

## An Experimental Study of the Antileprosy Activity of a Series of Thioamides in the Mouse<sup>1</sup>

Charles C. Shepard, Peter J. Jenner, Gordon A. Ellard,  
and R. Denise Lancaster<sup>2</sup>

Current interest in the potential use of thioamides in the combined treatment of lepromatous leprosy (<sup>25</sup>) stems from experimental evidence for the antileprosy activity of 2-ethyl-thioisonicotinamide (ethionamide, ETH) and 2-propyl-thioisonicotinamide (prothionamide, PTH) (<sup>2-4, 20-22</sup>), and from two clinical trials of ETH (<sup>16, 18</sup>).

The antituberculosis activity of the thioamides was first demonstrated in the mid 1950s. Substituting thioisonicotinamide (TH) with alkyl groups in the 2-position resulted in enhanced activity, maximal potency being achieved by the ethyl and propyl analogues ETH and PTH, respectively. Their minimal inhibitory concentrations (MICs) against *Mycobacterium tuberculosis* were identical, and they were equipotent against experimental murine tuberculosis. Increasing the size of the substituent further led to greatly reduced activity (<sup>8, 15, 17, 19</sup>). Although ETH was the first thioamide used in the treatment of tuberculosis, in many countries it has now been replaced by PTH due to the latter's apparently superior gastric tolerance.

The screening of compounds for potential antileprosy activity is best carried out *in vivo* by means of the mouse foot pad technique. However, since this usually requires 5–10 g quantities of material, it makes extensive structure-activity studies virtually impossible.

This paper describes a limited study to investigate whether or not the pattern of activity of the thioamides against *M. leprae*

is similar to that displayed against *M. tuberculosis* and, hence, whether ETH and PTH are also likely to be the most potent thioamides in the treatment of leprosy. To this end, the antileprosy activity and pharmacokinetics of seven thioamides, including pyrazine carbonic thioamide (PCT), were compared in the mouse. PCT was specifically selected in view of the key sterilizing role that pyrazinamide plays in the short-course chemotherapy of tuberculosis (<sup>7, 16</sup>). Pyrazinamide is apparently inactive against *M. leprae* (<sup>1, 24</sup>). It was reasoned that its inactivity could be caused by the absence in *M. leprae* of an amidase capable of converting pyrazinamide to the active moiety pyrazinoic acid, and that this might therefore be overcome by using the thioamide function as a potential carrier group.

The rates of elimination of the compounds in the mouse were compared in order to provide evidence as to whether the differences in their *in vivo* activities might have been due to differences in their pharmacological handling in the mouse rather than to their inherent relative antileprosy potencies. The most direct method of assessing the effects of potential differences in pharmacology on relative antileprosy activity would have been to measure the plasma concentrations achieved during continuous dietary administration. However, since ETH is eliminated very rapidly by the mouse (<sup>11</sup>), plasma concentrations are likely to vary greatly from hour to hour, according to the feeding pattern of individual mice. The relative rates of elimination of the thioamides were therefore compared after intravenous dosing.

The physicochemical characteristics of most of the thioamides studied suggested that they would be likely to be well absorbed after oral dosage. The excretion of unchanged drugs in the feces can sometimes provide a useful guide as to whether or not

<sup>1</sup> Received for publication on 8 March 1985; accepted for publication in revised form on 25 July 1985.

<sup>2</sup> C. C. Shepard, M.D. (deceased), Leprosy Section, Division of Bacterial Diseases, Centers for Disease Control, Atlanta, Georgia 30333, U.S.A. P. J. Jenner, B.Sc.; G. A. Ellard, Ph.D., National Institute for Medical Research, Mill Hill, London NW7 1AA, England. R. D. Lancaster, B.Sc., St. George's Hospital Medical School, London SW17 0RE, England.

Reprint requests to P. J. Jenner.

orally administered compounds are well absorbed (as, for example, in the case of thiambutosine<sup>5</sup>). It was appreciated that differences in the relative systemic availability of the thioamides could be due to differences in both oral absorption and clearance resulting from the first passage through the liver<sup>(6)</sup>. However, evidence from studies of the pharmacokinetics of orally and intravenously administered ETH and PTH in man suggests that they are well absorbed<sup>(10)</sup> and that their first pass clearance is small and similar (P. J. Jenner and S. E. Smith, unpublished observations). Therefore, the fecal elimination of the thioamides was determined by feeding combinations of the drugs to groups of mice.

