THE ANTITUBERCULOSIS AND ANTILEPROSY ACTIVITY OF DISUBSTITUTED THIOCARBANILIDES

IN PARTICULAR SU 1906[1]

R. L. MAYER, P. C. EISMAN
S. GEFTIC AND E. KONOPKA
Research Department, Ciba Pharmaceutical Products, Inc.
Summit, New Jersey

A. INTRODUCTION

Since the leprosy bacillus cannot, as yet, be cultivated, and since leprosy has not been transmitted to laboratory animals, there is only one certain approach in the search for new antileprosy compounds, namely, the testing of compounds known to possess antituberculosis activity, in leprosy patients.

In its relationships to its host, the Stefansky bacillus acts more like the tubercle bacillus than the Hansen bacillus; in fact, murine leprosy often more closely resembles murine tuberculosis than human leprosy. Consequently, the response of murine leprosy with respect to specific chemotherapeutic substances rather duplicates that of tuberculosis, and only to a lesser degree simulates that of human leprosy. Certain compounds with only slight antituberculosis activity, such as DDS or even chaulmoogra oil, are more useful in human leprosy than other compounds with excellent antituberculosis action, as for instance isoniazid or streptomycin. This difference is probably not due to intrinsic differences in the bacterial response to the various chemotherapeutic agents, but rather to differences in the rapidity with which bacterial resistance develops. In spite of these shortcomings, the search for new antileprosy compounds remains, at least for the present, confined to antituberculosis agents.

In 1941 one of us (RLM) ([17]) undertook a search for new antituberculosis agents on the basis of the following reasoning: The tubercle and leprosy bacilli are members of the order of Actinomycetales, and as such are closely related to the fungi in their structural and cultural characteristics. Regularly, or under certain growth conditions, *Mycobacterium tuberculosis* forms branched filaments, a characteristic associated with fungi. In addition, there are certain close similarities between the disease produced by the tubercle bacillus and those systemic diseases brought about by certain fungi, insofar as pathology and immunology response are concerned. It was thus deemed

[1] Read in condensed form at the VIIth International Congress of Leprology, held in Tokyo, Japan, November 12-19, 1958.

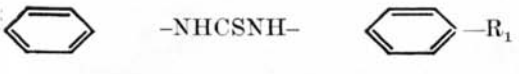
of interest to investigate whether a chemotherapeutic-response relationship would accompany this taxonomical and host-parasite relationship between tubercle bacilli and the fungi. We have consequently searched for new antituberculosis compounds among the antifungus agents, and found indeed that various such substances did possess strong tuberculostatic and tuberculocidal properties. Among the antifungus agents with antituberculosis activity, the thiourea group was particularly interesting.

While *monosubstituted* thioureas usually possessed only *in vitro* tuberculostatic activity and were inactive *in vivo,* at least one of them, sulfamidothiourea (Fontamide), displayed definite chemotherapeutic activity in human tuberculosis ([20, 7]). Lately, in a group of new thioureas, we have found more potent antituberculosis agents, namely, *disubstituted* thioureas, particularly the thiocarbanilides ([18, 12, 13, 15, 16, 19]). More recently, other members of this chemical group have been described by Buu Hoï *et al.* ([2, 3, 4, 5, 6, 22]), Doub *et al.* ([11]), and Youmans *et al* ([13]).

Obviously, all of these antituberculosis compounds were of interest, *a priori,* as possible therapeutic agents for leprosy, and we have tested numerous representatives of them in murine leprosy. In the meantime, good clinical results in human leprosy with certain thioureas have been reported by Buu Hoï ([3, 4]), Davey ([9, 10]), and Ross Innes ([21]).

Here we report on our results in experimental tuberculosis and murine leprosy with certain selected disubstituted thiocarbanilides, which were particularly promising representatives of this group of new chemotherapeutic substances. All of these active thioureas are

TABLE 1.—*General structure of the thioureas (disubstituted thiocarbanilides) discussed, and examples of substitution conferring good* in vivo *antituberculosis activities in mice and guinea-pigs.*



Compound	R	R_1
Su 1380	$-OCH_2CH_3$	$-OCH_2CH_3$
Su 1748	$-OiC_5H_{11}$	$-OiC_5H_{11}$
Su 1795	$-OCH_2CH_3$	$-OCH_2CH(CH_3)_2$
Su 1814	$-(CH_2)_3CH_3$	$-(CH_2)_3CH_3$
Su 1906 [a]	$-O(CH_2)_3CH_3$	$-N(CH_3)_2$
Su 2358	$-OiC_4H_9$	$-O(CH_2)_2N(C_2H_5)_2$

[a] The trade name of this compound is CIBA 1906.

thiocarbanilides with the general structure shown in connection with Table 1. Among several hundreds of these compounds tested, many showed activity *in vitro* and/or *in vivo*, depending upon the substitution in the R and R_1 positions. The compounds listed in Table 1 are examples of various substitutions leading to compounds of good *in vivo* activities.

B. ACTION OF THIOCARBANILIDES IN TUBERCULOSIS

in vitro ACTIVITIES

The *in vitro* antituberculosis activities of 307 of these compounds were evaluated with the virulent *M. tuberculosis* var. *hominis*, H37Rv, grown in a modified Kirchner's liquid medium containing 0.03 per cent Tween and 0.5 per cent serum albumin. The *in vitro* activities of the different compounds varied over a wide range. The minimal inhibitory concentration observed was 1 mcm. per cc. (10^{-6}), 27 of them being of this class, while many were tuberculostatic only in concentrations of 50 to 125 mcm./cc. (5×10^{-5} to 1.25×10^{-4}); others had still lower activity.[2]

There was a close parallelism between the antimycobacterial activity of the thiocarbanilides and their activity against actinomyces and fungi, while their antibacterial activity, on the other hand, was almost without exception very low ([8, 17]).

