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The Activity of Thiacetazone, Thiambutosine, Thiocarlide and Sulphamethoxypyridazine Against *Mycobacterium leprae* in Mice

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The mouse footpad model has been used to evaluate the activity of thiambutosine, thiocarlide, thiacetazone and sulphamethoxypyridazine against *Mycobacterium leprae*. The minimum effective doses of thiambutosine and thiocarlide were found to be approximately 0.05% and of thiacetazone 0.03%, although different strains of *M. leprae* displayed varying sensitivity to all 3 drugs. The minimal inhibitory concentrations of thiambutosine, thiacetazone and sulphamethoxypyridazine were estimated to be about 0.5 μg/ml, 0.2 μg/ml and 30 μg/ml, respectively. Evidence was obtained indicating that the antileprosy activity of thiambutosine, thiocarlide and thiacetazone was essentially bacteriostatic. The clinical relevance of these findings is discussed.

Introduction

The enormous problems posed by the widespread emergence of dapsone-resistant strains of *Mycobacterium leprae* among lepromatous patients who have been treated with dapsone monotherapy, and among those whom they may subsequently infect, are now widely recognized and it is generally agreed that the only potentially successful method of preventing the situation from becoming yet more serious is to initiate the treatment of all new lepromatous patients with combinations of antileprosy drugs (Pattyn, 1972; Ellard, 1974, 1975; Pearson *et al.*, 1975; Gelber, 1976; Shepard, 1976; Browne, 1977; Dharmendra, 1977; ILEP, 1977; Jacobson, 1977; Pearson *et al.*, 1977; Pearson, Haile and Rees, 1977, Sansarricq, 1977; Waters, 1977; WHO Expert Committee on Leprosy, 1977; Waters *et al.*, 1978). For the treatment of new lepromatous patients who are assumed to be infected with dapsone-sensitive

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strains of *M. leprae*, dapsone should be given at full dosage (50–100 mg/day) together with a companion drug. The most widely recommended companion drugs are rifampicin and clofazimine. However the use of rifampicin is severely limited by its high cost, while most lighter skinned patients find clofazimine unacceptable because of skin discoloration. Alternative antileprosy compounds are therefore required for use in combination with dapsone for the treatment of patients infected with dapsone-sensitive *M. leprae*, and with other drugs in the management of dapsone-resistant patients.

The mouse footpad model, originally introduced by Shepard and Chang (1964) for assessing the potential antileprosy activity of compounds, has made a major contribution to placing the chemotherapy of leprosy on an objective bacteriological and pharmacological basis (Shepard, 1971; Committee on Experimental Chemotherapy, 1976). Using this model the minimal inhibitory concentrations (MICs) of dapsone (Levy and Peters, 1976), rifampicin (Holmes, 1974), sulphadimethoxine and sulphadoxine (Ellard, Gammon and Rees (1970) against *M. leprae* were determined by measuring the serum concentrations present in mice fed with the minimum effective dose (MED) required to prevent multiplication of leprosy bacilli in the footpad. Subsequently Shepard (1967) described a "kinetic method" to ascertain whether the action of drugs against *M. leprae* in the mouse footpad was predominantly bacteriostatic or bactericidal. In this paper we report determination of the MED of thiambutosine, thiocarlide and thiacetazone against several strains of *M. leprae* and of sulphamethoxypyridazine against a single strain. Estimations of the MICs of thiambutosine, thiacetazone and sulphamethoxypyridazine are also reported together with studies to assess the degree of bactericidal activity of thiambutosine, thiocarlide and thiacetazone.

Thiambutosine (*p*-butoxy, *p'*-dimethylamino-diphenylthiourea, Ciba 1906) has been quite widely used in the treatment of leprosy but is no longer being manufactured. Despite initially encouraging results (Davey, 1958; Garrod, 1959), many patients relapsed clinically and bacteriologically after 2–4 years treatment due to the emergence of thiambutosine-resistant strains of *M. leprae* (Davey, 1960; Rees, 1967*a*,*b*; Garrod and Ellard, 1968; Waters, Pearson and Rees, 1976). Although Shepard and Chang (1964) originally concluded that thiambutosine fed at a dietary concentration of 0.1% did not prevent the multiplication of 2 strains of *M. leprae* in the mouse footpad, Rees (1965) subsequently reported that 0.1% thiambutosine completely suppressed the growth of 6 strains of *M. leprae*. The antileprosy activity of thiambutosine in the mouse footpad has since been confirmed by a number of workers (Pattyn and Royackers, 1965; Gaugas, 1967; Shepard, 1967; Levy and Ullman, 1975). Thiocarlide (*p*,*p'*-di-isoamyloxy-diphenylthiourea, Isoxyl), has also been used clinically, but although initial experience with the drug was favourable (Buu-Hoi *et al.*, 1961; Griffiths, 1965) there has been no report of extended observations. The MED of thiocarlide against a single strain of *M. leprae* was found by Hilson, Banerjee and Holmes (1971) to be 0.03%.

