$	$-OCH_2CH_2CH_2CH_3$	$-OCH_2CH(CH_3)_2$
I	Mycobacterium tuberculosis, $H_{37}Rv$	tube*	1:1000000	1:256000	1:1000000	1:1000000
II	*Trichophyton interdigitale*	tube	1: 50000	1: 1000	1: 1000	1: 10000
		disc*		>90 mm		>90 mm
	" *rubrum*	tube	1: 50000	1: 1000	1: 1000	1: 10000
		disc		>90 mm		>90 mm
	Candida albicans	tube	1: 50000			<1: 1000
		disc		>90 mm		
	Torula lipofera	tube		1: 1000		1: 10000
		disc		>90 mm		
III	*Staphylococcus aureus*	tube	<1:500	<1:500	<1:500	<1:500
	Streptococcus pyogenes	tube	"	"	"	"
	Escherichia coli	tube	"	"	"	"
	Salmonella schottmüelleri	tube	"	"	"	"
	Antituberculär activity *in vivo* (mice & guinea pigs)		active	active	active	active

* "Tube": refers to tube-dilution broth method; values shown are highest dilutions completely inhibiting growth of organism. "Disc": indicates diameter of zone of inhibition obtained on Sabouraud's agar by the agar-disc method, using 1/50 molar conc.

reas, as represented by Su 1795, is evident from the following additional experiments.

a) *Delayed therapy.* As is shown in Table III, considerable therapeutic activity is obtained when diet-therapy with 0.5% Su 1795 is delayed for as long as 15 days or with 0.1% for 9 days.

b) *Limited therapy.* According to the data presented in Table IV, a total treatment period of only 6 days with a diet containing 0.1% Su 1795 or only 12 days with 0.05% resulted in effective antituberculosis action.

Chemotherapeutic activity in guinea pigs; Experiment Bk 5/214. Procedure: Female guinea pigs weighing approximately 600 g were each infected subcutaneously with 1.0 ml of a 1:200 dilution of a 7-day-old culture of $H_{37}Rv$ in the modified Kirchner's liquid medium. Twenty-one days after infection, the tuberculin-positive animals were treated with medicated diets. Therapy was maintained for the following 115 days and the animals then sacrificed. The evaluation of the gross organ involvement was based on the method described by Feldman(4) who assigned maximum values of 40, 70 and 100 for slight, moderate and extensive degrees of total organ involvement respectively. In Table V are reported results obtained with 5 different thiourea-derivatives; 4 of the 5 substances possessed good tuberculostatic activities in guinea pigs at doses as low as 0.05% in the diet.

Experiment Bk 12/1. In a delayed therapy experiment with guinea pigs, similar to the one performed with mice (Table IV), diet-therapy with Su 1795 was started 60 days after infection and continued for 100 days. The results are given in Table VI and Fig. 1 which is a schematic representation showing the extent of tuberculous involvement for each animal at time of necropsy(5). According to this data, Su 1795 compares favorably with Isoniazid at the same dose level in exerting a therapeutic effect in a well-established guinea pig infection.

Discussion. In an extension of earlier studies(1) on antifungal and antimycobacterial agents, a large number of synthetic sulfur-containing compounds, particularly thiourea derivatives, have been investigated. Many of these newly tested substances were active

TABLE II. Antitubercular Activities in Mice of Selected Thioureas.

Compound*	R	R_1	Approx. MTD (%)	% conc. in diet	T50	% survival	Infecting organism
Su 1495/E	H–	–H	.2	.1	19	10	H37Rv
1380	CH_3CH_2O–	$-OCH_2CH_3$	5	.1	>35	80	"
				.05	32.8	50	"
1515	$CH_3CH_2CH_2CH_2O$–	$-OCH_2CH_2CH_2CH_3$	5	.025	>35	60	"
				.01	30	50	"
1618	"	$-OCH_2CH_3$	4	.05	>35	100	"
				.025	25	20	"
1795	CH_3CH_2O–	$-OCH_2CH(CH_3)_2$	5	.05	>35	70	"
				.025	20	0	"
1814	$CH_3CH_2CH_2CH_2$–	$-CH_2CH_2CH_2CH_3$	>.5	.05	>35	100	"
1607	H_2N–	$-NH_2$	.05	.05	19.2	0	Ravenel
1566	$(CH_3)_2N$–	$-N(CH_3)_2$	.1	.1	21.5	0	"
1906	$CH_3CH_2CH_2CH_2O$–	"	.1	.025	>35	90	H37Rv
				.01	31	40	"
2211	$CH_3CH_2CH_2CH_2$–	$-OCH_2CH_2N(CH_2CH_3)_2$	>.1	.1	>35	90	"
				.05	>35	60	"