#### MATERIALS AND METHODS

**Chemicals.** 2-t-Butyl-thioisonicotinamide (BTH) and 2-dimethylamino-thioisonicotinamide (DMTH) were synthesized for the study by Pfizer Ltd., Sandwich, Kent, England, through the courtesy of Dr. M. S. Tute, while N-hydroxy-methyl ethionamide (HTH) and PCT were kindly prepared by Prof. J. K. Seydel at the Forschungsinstitut, Borstel, West Germany. ETH and PTH were donated by May and Baker Ltd., Dagenham, Essex, England; thioisonicotinamide was purchased from Aldrich Chemical Co., Ltd., Gillingham, Dorset, England; and a small amount of 2-methyl-thioisonicotinamide (MTH) was a gift of Dr. N. Rist.

**Evaluating antileprosy activity of compounds in the mouse.** These investigations were carried out in two stages. In the first, the activities of the seven thioamides that were available in gram amounts were compared using the kinetic method. The protocol employed has been described in detail elsewhere<sup>(23)</sup>. In brief, CFW mice were inoculated into the foot pad with 5000 mouse-passaged *M. leprae*. The drugs were administered at a concentration of 0.1% w/w in the diet from the 70th to the 130th day after infection. The growth curves in each group and in four control (no drug) groups were monitored by counts of acid-fast bacteria in pools of foot pad tissue from four mice at 28-day intervals. By comparison with the average for the control groups, the amount of growth delay in each treated group was

estimated graphically. The statistical significance of the results was estimated by the method described previously<sup>(23)</sup>. In the second experiment, the antileprosy activities of the most potent thioamides, TH, ETH, and PTH, were compared by feeding them continuously from day 0 in dietary concentrations of 0.001%, 0.003%, 0.01%, and 0.03% respectively, to groups of five mice. The counts in the control (no drug) mice reached plateau levels at about 150 days and the foot pads of controls and drug-treated mice were harvested individually at 180 and 181 days.

**Pharmacokinetic studies.** To standardize the comparisons of the relative rates of elimination of the thioamides after intravenous dosing, groups of 25 adult female CD1 or BALB/c mice were dosed with a combination of the test thioamide together with the same dose of ETH. TH and PTH were administered at 25 mg/kg and 50 mg/kg, and the other thioamides at 25 mg/kg. Doses were injected in 0.05 ml ethanol/polyethylene glycol (3:7 by volume) into the tail vein under light anesthesia. Blood was then obtained from groups of five mice at 0.5-hr intervals from 0.5 to 2.5 hr by cardiac puncture under deep ether anesthesia. After heparinization, plasma was separated and stored at -20°C prior to analysis.

The fecal elimination of the thioamides was determined by feeding combinations of two or three compounds to groups of ten adult female CD1 mice at dietary concentrations of either 0.05% or 0.1% by weight for four-day periods. Each course of drug administration was separated by an interval of at least a week. The mice were caged on grids which allowed the collection of feces without contamination from urine. On each of the last two days of drug administration, complete collections of feces were made and drug ingestion was estimated from the differences between weights of administered and uneaten food.

**Estimation of thioamides.** Weighed portions of mouse plasma (about 0.4 g) or feces (1 g) were diluted to 3 ml or 5 ml, respectively, with water and 0.1 ml of a solution containing 1.5 µg of an appropriate internal standard added. PTH was used for assaying mixtures of ETH and MTH or BTH, DMTH for ETH/PCT, and MTH for the remaining

TABLE 1. *Antileprosy activity of thioamides in the mouse.*

Compound	Growth delay (days)
Thioisonicotinamide (TH)	>214 <sup>a,b</sup>
2-Methyl-thioisonicotinamide (MTH)	— <sup>c</sup>
2-Ethyl-thioisonicotinamide (ETH)	>258 <sup>a,b</sup>
2-Propyl-thioisonicotinamide (PTH)	>258 <sup>a,b</sup>
2-t-Butyl-thioisonicotinamide (BTH)	12
2-Dimethylamino-thioisonicotinamide (DMTH)	2
N-Hydroxy-methyl ethionamide (HTH)	110 <sup>a</sup>
Pyrazine carbonic thioamide (PCT)	0

<sup>a</sup> Significantly different from controls ( $p < 0.001$ ).

<sup>b</sup> No multiplication detected.