In vitro and *in vivo* activity were closely related: All 27 compounds that were active *in vitro* in the 1 mcm./cc. concentration were also active *in vivo*. Of all that were tuberculostatic in concentrations as low as 2 to 5 mcm./cc., 75 per cent were active in mice. On the other hand, a considerable number of compounds with very low *in vitro* activity, inhibiting growth of the tubercle bacillus only in concentrations of over 100 mcm./cc., showed significant *in vivo* activity. Similar observations have since been reported by Doub *et al.* ([11]).

in vivo EXPERIMENTS WITH MICE

Mice were inoculated intravenously with a culture of the H37Rv tubercle bacillus so standardized that 50 per cent of the control mice (T50) died of infection on approximately the 20th day. Immediately upon inoculation, the animals received food containing the finely pulverized test compound in concentrations varying from 3 to 0.005 per cent. Drug treatment was maintained for 21 days, at which time the normal diet was restored for an additional 15 days. On the 36th day after inoculation all of the surviving mice were sacrificed. The evaluation of activity was based upon: (1) the number of mice surviving on

[2] The table supplied to demonstrate these data, as well as certain other tables and certain text-figures, have had to be omitted, for consideration of space.—EDITOR.

the 36th day; (2) the calculated T50 values,[3] and (3) the gross pathology of the lungs and speen of all mice succumbing to the infection or sacrificed at the end of the experiment.

TABLE 2.—In vivo *(mouse) activities of four representative thioureas; extension of T50 values (over controls) and percentages of surviving mice on the 36th day.*[a]

Drug in diet (%)	Su 1380		Su 1795		Su 1906		Su 2358		PAS	
	T 50 (days)	Surv. (%)	T 50 (days)	Surv. (%)	T 50 (days)	Surv. (%)	T 50 (days)	Surv. (%)	T 50 (days)	Surv. (%)
0.1	>15	80	>15	100	>15	100			5	20
0.05	4	20	>15	80	>15	100	>15	100	0	0
0.025	0	0	1.5	0	>15	100	>15	90		
0.01					>15	60	0	0		
Controls	0	0								

[a] The abbreviation "surv." = survival.

In Table 2 are recorded the percentage survival rates and the T_{50} values of groups of tuberculous mice treated with representative thioureas at varying concentrations in the diet, with PAS serving as a reference standard. These results, which represent an average of four experiments, indicate that the antituberculosis activity of Su 1906 in mice was approximately 5 times that of Su 1795 and 10 times that of Su 1380 (Figs. 1 and 2). PAS was considerably less active, as indicated in Table 2. The results of a more detailed comparison of Su 1906 and PAS, not included here, showed that Su 1906 is approximately 50 times more active than PAS in tuberculosis mice.

Activity and chemical constitution. — We have tested a great variety of substituents in the R and R_1 positions, but it is not possible to indicate here the different correlations which exist between chemical constitution and antituberculosis activity. Restricting ourselves to only one example, we found among many substitutions with phenol ether that maximum activity was reached when R and R_1 had 3 to 5 carbon atoms each, whereas above and below that number activity was completely abolished. Detailed indications are found in reports by Mayer *et al.* ([18, 19]), Eisman *et al.* ([12]), and Doub *et al.* (11).

[3] This latter value represents the calculated survival time, in days, of 50 per cent of the mice, as obtained by plotting the cumulative percentage dead on a probability scale against time on an arithmetic scale.

Limited and delayed therapy. — The comparative activities of the various compounds were further assessed in limited-therapy and delayed-therapy experiments, the drugs as usual given in the diet.

In the limited therapy experiments the mice were given medicated food immediately after inoculation, but the treatment was maintained for only 24 hours, or 2, 3 or 4 days, instead of the usual 15 days. After withdrawal of the medicated food, the animals were kept on their normal diet until they were sacrificed on the 36th day.

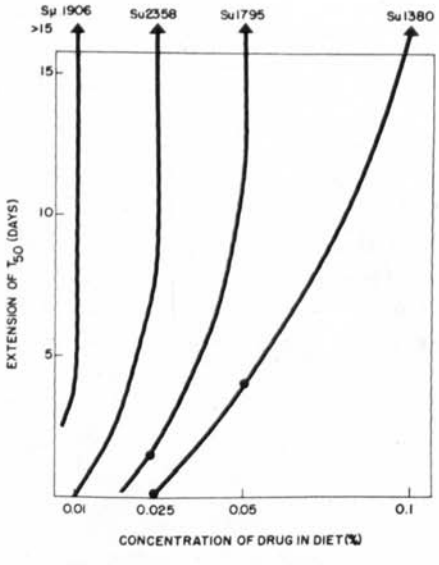


FIG. 1. Mouse tuberculosis. Treatment with thioureas in the diet; extension of T_{50} over that of the controls.

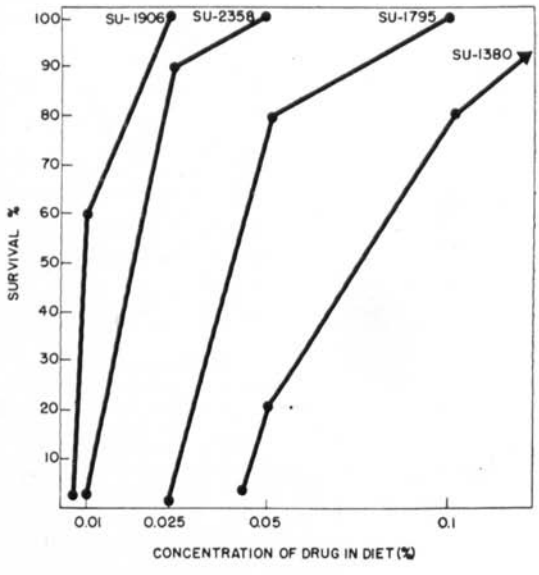


FIG. 2. Survivals of treated tuberculous mice (percentages) in relation to the dosage of thioureas.