The use of thiacetazone (*p*-acetylamino-benzaldehyde thiosemicarbazone, TB1) in the treatment of leprosy was originally described by Lowe (1954). Although it produced an initial clinical improvement, after continuing

treatment for about 4 years many patients relapsed, presumably through the appearance of drug-resistant bacilli. In the mouse footpad it was originally reported as being partially active when fed at a dietary concentration of 0.1% (Shepard and Chang, 1964) and subsequently reported as fully active at 0.2% (Rees, 1965) or 0.1% (Gaugas, 1967).

The long acting sulphonamide, sulphamethoxypyridazine (3-methoxy-6-sulphanilamido-pyridazine, Lederkyne, Midicel) has been used by a number of workers for the treatment of leprosy (Hirako and Sakurai, 1963; Mohr, 1971; Opromolla, 1971; Languillon, 1975). Pattyn and his colleagues have demonstrated that the multiplication of 5 dapsone-sensitive strains of *M. leprae* was fully inhibited by feeding mice with 0.05% sulphamethoxypyridazine but only partially inhibited by 0.01% (Pattyn *et al.*, 1975).

Methods

DETERMINATION OF THE MINIMUM EFFECTIVE DOSES

Six strains of *M. leprae* were used, all of which were originally derived from untreated lepromatous patients. The methods used for the inoculation of mice, the incorporation of drugs into mouse diet, its administration to mice, and the harvesting and counting of *M. leprae* from mouse footpads were based on those previously described (Rees, 1965, 1967*b*; Holmes and Hilson, 1972). Mice were inoculated in a single hind footpad with approximately 10^4 *M. leprae* and treatment started on the day of infection and continued throughout the experiment. When the numbers of *M. leprae* in the footpads of untreated controls had reached approximately 10^6, the drug-treated mice were sacrificed and numbers of acid-fast bacilli in the footpads counted to determine whether or not multiplication of *M. leprae* had occurred. In one laboratory [M.J.C., designated (a) in the Tables], groups of 6 mice were administered thiambutosine, thiocarlide and thiacetazone at dietary concentrations of 0.1%, 0.03%, 0.01% and 0.003%, whilst at the other laboratory [R.J.W.R., designated (b) in the Tables], groups of 12 mice were administered thiambutosine at 0.1%, 0.02% and 0.004%; thiacetazone at 0.2%, 0.04% and 0.008%, and sulphamethoxypyridazine at 0.1%, 0.01% and 0.001%.

ASSESSMENT OF BACTERICIDAL ACTIVITY

The bactericidal activity of thiambutosine, thiocarlide and thiacetazone was evaluated using the kinetic technique of Shepard (1967). In the first series of experiments, the effect of treating mice with graded doses of drugs for a fixed period during the lag or early exponential phase of growth was assessed. Thiambutosine and thiacetazone were tested against 2 strains of *M. leprae* at dietary concentrations of 0.03%, 0.1% and 0.2%, the drugs being given for a period of 60 days immediately after inoculating mice with 10^4 *M. leprae* for strain SBL 16220, and from days 75 to 135 *post inoculum* for strain TG. Thiocarlide was tested against 3 strains of *M. leprae*; against strain SBL 16220 administration was for 60 days from day 56 at dose levels of 0.01% and 0.03%, against strain TG for 30 days from day 66 at 0.03% and 0.1%, and against strain SBL 16220 for 30 days from day 26 at 0.01%, 0.03% and 0.1%.

In the second series of experiments thiambutosine, thiocarlide and thiacetazone were fed at a dietary concentration of 0.1% for periods of from 7–85 days commencing 60 days after inoculating with strain TG.