<sup>c</sup> Not tested.

estimations. With the exception of PCT, all of the thioamides were then extracted by procedures based on those described previously (<sup>9, 10</sup>). Compounds were initially extracted into diethyl ether, then into 0.1 N hydrochloric acid and, after neutralization, back-extracted into either ethyl acetate (for normal phase chromatography) or diethyl ether (for reverse phase chromatography). Because of its greater polarity and lack of basicity, PTC was first extracted into ethanol/diethyl ether (1:4), and the extract was then directly evaporated to dryness in silanized tapered tubes to avoid losses onto the glassware.

Mixtures of MTH, ETH and PTH were separated by high pressure liquid chromatography, using a normal phase  $\mu$ -Porasil silica column with diethyl ether/methanol (96:4) as the mobile phase (retention times 5.8, 4.5, and 4.1 min, respectively). All of the other analyses were carried out using a reverse-phase ODS Hypersil column. Mixtures of TH, MTH, and ETH were separated using acetonitrile/water (3:7) as the mobile phase (flow rate 1.5 ml/min ca. 1500 p.s.i.; retention times = 2.9, 3.3, and 4.5 min, respectively), while ETH/PTH/BTH mixtures were separated using acetonitrile/water (9:11) (retention times = 2.9, 3.5, and 5.0 min, respectively). Mixtures of PCT, MTH, ETH, DMTH, and/or PTH were separated using acetonitrile/0.02 M pH 7 phosphate buffer (1:3) (retention times = 3.4, 3.8, 5.6, 6.4, and 10.1 min, respectively). Calibration curves (<sup>9, 10</sup>) were prepared by extracting and chromatographing duplicate 3 ml aliquots of aqueous solutions of 0.05–0.5  $\mu$ g/ml ETH plus the companion thioamide

after adding 1.5  $\mu$ g of the appropriate internal standard.

## RESULTS

**Antileprosy activity of thioamides in the mouse.** The periods of growth delay engendered by feeding 0.1% of the various thioamides in the diet for 60 days are summarized in Table 1. The most active compounds were ETH and PTH, which caused growth delays greater than 258 days. TH was also very active; only a few acid-fast bacilli (AFB) were seen in the 336-day harvest, and the growth delay was not significantly different from that of ETH and PTH. HTH was also active, resulting in a growth delay of 110 days. Although such a delay was not significantly less than that caused by ETH and PTH, past experience with the kinetic method indicates that this shorter growth-delay period was probably due to reduced antileprosy activity. By contrast BTH, DMTH, and PCT were inactive.

The results obtained when TH, ETH, and PTH were fed continuously in the diet at concentrations of 0.001–0.03% are illustrated in Figure 1. The activities of ETH and PTH were effectively identical, growth being entirely suppressed when concentrations of 0.01% were administered in the diet and scarcely impeded by giving 0.003%. TH was considerably less active, multiplication being uninhibited when 0.01% was given and being only partially suppressed when 0.03% was employed. A more precise estimate of the relative antileprosy activities of TH and ETH was interpolated using the results obtained when ETH was fed at dietary concentrations of 0.003% and 0.01%

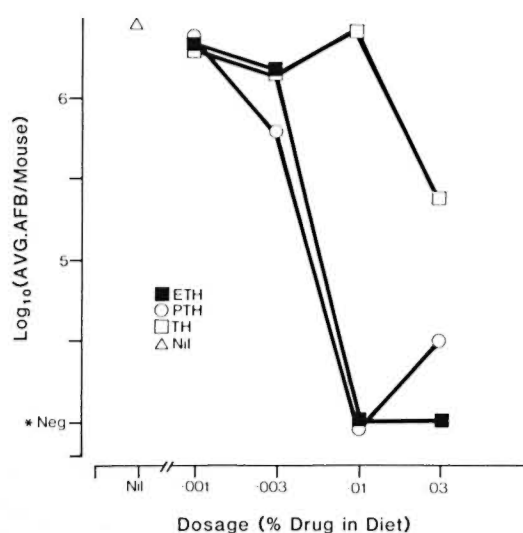


FIG. 1. Results of second mouse experiment, in which ETH, PTH, and TH were compared by continuous administration for 180–181 days in varying dosages. Values shown are averages of AFB counts for five individual mice in each group (\*Negative = less than  $10^{4.2}$  bacilli).