In the delayed therapy experiments, mice were kept on their normal unmedicated diet for periods of 9, 12 or 15 days after inoculation, at which time treatment with medicated food was begun and was maintained for 15 consecutive days. The animals were then returned to the nonmedicated diet for the remainder of the experiment.

Results of limited therapy: Given for only one day, both Su 1906 and isoniazid exhibited a marked degree of activity, in that the T_{50} values exceeded that of the controls by six days, with 50 per cent of the animals surviving. Treatment for an additional day resulted in the maximum activity recorded in this type of experiment, i.e., more than 15 days' survival beyond the controls. This therapeutic effect was obtained with amounts of Su 1906 and isoniazid totaling only 2.5 mgm.

Results of the delayed therapy: Even when there was a lapse of 15 days between the time of inoculation and commencement of therapy, treatment with medicated food containing 0.1 per cent Su 1906 or 0.05 per cent isoniazid resulted in a high degree of protection, as shown by data not reproduced here.

in vivo EXPERIMENTS WITH GUINEA-PIGS

Female guinea-pigs weighing 500-600 gm. were inoculated subcutaneously with 1.0 cc. of a 1:100 saline dilution of an H37Rv culture grown for seven days in a modified Kirchner medium. Treatment was started 23 days after inoculation in all animals which presented, at that time, strongly positive tuberculin reactions. As in the mouse experiments, the test substances were administered mixed with a finely-ground guinea-pig food. The animals were treated for 60 days, and the animals which survived at the end of that period were sacrificed. The gross organ involvement was determined according to the procedure described by Karlson and Feldman ([14]), in which numerical values of 40, 70 and 100, respectively, correspond to the more general evaluation as slightly, moderately and extensively diseased.

The composite results obtained in chemotherapy experiments in which tuberculous guinea-pigs were treated with three thiocarbanilides found active in mice are summarized in Table 3. The values listed are based on a gross evaluation of the lungs (maximum titer 25, according to Karlson and Feldman) and the total of all organs inspected (lungs, spleen, site of infection, maximum titer 100), are shown in Fig. 3.

TABLE 3.—*Activities of three representative thioureas in guinea-pig tuberculosis; treatment 60 days, duration of experiment 82 days. Average score of microscopic involvement of lungs, and total score (sum of spleen, lungs, liver and inoculation site).*

Drug in diet	Su 1795		Su 1906		Su 2358		Isoniazid	
(%)	Lungs	Total	Lungs	Total	Lungs	Total	Lungs	Total
0.2	2.5	15.0						
0.1			4.2	17.5			1.5	6.2
0.05	10.0	22.0	5.0	23.0	8.5	26	4.4	18.8
0.01	2.0	32.0	7.6	26.9	12.3	50.8	7.3	27
Untreated controls	25	83.5						

All of the compounds tested demonstrated a considerable degree of chemotherapeutic activity. With a daily intake of approximately 2.5 mgm. Su 1906, corresponding to a diet concentration of 0.1 per cent, the lung involvement was only one-fifth as severe as in the controls, and total organ involvement (lungs + spleen + liver + site of infection) was approximately one-third of that of the controls. As with mice, Su 1906 demonstrated a definite superiority over the other thiocarbanilides tested.