* The compounds were prepared by Drs. C. Huebner, J. Marsh, D. C. Schroeder and C. R. Scholz of our Chemical Research Division(2).

TABLE III. Effect of Delayed Therapy with Su 1795 on Mouse Tuberculosis.

Conc. of Su 1795 in diet, %	Treatment started	No. mice used	T50 (days)	% survivors
.5	At time of infection	10	>37	90
	3 days post-infection	10	>37	100
	6 " " "	9	>37	90
	9 " " "	10	>37	90
	12 " " "	10	>37	90
	15 " " "	9	>37	70
	18 " " "	10	19.5	30
.1	At time of infection	9	>37	80
	3 days post-infection	10	>37	90
	6 " " "	9	>37	100
	9 " " "	10	34	50
	12 " " "	10	24	40
	15 " " "	10	17	10
	18 " " "	8	17	0
Untreated controls	—	19	20.5	0

in vitro against the pathogenic mycobacteria and several species of actinomyces and fungi, but exhibited only insignificant activities against staphylococci, *E. coli, Shigella paradysenteriae,* Salmonella and other common bacteria, as was the case with the substances reported in the earlier study. However, this parallelism between anti-mycobacterial and antifungal action is not evident in all instances, and such irregularities are not unexpected. Substances commonly characterized as antibacterial, antifungal or as antiparasitical agents do not necessarily act upon all bacteria, fungi or parasites respectively but as a rule only affect certain species or more often, sub-species. It is probable that biochemical specificities, differences in permeability of the cell walls and many other factors play very important contributing roles. We feel that the observations reported in this paper support the theory that the botanical relationship between the mycobacteria and fungi is based not only upon superficial morphological characteristics but rather on profound biochemical re-

TABLE IV. Effect of Limited Therapy with Su 1795 on Mouse Tuberculosis.

Conc. of Su 1795 in diet, %	Total time of treatment, days	No. mice used	T50	% survivors
.1	3	10	27.5	30
	6	8	>44	70
	9	9	>44	80
	12	10	>44	100
	15	8	>44	80
.05	3	10	23.5	30
	6	10	33	50
	9	10	31	30
	12	9	>44	80
	15	10	>44	80
Untreated controls	—	19	29.0	10

lationships. As evident by this study, this theory has proven to be useful as a rational approach in the search for new antitubercular agents.

Certain disubstituted thioureas exhibited strong antitubercular activity in mice and guinea pigs. A number of compounds were highly active *in vitro* but inactive *in vivo*, while others had very low *in vitro* activity but considerable *in vivo* activity, suggesting that these latter compounds undergo a metabolic transformation into more active products. From these data it is obvious that within this class of compounds little relationship exists between *in vivo* and *in vitro* tuberculostatic activities.

The activities of certain substituted thioureas such as Su 1795 and Su 1906 in guinea pig experiments exceeded those of PAS or streptomycin, and approached that of Isoniazid.

Preliminary studies thus far have indicated that *in vitro* resistance develops slowly and that apparently no resistant forms of the tubercle bacillus arise in the course of animal

TABLE V. Summary of Mean Gross Organ Involvement in Guinea Pigs Treated with Four Thiourea Derivatives.