(Fig. 1). The dosage of ETH which would have reduced the  $\log_{10}$  AFB count to those found for the five individual mice fed with 0.03% TH was calculated, giving a mean ETH dosage of  $0.0059\% \pm 0.0024\%$ . This suggests that ETH is some 5.1-fold (range 3.6 to 8.6-fold for one standard deviation) more active than TH. In this calculation the number of *M. leprae* in mice fed 0.01% ETH was assumed to be  $10^4$  (Fig. 1). However, even if there had been as few as one bacillus, the estimate of the relative activities of ETH to TH would have been only slightly modified.

**Estimation of plasma thioamide concentrations.** A representative normal-phase chromatogram of an extract of mouse plasma obtained 1 hr after dosing with 50 mg/kg ETH plus PTH is illustrated in Figure 2a and Figure 2b shows a typical reverse-phase chromatogram of an extract of mouse plasma obtained 2 hr after combined dosing with 50 mg/kg ETH and TH using acetonitrile/water (3:7) as the mobile phase. All of the calibration curves relating the mean peak height ratios to the concentrations of 2-substituted thioamides gave calculated intercepts that did not differ significantly from zero, and the mean replicate errors for the five pairs of calibration curves were very similar and averaged 6%.

**Elimination of thioamides from the mouse.** All of the thioamides studied were rapidly eliminated by the mouse after intravenous injection. Thus, the plasma half-life of ETH averaged  $30 \pm 6$  min (mean  $\pm$  S.E.) for the nine combined drug administrations. The plasma half-lives of the other thioamides expressed as a ratio to that of the concomitantly administered ETH are summarized in Table 2.

TH, MTH, ETH, and PTH were eliminated at very similar rates, and the ratios of the plasma half-lives of PTH or TH to ETH were not influenced by the dosages (25 mg/kg or 50 mg/kg) employed. DMTH and PCT were eliminated a little more rapidly than ETH, while BTH was cleared at approximately half the rate (Table 2). The slower elimination of BTH compared with ETH is illustrated in Figure 3, while the more rapid clearance of DMTH is convincingly demonstrated by plotting the ra-

TABLE 2. *Relative rates of plasma elimination of thioamides in the mouse.*

Compound	Half-life relative to ETH
Thioisonicotinamide (TH)	1.16 <sup>a</sup>
	1.15
2-Methyl thioisonicotinamide (MTH)	0.87
2-Ethyl thioisonicotinamide (ETH)	1.00 <sup>b</sup>
2-Propyl thioisonicotinamide (PTH)	0.95, <sup>a</sup> 0.84 <sup>a</sup>
	0.90
2-t-Butyl thioisonicotinamide (BTH)	1.80
2-Dimethylamino thioisonicotinamide (DMTH)	0.79
N-Hydroxymethyl ethionamide (HTH)	— <sup>c</sup>
Pyrazine carbonic thioamide (PCT)	0.69

<sup>a</sup> Given intravenously at a dose of 50 mg/kg, remaining experiments were at a dose of 25 mg/kg.

<sup>b</sup> By definition (see text).

<sup>c</sup> Broke down rapidly to ETH both *in vitro* and *in vivo*.

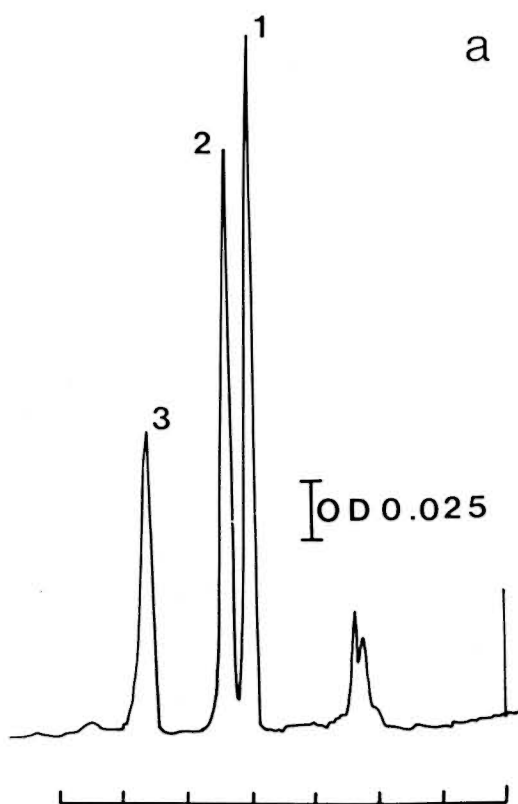


FIG. 2a. Normal phase chromatogram of an extract of mouse plasma 1 hr after i.v. dosing with 50 mg/kg ETH plus PTH. Peaks: 1 = PTH, 2 = ETH, 3 = MTH (internal standard).