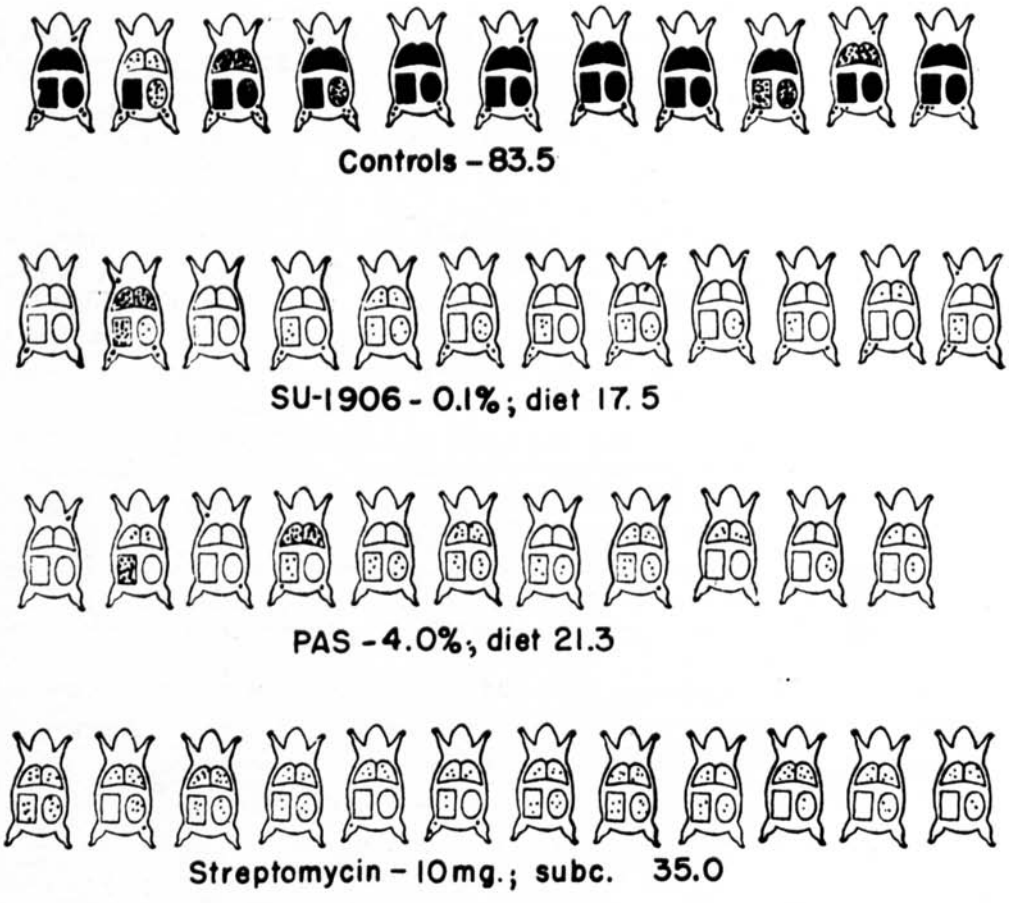


FIG. 3. Guinea-pig tuberculosis, experimental infection with strain H37Rv. Comparison of the effects of treatment with Su 1906, PAS, and streptomycin at M.T.D. dosage as indicated. The numerical values of the gross organ involvement are 83.5, 17.5, 21.3, and 35.0, respectively.

The activity of Su 1906 in guinea-pigs as established by gross pathology is comparable to that of isoniazid. As in all the experiments with mice, Su 1906 was considerably superior to PAS; only at a dose 400 times greater did PAS yield comparable results.

Therapeutic effect on severely infected animals.—In the usual chemotherapy experiment with mice, treatment is instituted immediately after infection, and the experimental conditions intentionally favor the drug; any therapeutic effect indicates *suppressive,* or prophylactic activity. In the usual guinea-pig experiment, on the other hand, treatment is initiated 22 days after inoculation, when the positive tuberculin reaction proves the presence of a well-established infection. Here, actual *chemotherapeutic* effects are established.

We have examined the activity of Su 1906 in guinea-pigs with considerably more advanced tuberculous infection. The animals were infected as usual but left untreated for two months, at which time they presented almost maximal tuberculous involvement of lungs and spleen, established by autopsies of "pretreatment controls." Therapy was maintained for 100 consecutive days.

Results: From the results presented in Table 4 it is evident that, in spite of the 2-months delay in starting treatment, Su 1906 in a daily dose of approximately 25 mgm. exercised a considerable degree of antituberculosis effect. INH, under the same conditions, was more active.

TABLE 4.—*Influence of delayed therapy (delayed 60 days) with Su 1906 on guinea-pig tuberculosis.*

Compound	Drug in diet (%)	Index of average involvement				Total involvement (index)
		Spleen	Lungs	Liver	Site of inoculation	
Su 1906	0.1	10.3	12.2	9.0	3.9	35.4
Isoniazid	0.1	2.8	5.0	6.1	1.1	15.0
Controls						
Pre-treatment	—	35	22	24	10	91
Post-treatment	—	27	21	24	10	83

Persistence of therapeutic effects.—None of our present antituberculosis drugs has achieved, as yet, complete sterilization of tuberculous animals. Recurrences are regularly observed even after thorough treatment with the most active compounds, such as isoniazid. We have not achieved sterilization—nor did we expect to—with Su 1906. It was, however, of interest to investigate the duration of clinical improvement after cessation of therapy with Su 1906 as another indicator of therapeutic potency and usefulness.

Groups of tuberculous guinea-pigs were treated with Su 1906 or INH, 0.1 per cent in the diet, for three different periods of time, 3, 6 and 9 months, and the degree of tuberculosis at the end of each treatment period was determined in a number of the animals. The remaining ones of each group were then maintained on a normal diet for a period of time equal to the respective treatment period: 3 months for the animals treated for 3 months, 6 months for those treated for 6 months,

and so on. The longest total observation period was 18 months. The extent of tuberculosis in the guinea-pigs surviving at the end of each total period was then compared with the degree of tuberculosis that had been found in the animals killed at the end of the treatment periods.

Results: The results, illustrated in Fig. 4, revealed that Su 1906 as given exerted a high curative effect when administered for 3 or 6 consecutive months. The additional 3 months' treatment of the 9-months group did not increase the therapeutic effect further. During