Bacilli were harvested from groups of 3 untreated control mice 90 days after inoculation and at 30 day intervals thereafter. When bacilli were multiplying exponentially in the controls, the counting of *M. leprae* in the footpads of groups of 3 drug-treated mice was commenced. The activity of the drugs was assessed by comparing the growth curves of *M. leprae* in the treated and control mice. The growth delay engendered by drug treatment was defined as the period in days during which multiplication to $10^{5\cdot3}$ bacilli was delayed in the treated animals as compared to the controls. The number $10^{5\cdot3}$ was chosen because at this point bacilli were multiplying exponentially and accurate numbers of *M. leprae* could be obtained (Colston, 1977). The excess growth delay was defined as the growth delay minus the period of drug administration. Drugs causing significant excess growth delays by this method are regarded as having bactericidal-type activity, indicating that they are capable of killing *M. leprae*, or of inducing prolonged bacteriostasis or both (Committee on Experimental Chemotherapy, 1976).

DETERMINATION OF DRUG CONCENTRATIONS IN MOUSE SERUM

Thiambutosine

A novel method was devised for the determination of thiambutosine serum concentrations of down to about 0.05 μg/ml. The method is based on the conversion of thiambutosine to *p*-butoxy-aniline and its derivatization to a product capable of quantification by gas–liquid chromatography with electron capture detection.

A 2 ml aliquot of serum, diluted to contain between 0.1 and 0.5 μg/ml thiambutosine, was pipetted into a small centrifuge tube, together with 0.1 ml of a methanolic solution of 5 μg/ml *p*-n-propoxy-*p'*-dimethylamino-diphenylthiourea to act as an internal standard, and gently shaken. Serum proteins were precipitated by adding 6 ml methanol and removed by centrifugation. The methanolic supernatant was then transferred by Pasteur pipette to a large centrifuge tube, evaporated to dryness under reduced pressure using a rotary evaporator and the residue dissolved by shaking with 5 ml 0.1 N sodium hydroxide and 20 ml n-heptane/*iso*amyl alcohol (98.5 : 1.5 v/v). After centrifugation the lower aqueous phase was removed and the heptane extract rewashed by shaking with a further 5 ml 0.1 N sodium hydroxide to ensure the complete removal of all metabolites of thiambutosine (Ellard, 1964).

Thiambutosine and the internal standard were then recovered by extracting the heptane phase with 3 ml N sulphuric acid and the sulphuric acid extract transferred to a small centrifuge tube and heated for 15 min at 100°C with about 50 mg zinc powder to liberate *p*-butoxy-aniline and *p*-propoxy-aniline from thiambutosine and the internal standard, respectively. After cooling and neutralizing with 0.3 ml 10 N sodium hydroxide, 1 ml M/15 pH 7.0 phosphate buffer was added and the liberated anilines extracted into 3 ml ethyl acetate. The ethyl acetate extract was decanted, shaken with 0.5 g anhydrous sodium sulphate, transferred to a small tapered centrifuge tube and evaporated to dryness at 50°C under a stream of nitrogen. The butoxy and propoxy anilines were then converted to their pentafluoropropionic derivatives by shaking with 100 μl of a freshly prepared solution containing 0.1% (v/v) pentafluoropropionic anhydride (Pierce Chemical Co.) in dry ethyl acetate, and standing at room temperature for 15 min. Excess pentafluoropropionic anhydride was then removed by drying to dryness under a stream of nitrogen and the residue redissolved in 20 μl ethyl acetate, from which aliquots of 1 μl were injected onto the gas–liquid chromatography column.

Chromatography was carried out at 160°C on a coiled glass column (9 ft long × 2 mm i.d.) silanized with dimethyldichlorosilane and packed with 3% OV-1 on Gas Chrom Q (100–120 mesh), using nitrogen (50 ml/min) as the carrier gas with a Pye 104 Chromatograph equipped for electron capture detection. The detector was maintained at 250°C and operated on the pulse mode with a pulse period of 150 μs. Under these conditions the pentafluoropropionic derivatives of *p*-butoxy-aniline and *p*-proxy-aniline eluted with retention times of about 6 and 4 min, respectively. Their peak areas were calculated by multiplying their peak heights by their widths at half peak height, and a linear relationship was obtained between the ratios of the areas of the peaks of the pentafluoropropionyl derivatives of the anilines formed from thiambutosine and the internal standard respectively, and the initial thiambutosine concentration.

Thiacetazone

Because of the insensitivity of currently available ultraviolet and colorimetric methods for determining thiacetazone in the serum (Ellard *et al.*, 1974) and the small amounts of serum obtainable from mice, a novel radiochemical method was devised for measuring mouse serum thiacetazone concentrations. In this method mice were fed with radioactively labelled thiacetazone and the unmetabolized drug recovered from the serum using a combined solvent extraction/thin layer chromatographic procedure.