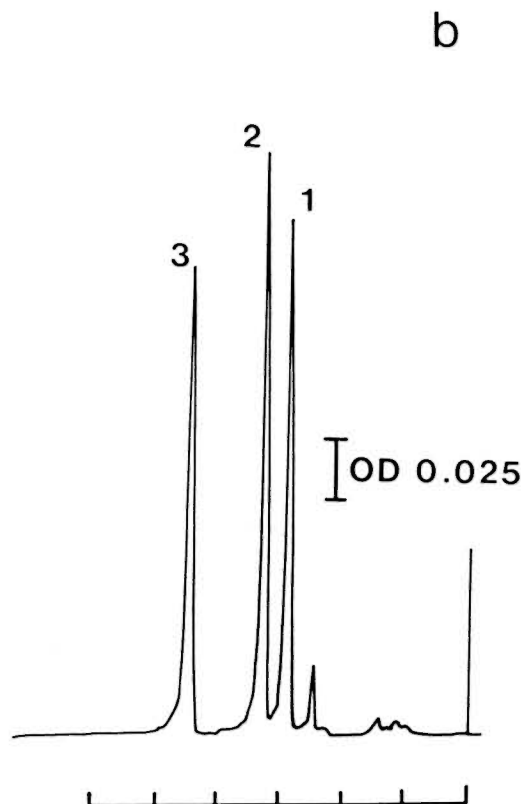


FIG. 2b. Reverse phase chromatogram of an extract of mouse plasma 2 hr after i.v. dosing with 50 mg/kg ETH plus TH. Peaks: 1 = TH, 2 = MTH (internal standard), 3 = ETH.

tios of the concentration of ETH to DMTH as a function of time (Fig. 4). These ratios increased approximately sevenfold over the period from 0.5 to 2.5 hr. HTH proved to be very unstable in aqueous solution, breaking down to give ETH at rates equivalent to half-lives of about 35 and 110 min at pHs of 6.8 and 5.5, respectively, at room temperature. After intravenous co-administration with ETH, traces of HTH were only seen in the first plasma (15 min) sample.

When the compounds were given in the diet, less than 0.5% of the administered doses were recovered unchanged in the feces. The proportions recovered were 0.40%, 0.45%, 0.32%, and 0.27% for TH, PTH, BTH, and DMTH, respectively, together with 0.27–0.33% of the co-administered ETH. It was therefore concluded that dietary doses of up to 0.1% of the thioamides were probably well absorbed by the mouse and that, as a consequence, the relative plas-

ma half-lives of the compounds provided a fairly good indication of the relative plasma and tissue levels achieved in the therapeutic studies.

## DISCUSSION

The potential place of a drug in the treatment of leprosy is critically dependent on the type of activity that it displays. Whether an antileprosy drug is only bacteriostatic or whether it possesses bacteriopausal/bactericidal activity can be effectively assessed in the mouse foot pad using the kinetic method (<sup>23</sup>). However, for each drug being evaluated a group of some 30 mice are usually needed to assess the pattern of growth over periods of 6 to 12 months, and the large amounts of test drugs required for a compound that must be tested in a dosage of 0.1% in the diet (about 10 g in this study) may necessitate the commissioning of special syntheses. As a consequence it is only practical to



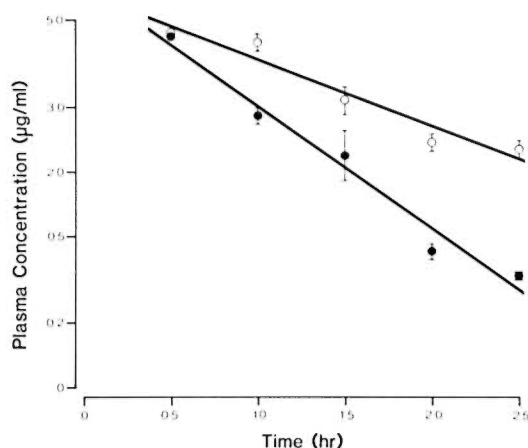


FIG. 3. Elimination of ETH (●) and BTH (○) by the mouse. Points represent geometric means and bars are standard errors from five individual mice at each time.

test small numbers of compounds. In this study the activities of seven compounds were compared. The demonstration of the rapid breakdown of HTH to ETH both *in vitro* and *in vivo* strongly suggests that its antileprosy activity in the mouse was due to ETH rather than to the administered derivative. The complete lack of activity of PCT in the mouse mirrors its inactivity against *M. tuberculosis*, both *in vitro* and *in vivo* (12, 13). It is most unlikely that its complete lack of activity in the mouse foot pad was simply due to its being slightly more rapidly eliminated than ETH (Table 2).