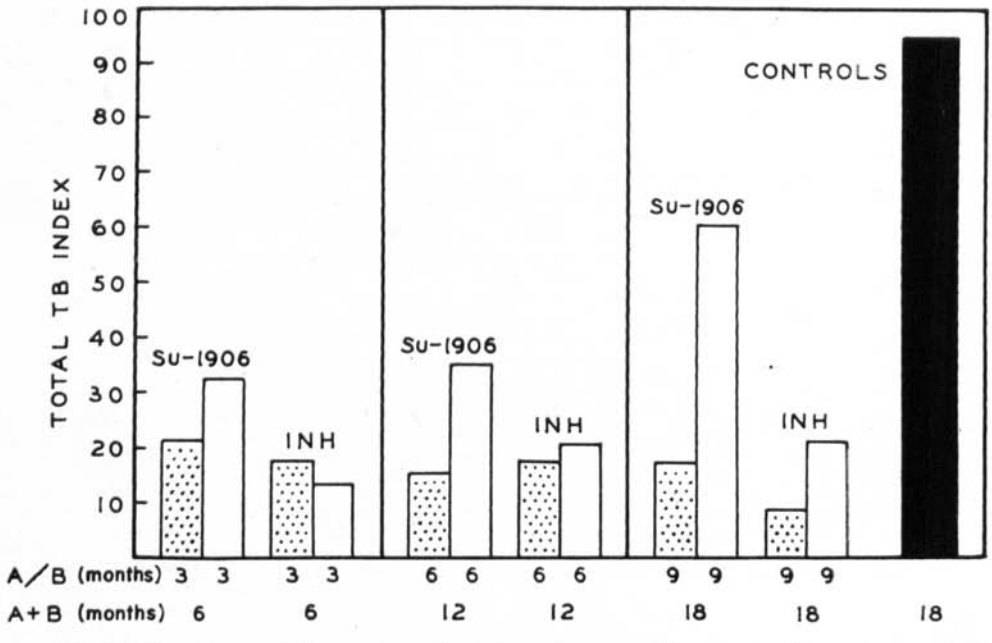


FIG. 4. Persistence of therapeutic effects in guinea-pig tuberculosis after various periods of treatment and equal periods after cessation of treatment. A = the time of treatment, B = the period after treatment before examination, and A + B = the total period.

the drug-free observation periods of 3 to 9 months, the infection progressed in all instances, although only slowly, and even after 9 months it had not reached the degree of infection observed in the untreated controls. In the same manner, the tuberculous infection increased regularly after therapy with isoniazid was interrupted, but this drug was definitely superior to Su 1906.

OTHER OBSERVATIONS WITH SU 1906

The data presented indicate that the antituberculosis activity of Su 1906 in experimental tuberculosis ranges between that of PAS and INH. Consequently, it can be expected that its place in the treatment of clinical tuberculosis will be similar to that of PAS, preferentially in combination with some other chemotherapeutic agent with a different

mechanism of action. We have therefore investigated whether the administration of Su 1906 with one of the established antituberculosis substances will result in an additive therapeutic effect.

Enhancement of activity by combination with isoniazid or streptomycin.—In the usual guinea-pig experiment, Su 1906 was administered in combination with either isoniazid or streptomycin, beginning 22 days after inoculation, when the tuberculin reaction had become positive. Su 1906 and isoniazid were mixed in the diet, while streptomycin was given by subcutaneous injection.

The results of this experiment, shown in Table 5 and Fig. 5, indicate that the combination of suboptimal doses of Su 1906 with suboptimal doses of either isoniazid or streptomycin resulted in a considerable enhancement of the therapeutic effect.

TABLE 5.—*Enhancement of therapeutic activities by combination of Su 1906 with isoniazid or streptomycin.*

Treatment	Dosages [a]		Tuberculosis indices [b]	
	I	II	I	II
Su 1906 alone	0.005%	0.01%	81.9	77.8
Isoniazid alone	0.005%	0.005%	52.3	52.3
Combination of both	(as above)	(as above)	32.6	23.6
Su 1906 alone	0.01%		77.8	
Streptomycin alone	1.0 mgm. (subcut.)		56.6	
Combination of both	(as above)		36.5	
Untreated controls			79.3	

[a] In food, except for streptomycin, which was administered subcutaneously.

[b] Total gross organ involvement.

Bacterial resistance to Su 1906.—A study conducted in our laboratory ([15]) revealed that the virulent tubercle bacillus H37Rv develops resistance to thiocarbanilides slowly, as compared with the rapidity with which resistance against streptomycin or isoniazid emerges especially the former. A maximal 16-fold increase in resistance to Su 1906 was attained after 6 transfers, and this level of resistance remained unchanged during 10 subsequent transfers. In the same experiment, resistance to streptomycin of more than 1,500-fold developed after only 6 transfers, and a 3,500-fold resistance to isoniazid was obtained after only 5 transfers.

FIG. 5. Showing the enhancing effects of combining a suboptimal dose of Su 1906 with a suboptimal dose of streptomycin or of isoniazid. The gross organ involvement of a sample of the animals examined before treatment was 61.0, and of the controls at the end of the experiment was 80.0.

Delay in the emergence of resistance by combinations.—Combination therapy serves several purposes. In addition to increasing effectiveness, while at the same time reducing the toxic effects of the components, it is used to retard the emergence of resistance. In order to investigate the influence of Su 1906 upon the resistance of *M. tuberculosis* H37Rv to isoniazid and streptomycin, serial transfers were made in media containing those substances, with and without a very small amount of Su 1906. As seen from the results shown in Table 6, the presence of only 2 mcg./cc. of Su 1906 in the medium appreciably delayed the development of resistance to streptomycin and almost completely suppressed it in the case of isoniazid.

TABLE 6.—*Influence of Su 1906 (2 mcg./cc.) on the emergence of resistance to streptomycin or isoniazid.*

Serial transfer No.	Minimal inhibiting concentrations				
	Su 1906 (mcg./cc.)	Streptomycin (mcg./cc.)	Su 1906 and streptomycin combined	Isoniazid (mcg./cc.)	Su 1906 and isoniazid combined
1	3.9	1.0	0.5	0.016	0.031
2	—	—	—	0.063	0.031
3	3.9	3.9	0.3	0.13	0.063
4	—	—	—	1.0	0.063
5	15.6	7.8	0.3	7.8	0.25
6	—	—	—	30.0	0.25
7	15.6	31.2	1.0	>125.	1.0
10	15.6	62.4	2.0	—	—
13	15.6	—	31.2	—	—

Cross resistance between thiocarbanilides and thiosemicarbazones.—Cross resistance exists between the thiocarbanilides and thiosemicarbazones, indicating that both classes of compounds probably act in similar ways. It is a two-way type of resistance, regardless of which compound was used to produce the initial resistance. Strains resistant to other known antituberculosis agents are sensitive to the thiocarbanilides.