Compound	Approx.* MTD, %	% conc. in diet	No. of animals	Mean organ involvement: Spleen	Lungs	Liver	Site of inoculation	Total organ involvement
Su 1380	2.0	.05	12	5.8	5.0	6.7	5.8	23.3
		.2	13	4.6	2.3	5.4	1.5	13.8
Su 1795	1.0	.05	14	7.2	8.6	7.9	1.4	25.1
		.2	12	5.8	2.5	5.8	.8	14.9
Su 1814	>.2	.05	13	13.1	13.1	13.5	6.9	46.6
		.2	14	13.6	13.6	10.7	6.4	44.3
Su 1906	.1	.05	10	2.0	4.0	6.0	4.0	16.0
		.1	12	4.2	4.2	6.7	2.5	17.6
Isoniazid	.1	.05	13	3.1	3.1	3.1	2.3	11.6
		.1	13	1.5	1.5	2.3	.8	6.1
Tibione	.1	.05	12	9.2	10.8	12.9	5.0	37.9
		.1	12	2.5	7.5	9.2	5.0	24.2
Untreated controls	—	—	13	23.7	25.3	24.2	10.0	83.2
Pretreatment controls†		—	5	21.0	10.0	13.0	10.0	54.0

* The MTD is based on a 60-day feeding period. Values indicate percentage of drug in diet.
† These animals sacrificed on 21st day post-infection to ascertain degree of tuberculosis involvement at commencement of therapy.

TABLE VI. Influence of Delayed Therapy with Su 1795.

Compound	% conc. in diet	No. animals used	Avg values assigned to: Spleen	Lungs	Liver	Site of inoc.	Total score
Su 1795	.1	17	1.8	6.5	8.8	1.2	18.3
Isoniazid	.1	18	2.8	5.0	6.1	1.1	15.0
Untreated controls	—	17	27.4	21.2	24.1	10.0	82.7
Pretreatment controls	—	5	35.0	22.0	24.0	10.0	91.0