The antileprosy activities of TH, ETH, PTH, BTH, and DMTH were compared to evaluate whether changing the size of the group in the 2-position leads to similar changes in activity to those encountered with *M. tuberculosis*. The high potencies of TH, ETH and PTH and the complete lack of activity of the t-butyl analogue, despite its slower rate of elimination from the mouse, suggests that increasing the bulk of the 2-substituent has a broadly similar effect against *M. leprae* and *M. tuberculosis*. The inactivity of the dimethylamino analogue is also in accord with such a conclusion, although its more rapid elimination in the mouse could have been a contributory factor.

The rates of elimination of TH, ETH, and PTH from the mouse varied little, so that any differences in their activity *in vivo* could probably be ascribed to differences in in-

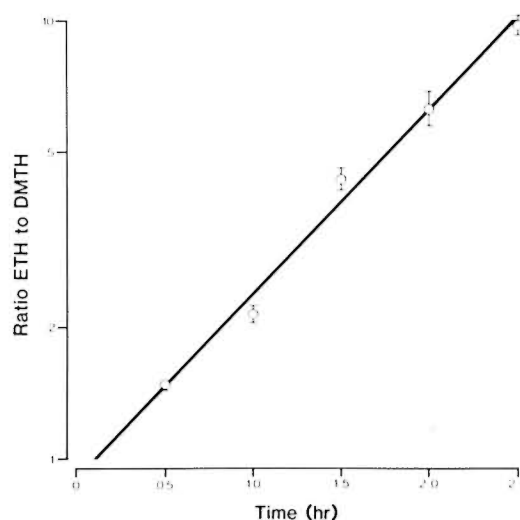


FIG. 4. Relative elimination of ETH and DMTH by the mouse. Points represent geometric means and bars are standard errors from five individual mice at each time.

herent potency. The fact that, as in tuberculosis, ETH and PTH were considerably more active than TH against *M. leprae* further supports the conclusion that the structure-activity pattern of 2-substituted thioamides against both mycobacteria may be quite similar. Indeed, in the treatment of experimental tuberculosis in the mouse, TH was found to be four times less active than ETH or PTH (15, 17), which is very similar to the estimate (five times less active) for its potency against *M. leprae* in the current study.

The identical antileprosy potencies of ETH and PTH demonstrated in the current study parallel not only the findings against *M. tuberculosis* but also the results of previous studies on their activity against *M. leprae* in the mouse foot pad. Thus, investigations of the minimal effective doses of ETH and PTH, when administered continuously in the diet, and of their bactericidal activity, using both the kinetic and proportional bactericidal test methods, also failed to reveal significant differences in their potencies (2, 3). However, in every case the inherent limitations of the test systems were such that only a small number of drug dosages were investigated, with the consequence that a precise comparison of their potencies was impossible.

Recent studies of the comparative pharmacokinetics of ETH and PTH in man in-

dicate that both are well absorbed and that they are then eliminated at closely similar rates from the body (<sup>9, 10</sup>). A current pilot study has also found that when they were prescribed to leprosy patients at dosages of 125 mg or 250 mg a day, compliance was reasonably satisfactory (Stanley, Pearson and Ellard, unpublished data). Thus, as far as their antileprosy potencies and pharmacokinetics are concerned, ETH and PTH can be considered as effectively interchangeable. Direct evidence, however, should be available when the pilot trial currently being conducted in Cebu, Philippines, has been completed. For the present, the choice as to which should be used in any given situation will probably be governed primarily by their relative availability and cost.

### SUMMARY

A series of substituted thioamides have been studied to establish whether their structure-activity pattern against *Mycobacterium leprae* is similar to that displayed against *M. tuberculosis*. Antileprosy activity was evaluated in the mouse foot pad using both the kinetic and continuous methods. Ethionamide and prothionamide were found to be the most active compounds and to be of approximately equal potency. Thioisonicotinamide was about five times less active. 2-t-Butyl-thioisonicotinamide, 2-dimethylamino-thioisonicotinamide, and pyrazine carbonic thioamide were inactive at the dosages tested. High-pressure liquid chromatographic methods were devised to study the potential influence of pharmacological factors on their *in vivo* activity. Fecal measurements suggested that all of the thioamides were well absorbed when fed in the diet. After intravenous administration, all of the thioamides were rapidly eliminated from the mouse. The differences in their elimination rates probably played only a minor role in affecting their relative antileprosy activities. It was concluded that the structural requirements for antileprosy and antituberculosis activity of the thioamides are probably similar.