ACTION ON PHOTOCHROMOGENIC MYCOBACTERIA

Clinical reports indicate that the incidence of tuberculosis-like infections due to the so-called atypical, photochromogenic mycobacteria is increasing. Most strains of these chromogens as reported are resistant to PAS, and in many instances also to streptomycin. We have therefore evaluated the activity of selected thiocarbanilides against 9 dif-

ferent strains, pathogenic as well as nonpathogenic to mice, all isolated from human tuberculous infections ([19]). In the following account, we shall restrict our discussion to the results obtained with 6 of the pathogens tested.

(*a*) *In vitro* activity against photochromogens: All of the 6 chromogens referred to were sensitive to Su 1906 *in vitro*, but they were resistant to PAS and streptomycin to a considerable degree. Two of them were sensitive to INH, and the other 4 were less resistant to it than to PAS or SM. These chromogens represent a most heterogenous group of mycobacteria. Derived from human tuberculosis, they had undoubtedly been subjected to intensive chemotherapy, and it is therefore not known to what extent their present sensitivities are intrinsic or acquired. That they are regularly sensitive to the thiocarbanilide Su 1906 may well be due to the fact that they have never had previous contact with it, and may well reflect an intrinsic sensitivity to this compound, toward which resistance develops only slowly and to only a limited extent.

(*b*) *In vivo* activity against photochromogens: Groups of mice were infected, each with one of five strains of photochromogenic mycobacteria: P_1, P_4, P_{18}, P_{21}, P_{26}, and treated immediately after inoculation as in the experiment with H37Rv. The antituberculosis agents used were Su 1906 (0.1% in the diet), isoniazid (0.1% in the diet), PAS (1.0% in the diet), and streptomycin (3.0 mgm. subcutaneous). The treatment was continued for 30 consecutive days, after which the results were evaluated. It turned out that, compared with the other drugs used, Su 1906 was superior in the two groups of mice infected with the P_1 and P_{21} strains, slightly less effective than the best (INH) in the group infected with strain P_{26}, and inferior but second to INH in the two groups infected with strains P_4 and P_{18}. The order of *in vivo* activities was as follows:

Strain P_1	Su 1906 > INH > SM > PAS
Strain P_4	INH > Su 1906 > SM > PAS
Strain P_{18}	INH > Su 1906 = SM > PAS
Strain P_{21}	Su 1906 > INH > SM > PAS
Strain P_{26}	INH > Su 1906 > SM > PAS

There was no direct relationship between *in vitro* and *in vivo* activity. PAS was virtually inactive against all 5 strains.

C. ACTION OF THIOCARBANILIDES IN MURINE LEPROSY

In spite of the deficiencies noted at the outset of this report, the only chemotherapeutic laboratory test object for antileprosy compounds is murine leprosy, and we have used it. Previously, Chang ([8]) had tested three of our more active antituberculosis thiocarbanilides

(Su 1906, Su 1795, Su 2358) in mouse leprosy, and obtained negative results. In a number of our own experiments, however, Su 1906 exhibited definite activity. Such discrepancies in results obtained by different observers using the same compound are not uncommon in chemotherapy experiments involving either mouse or rat leprosy; positive as well as negative results have been reported with such compounds as sulfones, thiosemicarbazones and INH ([1]).

Our tests in murine leprosy have been made by two different methods. (1) One was the usual procedure, in which infected animals are treated for a prescribed period of time with the test substances usually administered in medicated diet. (2) The other method was one which we can conveniently designate as a neutralization type of test. In this procedure fresh rat leproma was homogenized with a given concentration of the test substance and incubated at 37°C for 24 hours. After incubation, 0.5 cc. aliquots of the mixture were injected subcutaneously into female CF1 mice, and the animals were observed without further treatment for 10 weeks. When rats were used, the observation period was 18 weeks. This second procedure was adopted after systematic investigation of the various conditions (temperature, length of incubation, and most suitable receptive animal), and a statistical evaluation of the data was obtained. In both types of test the excised lepromatous mass served as an index of the relative effectiveness of the drugs.

Treatment with medicated diet.—The results of a number of experiments, in all of which DDS and INH were used as reference substances, are given in Table 7. In three of four experiments in which Su 1906 was used, it exerted definite activity, which was about one-half of that of isoniazid or DDS.

Neutralization test.—In two of three tests of this type, Su 1906 showed complete lack of activity, as did DDS in the two tests in which it was used, whereas isoniazid displayed good activity in both tests performed.

Further experiments are necessary to establish the significance of the negative results obtained with Su 1906 and DDS in this type of experiment. They may well indicate that the activities of Su 1906 and DDS are not due to a direct action of the unmetabolized compound; rather, they may depend on metabolites formed within the intact body but not in isolated lepromatous tissue. This is apparently the case also with respect to antituberculosis activity.

COMMENTS ON THE ANTILEPROSY ACTIVITY OF SU 1906

Su 1906 has considerable activity in murine tuberculosis, but it is only moderately active in murine leprosy. Moreover, in murine leprosy its activity is irregular. In one of our experiments reported in Table 7, its suppressive effect was comparable to that of DDS; in two other

experiments it was approximately one-half to one-third as effective as DDS; and in a fourth experiment it showed no significant activity. Similar observations have been made with other thioureas. Chang reported only negative results in mouse leprosy with three different thioureas. This is in contrast to the consistently good results obtained by Davey and Ross Innes in treating human leprosy with Su 1906.