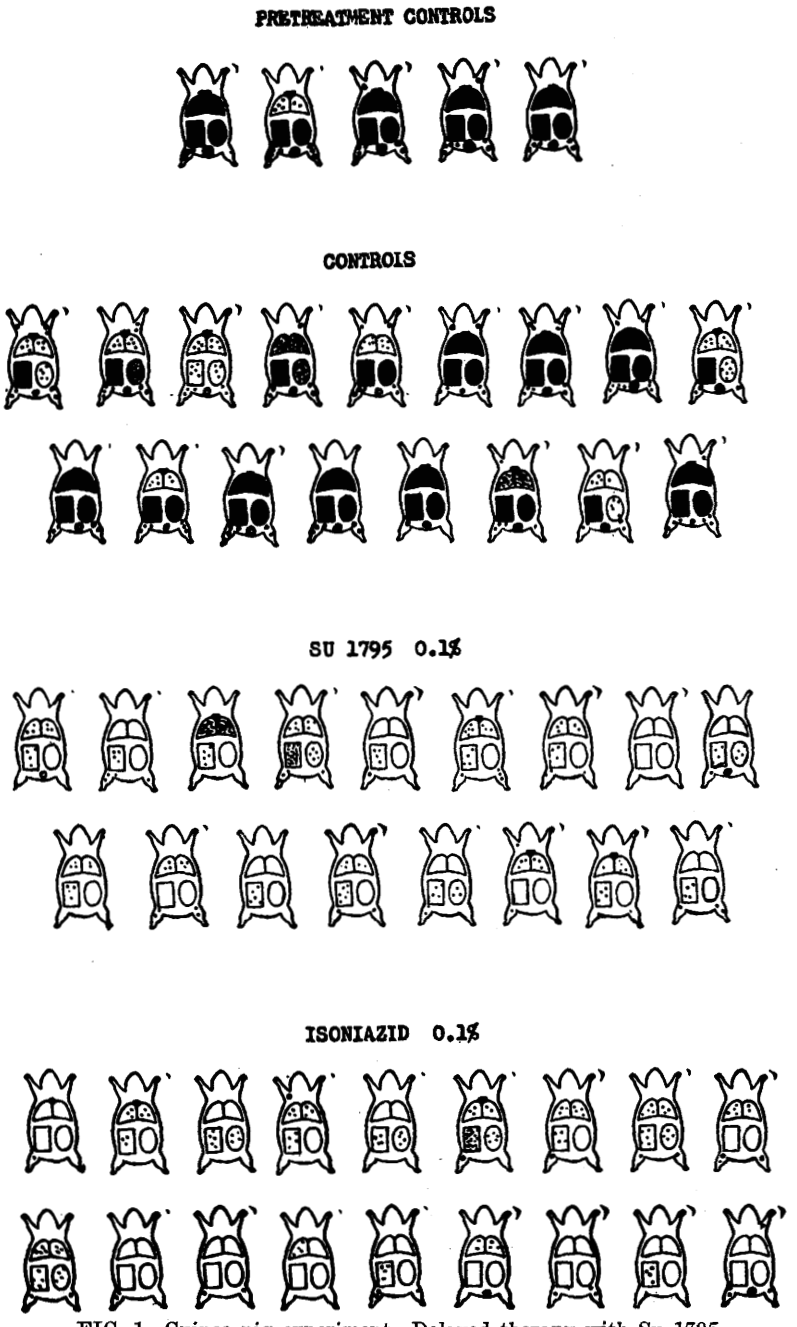

FIG. 1. Guinea pig experiment. Delayed therapy with Su 1795.

experiments. Cross-resistance studies have shown that infection of mice with a streptomycin-resistant strain of $H_{37}Rv$ yielded to the action of the thiocarbanilides, and Rimifon-resistant strains retained their sensitivity to the thiourea derivatives. The favorable results obtained in the delayed and limited therapy experiments together with the high therapeutic index suggest that certain of these compounds warrant consideration as therapeutic agents in the treatment of tuberculosis.

The mode of action of our compounds is still

unknown. With the collaboration of Dr. F. Kull and Miss M. Grimm of our laboratories, we have examined their influence upon various enzyme systems and found that their antituberculosis action is not based upon an antiphenoloxidase activity so pronounced with thiourea and certain monosubstituted thioureas nor due to an effect upon cytochrome C. A very high *in vitro* activity against different strains of torula, which contain as do the mycobacteria, a high percentage of lipids, may indicate a possible pathway for the tuberculostatic activity of these thioureas.

Summary. 1. Over 350 thioureas have been prepared in our laboratories and tested for antitubercular activity. 2. A number of disubstituted thioureas have demonstrated considerable protective and therapeutic activities in tuberculous mice and guinea pigs. 3. Little correlation existed between the *in vitro* and *in vivo* tuberculostatic action. 4. Many compounds possessed strong antifungal as well as antimycobacterial properties.

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Determination of Specific Gravity of Intact Animals by Helium Displacement; Comparison with Water Displacement.* (20242)

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Interest in the concept that the body is composed of a lean tissue mass of constant composition upon which is superimposed a variable amount of fat was stimulated by the work of Behnke(1). In normal men(2) and animals(3), the weight of fat-free tissue in the body as a whole or in individual tissues(4) is more closely correlated with the water and electrolyte content than is the weight of the total body or tissue. Since fat is relatively inert metabolically, the lean body mass may also be a more valuable referent for total metabolism than the surface area(5). Since the specific gravity of fat is about 0.92 and the specific gravity of the fat-free body is about 1.100(2), the proportion of body weight which is lean tissue and, conversely, the proportion which is fat, may be calculated from the whole body specific gravity. In man, specific gravity may be determined by underwater weighing, if correction is applied for the residual air in the lungs. This procedure is difficult to perform because it requires considerable training and cooperation on the part of the subject, and it is not feasible in incapacitated individuals. In intact animals this procedure is obviously impractical, and it is necessary to sacrifice and eviscerate animals in order to find their weight under water.

The method herein reported permits rapid determinations of specific gravity in intact, unanesthetized animals. Body volume is measured by gas displacement. Weight (g) divided by volume (cc) gives specific gravity, and no correction for residual air is needed.

Methods. Principle. A measured quantity of helium is added to a chamber containing the animal. After complete mixing of the gas with the air in the chamber and in the animal's lungs, a sample is obtained for analysis. Then, by the dilution principle: Volume of empty chamber $-\frac{\text{Vol. of gas added}}{\text{Final conc. of added gas}} =$ vol. of animal. Helium was selected as the referent

* The opinions expressed herein are those of the authors and should not be construed as necessarily representing the opinion of the naval service.