### RESUMEN

Se estudiaron una serie de tioamidas substituídas para establecer si su patrón de estructura-actividad contra el *Mycobacterium leprae* es similar al presentado contra el *M. tuberculosis*. La actividad antileprosa se evaluó en el cojinete plantar del ratón usando tanto

el método cinético como el método continuo. Se encontró que la etionamida y la protionamida fueron los compuestos más activos y tuvieron una potencia similar. La tioisonicotinamida fue casi 5 veces menos activa en tanto que la 2-t-butil-tioisonicotinamida, la 2-dimetil-amino-tioisonicotinamida y la tioamida carbónica de la pirazina fueron inactivas a las dosis probadas. Se diseñaron métodos de cromatografía de líquidos de alta presión para estudiar la influencia potencial de los factores farmacológicos sobre su actividad *in vivo*. Las determinaciones fecales sugirieron que todas las tioamidas fueron bien absorbidas cuando se administraron oralmente. Todas las tioamidas fueron rápidamente eliminadas después de su administración intravenosa. Las diferencias en sus velocidades de eliminación probablemente tuvieron poca influencia en su actividad antileprosa. Se concluyó que los requerimientos estructurales de las tioamidas para su actividad antileprosa y antituberculosa son probablemente similares.

### RÉSUMÉ

On a étudié une série de thioamides substituées en vue d'établir si les profils d'activités associés à leurs structures étaient, à l'égard de *Mycobacterium leprae*, similaires à ceux observés chez *M. tuberculosis*. L'activité antilépreuse a été évaluée dans le coussinet plantaire de la souris, en utilisant à la fois une méthode cinétique et une méthode continue. On a constaté que l'éthionamide et la prothionamide étaient les composés les plus actifs; leur activité était approximativement égale. La thioisonicotinamide était environ cinq fois moins active. La 2-t-butyl-thioisonicotinamide, la 2-diméthylamino-thioisonicotinamide, et la thioamide pyrazine carbonique, étaient inactives aux dosages étudiés. On a mis au point des méthodes de chromatographie liquide à haute pression pour étudier l'influence éventuelle des facteurs pharmacologiques sur l'activité *in vivo*. Des dosages dans les fèces ont suggéré que toutes les thioamides étaient bien absorbées lorsque on les ajoutait à l'alimentation. Après administration intraveineuse, chez la souris, toutes les thioamides étaient éliminées rapidement. Les différences notées dans le taux d'élimination ont probablement joué un rôle, mais qui n'était cependant que mineur, pour modifier les activités antilépreuses respectives. On en conclut que les conditions structurelles définissant l'activité antilépreuse et antituberculeuse des thioamides sont probablement semblables.

**Acknowledgment.** This investigation received support from the Chemotherapy of Leprosy (THELEP) component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

### REFERENCES

1. COLSTON, M. J. *The application and assessment of various techniques used to determine the activity of seven antituberculosis drugs against M. leprae*