TABLE 7.—*Activity against murine leprosy of Su 1906 in mice or rats; medicated diet administered for 6 weeks.*

Compound	Dose in diet (%)	Number of animals used	Leproma inhibition (per cent)
Su 1906	0.1	14 mice	15
	0.1	9 mice	58
	0.1	7 mice	28
	0.1	5 rats	25
			average 29
DDS	0.05	5 mice	73
	0.05	9 mice	56
	0.05	5 rats	63
			average 64
Isoniazid	0.1	10 mice	84
	0.1	10 mice	89
	0.1	5 rats	98
			average 90
Controls		10 mice	0
		10 mice	0
		5 rats	0
			average 0

Differences in activity against these various infections are also exhibited by DDS, which has excellent activity in murine leprosy, and is as effective as Su 1906 in human leprosy, but has only a very slight effect upon experimental or clinical tuberculosis. Isoniazid, in turn, although highly active in experimental and clinical tuberculosis and murine leprosy, has an inadequate therapeutic action in clinical leprosy. Thus, the results of the present study with Su 1906 confirm the general observation that the correlations between the sensitivities of *M. tuberculosis* and *M. leprae murium* (and, apparently, *M. leprae* also) to the various active chemotherapeutic agents are qualitative rather than quantitative.

This fact is not necessarily attributable in all instances to intrinsic differences in the susceptibility of the various species of mycobacteria. It seems quite probable that, with isoniazid and streptomycin, rapidly-developing resistance of the human leprosy bacillus nullifies the initially good activity. Mycobacterial resistance to Su 1906, however, is acquired very slowly and to a considerably less degree than is the case with isoniazid and streptomycin. In this respect Su 1906 obviously occupies a favored position, because even after prolonged treatment and repeated therapeutic cycles the infective agents remain sensitive to the drug.

SUMMARY

On the basis of the taxonomic relationship between mycobacteria and fungi, numerous thioureas known to be potent fungicides were tested for their activity against mycobacteria. Many disubstituted representatives of this group of drugs, among them especially certain thiocarbanilides, demonstrated excellent *in vitro* and *in vivo* activities against a virulent strain of *M. tuberculosis,* and Su 1906 showed moderate activity against murine leprosy.

Activity of the thiocarbanilides depends on the nature of the substituents in the R and R_1 positions. In the example given, those with 3 to 5 carbon atoms possessed most activity. Results of treatment of experimental tuberculosis in mice and guinea-pigs under various conditions are reported. Combinations of suboptimal doses of Su 1906 with suboptimal doses of isoniazid or of streptomycin resulted in considerable enhancement of activity. The tubercle bacillus developed, slowly, only a low grade of resistance to Su 1906, and when the latter was used in combination the rapid and high resistance to streptomycin was appreciably delayed, and that to isoniazid was almost completely suppressed. Su 1906 has been found to have activity against the photochromic "atypical" mycobacteria in some cases more than INH or streptomycin. Not a great deal of stress is laid on the results of treatment of murine leprosy, because that is not a good test object for assessing activity of drugs for human leprosy.

Clinical reports on the action of certain thiocarbanilides in human leprosy are encouraging. However, the chemotherapeutic assessment of new drugs, particularly in diseases like tuberculosis and leprosy, requires prolonged observation in all types of the infection. It is not only the immediate effectiveness of a new drug, or its lack of toxicity, or the paucity of untoward side effects, which make its introduction worthwhile. Factors such as persistence of beneficial effects, absence of rapidly-acquired resistance, and compatibility with other types of therapy are of equal importance. For the benefit of those who are in need of this therapy, it is to be hoped that future experience will prove that all the above conditions will be fulfilled by this new thiocarbanilide class of antituberculosis and antileprosy compounds.

RESUMEN

A base de la relación taxonómica entre las micobacterias y los hongos, se comprobaron numerosas tioureas conocidas como fungicidas potentes en cuanto a su actividad contra las micobacterias. Muchos representantes bisubstituídos de este grupo de drogas, entre ellos en particular ciertas tiocarbanilidas, desplegaron excelentes actividades *in vitro* e *in vivo* contra una cepa virulenta del *M. tuberculosis* y el Su 1906 reveló moderada actividad contra la lepra murina.

La actividad de las tiocarbanilidas depende de la naturaleza de los substituyentes en las posiciones R y R_1. En el ejemplo ofrecido, las que tenían de 3 a 5 átomos de carbono poseían la mayor actividad. Preséntanse los resultados del tratamiento de la tuberculosis experimental en ratones y cobayos en varias condiciones. Las combinaciones de dosis subóptimas del Su 1906 con dosis subóptimas de isoniacida o de estreptomicina dieron por resultado considerable aumento de la actividad. El bacilo tuberculoso manifestó, lentamente, sólo tenor bajo de resistencia al Su 1906, y cuando se usó el último en combinación, se demoró apreciablemente la rápida y alta resistencia a la estreptomicina y se suprimió casi absolutamente la manifestada a la isoniacida. Se observó que el Su 1906 poseía actividad contra las micobacterias "atípicas" fotocrómicas en algunos casos, superior a la de la INH o la estreptomicina. No se hace mucho hincapié en los resultados del tratamiento de la lepra murina, por no constituir ésta buen material de ensayo para justipreciar la actividad de las drogas en la lepra humana.

Las comunicaciones clínicas acerca de la acción de ciertas tiocarbanilidas en la lepra humana son alentadoras. Sin embargo, la valoración quimioterapéutica de nuevos medicamentos, en particular en dolencias tales como la tuberculosis y la lepra, requiere observación prolongada en todas las formas de la infección. No es sólo la eficacia inmediata de una droga nueva, o su atoxicidad, o la escasez de adversos efectos colaterales, lo que presta valor a la introducción de la misma. Revisten igual importancia factores tales como la persistencia de los efectos beneficiosos, la falta de resistencia adquirida rápidamente y la compatibilidad con otras formas de terrapéutica. Para bien de los que necesitan esta terapéutica, es de esperar que las futuras observaciones demuestren que cumplirá todas las condiciones anteriores esta nueva clase tiocarbanilídica de compuestos antituberculosos y antileprosos.