- infections in mice*. Ph.D. thesis, University of London, 1977.
2. COLSTON, M. J., ELLARD, G. A. and GAMMON, P. T. Drugs for combined therapy: experimental studies on the antileprosy activity of ethionamide and prothionamide, and a general review. *Lepr. Rev.* **49** (1978) 115–126.
  3. COLSTON, M. J., HILSON, G. R. F. and BANERJEE, D. K. "The proportional bactericidal test." A method for assessing the bactericidal activity of drugs against *M. leprae* in mice. *Lepr. Rev.* **49** (1978) 7–15.
  4. COLSTON, M. J., HILSON, G. R. F. and LANCASTER, R. D. Intermittent chemotherapy of experimental leprosy in mice. *Am. J. Trop. Med. Hyg.* **29** (1980) 103–108.
  5. ELLARD, G. A. *A biochemical study of the diphenyl thioureas used in the treatment of leprosy in man*. Ph.D. thesis, University of London, 1964.
  6. GIBALDI, M. and PERRIER, D. *Pharmacokinetics*. 2nd ed. New York: Marcel Dekker, 1982.
  7. GROSSET, J. Bacteriologic basis of short-course chemotherapy of tuberculosis. *Clinics Chest Med.* **1** (1980) 231–241.
  8. GRUMBACH, F., RIST, N., LIBERMANN, D., MOYEUX, M., CALS, S. and CLAVEL, S. Activité antituberculeuse expérimentale des certains thioamides isonicotiques substitués sur le noyau. *C. R. Acad. Sciences Sci. (III)* **242** (1956) 2187–2189.
  9. JENNER, P. J. and ELLARD, G. A. High-performance liquid chromatograph determination of ethionamide and prothionamide in body fluids. *J. Chromatogr.* **225** (1981) 245–251.
  10. JENNER, P. J., ELLARD, G. A., GRUER, P. J. K. and ABER, V. R. A comparison of the blood levels and urinary excretion of ethionamide and prothionamide in man. *J. Antimicrob. Chemother.* **13** (1984) 267–277.
  11. JOHNSTON, J. P., KANE, P. O. and KIBBY, M. R. The metabolism of ethionamide and its sulphoxide. *J. Pharm. Pharmacol.* **19** (1967) 1–9.
  12. KAKIMOTO, S., SEYDEL, J. K. and WEMPE, E. Zusammenhänge zwischen Struktur und Wirkung bei Carbothionamiden. *Jahrbuch Borstel* **5** (1961) 240–281.
  13. KUSHNER, S., DALALIAN, H., SANJURJO, J. L., BACH, F. L., SAFFIR, S. R., SMITH, V. K. and WILLIAMS, J. H. Experimental chemotherapy of tuberculosis. II. The synthesis of pyrazinamides and related compounds. *J. Am. Chem. Soc.* **74** (1952) 3617–3621.
  14. LEVY, L. cited in SHEPARD, C. C., ELLARD, G. A., LEVY, L., OPRMOLLA, V. A., PATTYN, S. R., PETERS, J. H., REES, R. J. W. and WATERS, M. F. R. Experimental chemotherapy of leprosy. *Bull. WHO* **53** (1976) 425–433.
  15. LIBERMANN, D., RIST, N., GRUMBACH, F., CAL, F., MOYEUX, M. and ROUIAX, A. Études dans le domaine de la chimiothérapie antituberculeuse—thioamides isonicotiques substitués en position 2 par un radical hydrocarboné. *Bull. Soc. Chim. Fr.* (1958) 687–694.
  16. MITCHISON, D. A. Basic mechanisms of chemotherapy. *Chest* **76S** (1979) 771S–781S.
  17. RIST, N. L'activité antituberculeuse de l'ethionamide (L'alpha-éthylthioisonicotinamide ou 1314 Th.). Étude expérimentale et clinique. *Adv. Tuberc. Res.* **10** (1960) 69–126.
  18. ROLLIER, R. and ROLLIER, M. Traitement de la lèpre lepromateuse par l'ethionamide. *Maroc Med.* **52** (1972) 148–166.
  19. Seydel, J., Wempe, E. and FETTING, R. Struktur und Wirkung bei Carbothionamiden. *Arzneimittelforsch* **13** (1963) 200–205.
  20. SHEPARD, C. C. Further experience with the kinetic method for the study of drugs against *M. leprae* in mice. Activities of DDS, DFD, ethionamide, capreomycin and PAM 1392. *Int. J. Lepr.* **44** (1969) 389–397.
  21. SHEPARD, C. C. Minimal effective dosages in mice of clofazimine (B663) and of ethionamide against *M. leprae*. *Proc. Soc. Exp. Biol. Med.* **132** (1969) 120–124.
  22. SHEPARD, C. C. Combinations involving dapsone, rifampin, clofazimine, and ethionamide in the treatment of *M. leprae* infections in mice. *Int. J. Lepr.* **44** (1976) 135–139.
  23. SHEPARD, C. C. Statistical analysis of results obtained by two methods of testing drug activity against *M. leprae*. *Int. J. Lepr.* **50** (1982) 96–101.
  24. SHEPARD, C. C. and CHANG, Y. T. Activity of antituberculosis drugs against *M. leprae*. *Int. J. Lepr.* **32** (1964) 260–271.
  25. WHO STUDY GROUP. *Chemotherapy of Leprosy for Control Programmes*. Geneva: World Health Organization, Tech. Rept. Ser. 675, 1982.