REFERENCES

1. Bushby, S. R. M. The chemotherapy of leprosy. Pharmacol. Rev. **10** (1958) 1-42.
2. Buu Hoï, N. P. The selection of drugs for chemotherapy research in leprosy. Internat. J. Leprosy **22** (1954) 16-21.
3. Buu Hoï, N. P., Ba-Khuyen, N. and Dat-Xuong, N. Six mois de chimothérapie antilépreuse au Sud-Vietnam avec le 4,4′-diaminodiphénylsulfoxyde et le 4,4′-diéthoxythiocarbanilide. Bull. Acad. nat Méd. **139** (1955) 275-280.
4. Buu Hoï, N. P., Ba-Khuyen, N. and Dat-Xuong, N. Chimiothérapie antilépreuse au Sud-Vietnam avec les thiocarbanilides seuls ou associés au 4,4′-diaminodiphénylsulfoxyde, et avec une thiosemicarbazone. Bull. Acad. nat. Méd. **141** (1957) 204-210.
5. Buu Hoï, N. P. and Dat-Xuong, N. Sur les composées tuberculostatiques du groupe de la thiourée et leur mecanisme d'action. Compt. rend. Acad. Sci. **237** (1953) 498-500.
6. Buu Hoï, N. P., Dat-Xuong, Nam, N. H., Cazave, J. M., Pillot, J., and Schembri, L. Activité tuberculostatique de nouvelles NN'diarylthiourées. Experientia 11 (1955) 97-98.

7. Celice, J. and Boudreaux, J. La sulfamido-thiourée. Gaz. Méd. France **53** (1946) 113-137.
8. Chang, Y. T. Chemotherapy of murine leprosy. IV. The effects of amithiazone (TB/698), *p*-aminosalicyclic acid (PAS), B283 (a phenazine pigment), five antibiotics and three diphenylthiourea compounds on mouse leprosy. Internat. J. Leprosy **23** (1955) 167-180.
9. Davey, T. F. The treatment of leprosy with diphenyl thiourea compound SU 1906 (DPT). A report on expanded trials in Nigeria. 1. A second report on the progress of the pilot trial with a review of the findings in expanded trials. Leprosy Rev. **29** (1958) 25-36.
10. Davey, T. F. and Currie, G. Clinical trial of diphenyl thiourea compound SU 1906 (Ciba 15095E) in the treatment of leprosy. Progress during the first year. Leprosy Rev. **27** (1956) 94-111.
11. Doub, L., Richardson, L. M., Herbst, D. R., Black, M. L., Stevenson, O. L., Bambas, L. L., Youmans, G. P. and Youmans, A. S. Some phenyl thiourea derivatives and their antituberculous activity. J. American Chem. Soc. **80** (1958) 2205-2217.
12. Eisman, P. C., Konopka, E. A. and Mayer, R. L. Antituberculous activity of substituted thioureas. II. Activity in mice. American Rev. Tuberc. **70** (1954) 121-129.
13. Huebner, C. F., Marsh, J. L., Mizzoni, R. H., Mull, R. P., Schroeder, D. C., Troxell, H. A. and Scholz, C. R. A new class of antitubercular compounds. J. American Chem. Soc. **75** (1953) 2274-2275.
14. Karlson, A. G. and Feldman, W. H. Use of guinea pigs in studying chemotherapy of experimental tuberculosis. Ann. New York Acad. Sci. **52** (1949) 637-645.
15. Konopka, E. A., Eisman, P. C., Mayer, R. L., Parker, Jr., F. and Robbins, S. L. Antituberculous activity of substituted thioureas. III. Activity in guinea pigs. American Rev. Tuberc. **70** (1954) 130-138.
16. Konopka, E. A., Gisi, T., Eisman, P. C. and Mayer, R. L. Antituberculous activity of substituted thioureas; studies with 4-butoxy-4′-diemethylaminothiocarbanilide (SU 1906). Proc. Soc. Exper. Biol. & Med. **89** (1955) 388-391.
17. Mayer, R. L. Recherches sur la chimiothérapie des mycoses et de la tuberculose. Rev. méd. France **9** (1941) 393-418.
18. Mayer, R. L., Eisman, P. C. and Konopka, E. A. Antituberculous activity of substituted thioureas. Proc. Soc. Exper. Biol. & Med. **82** (1953) 769-774.
19. Mayer, R. L., Eisman, P. C., Gisi, T. and Konopka, E. A. The chemotherapeutic activity upon chromogenic mycobacteria of certain derivatives of thiocarbanilide (SU 1906), thiazoline (SU 3068), and thiazolidinone (SU 3912). American Rev. Tuberc. & Pulmon. Dis. **77** (1958) 694-702.
20. Pichat, P. Action in vivo sur le bacille de Koch des épanchements pleuraux tuberculeux de la p. amino-benzène sulfonyl-thiourée injectée dans la cavité pleurale. Compt. rend. Soc. Biol. **139** (1945) 124.
21. Ross Innes, J., Smith, M. and Harden-Smith, W. An interim report on SU 1906 (diphenyl-thiourea) in leprosy. East African Med. J. **34** (1957) 395-402.
22. Welsch, M., Buu Hoï, N. P., Panthinne, P. and Dat-Xuong, N. Structure moléculaire et activité tuberculostatique dans le groupe des dérivés de la thiourée. Experientia **12** (1956) 102-103.
23. Youmans, G. P., Youmans, A. S. and Doub, L. The effect of thiocarbanidin and related compounds on *Mycobacterium tuberculosis* var. *hominis in vitro* and *in vivo*. American Rev. Tuberc. & Pulmon. Dis. **77** (1958) 301-310.