Batches of radioactive thiacetazone were synthesized from ^{35}S-labelled thiosemicarbazide (specific activity 11.6 mCi/mM, The Radiochemical Centre, Amersham), according to the method employed by Tattersall (1968), by dissolving 1 mCi of the compound (approximately 8 mg) in 0.5 ml water, adding 17.5 mg unlabelled thiosemicarbazide in 2.5 ml water, 55 mg of *p*-acetamidobenzaldehyde in 2 ml water and a drop of glacial acetic acid (D. J. Drain, pers. comm., 1974). After standing for 1 h the crystalline ^{35}S-labelled thiacetazone formed was filtered through an 0.45 μ membrane filter (Millipore Ltd), washed with a small quantity of distilled water and dried in a vacuum desiccator. These batches, with a specific activity of about 3.6 mCi/mM, were used for preparing mouse diets containing 0.03% and 0.04% thiacetazone. Other batches of ^{35}S-labelled thiacetazone, with specific activities of 0.72 and 0.36 mCi/mM for incorporation in diets containing 0.1% and 0.2% thiacetazone, respectively, were prepared by mixing appropriate volumes of methanolic solutions containing 1 mg/ml of the original ^{35}S-labelled preparation and of unlabelled ("cold") thiacetazone, and evaporating to dryness under reduced pressure.

A 1 ml aliquot of serum from mice fed for 24 h on ^{35}S-labelled thiacetazone was pipetted into a small separating funnel together with 0.1 ml of a methanolic solution containing 1 mg/ml "cold" thiacetazone to act as a "marker" to estimate the recovery of thiacetazone in the separation system. After the addition of 1 ml M dipotassium hydrogen phosphate, thiacetazone was extracted by shaking with 4 ml chloroform/propan-2-ol (4 : 1 v/v) (Ellard *et al.*, 1974). The lower organic phase was then filtered through a small amount of anhydrous sodium sulphate into a tapered centrifuge tube and the extract concentrated to a volume of about 0.1 ml by warming under a stream of nitrogen.

The concentrated extract was dried onto a silical gel thin layer chromatography plate (Merck) and then developed with ethyl acetate for 1 h. After drying and examining under ultraviolet light, the u.v.-absorbing thiacetazone spot was scraped off and eluted by shaking with 2 ml methanol in a small centrifuge tube. After centrifugation, 1.5 ml of the methanol extract was dried down in a scintillation vial, 5 ml of 0.6% butyl-PBD in toluene added and counted in a liquid scintillation counter. The overall recovery of thiacetazone in the combined separation procedure was determined by diluting a further sample of the methanol extract 5-fold with methanol and measuring its extinction at its absorption maximum (approximately 333 nm). The concentration of thiazetazone in the original mouse serum could then be estimated by calculating the ratio of the extract's radioactivity (counts per min) to its absorbance, and comparing it with the ratio for standards containing 1 μg/ml of the appropriate ^{35}S-labelled thiacetazone preparation in blank serum taken through the same procedure.

Sulphamethoxypyridazine

Sulphamethoxypyridazine was determined, after extraction into ethyl acetate and thence into 2 N hydrochloric acid, by the colorimetric method employed previously for the estimation of sulphadimethoxine and sulphadoxine in mouse plasma (Ellard *et al.*, 1970).

Results

The results of the studies to determine the minimum effective doses of thiambutosine, thiocarlide, thiacetazone and sulphamethoxypyridazine are summarized in Tables 1–4. Thiambutosine at a dietary concentration of 0.01% failed to inhibit any of the strains, feeding 0.03% almost completely suppressed the growth of 3 strains, while 0.1% was required to suppress the growth of the other 3 strains (Table 1). These findings indicate that there may be small differences in the natural sensitivity of wild strains of *M. leprae* to thiambutosine, but that a representative value for its MED would be about

TABLE 1

Estimation of the minimum effective dose (MED) of thiambutosine against M. leprae

M. leprae strain	Percentage thiambutosine in the diet						MED
	0.003	0.004	0.01	0.02	0.03	0.1	
SBL 16325[a]*	6/6†	—	5/6	—	1/6	0/6	0.03%
SBL 16220[a]	5/5	—	6/6	—	0/6	0/6	0.03%
SBL 15337[a]	6/6	—	6/6	—	4/6	0/6	0.1%
TG[a]	6/6	—	5/6	—	1/6	0/6	0.03%
9593[a]	6/6	—	6/6	—	3/6	0/6	0.1%
SBL 16509[b]	—	11/12	—	12/12	—	0/12	0.1%

* Laboratories:
(*a*) St George's Hospital Medical School (M.J.C.).
(*b*) National Institute for Medical Research (R.J.W.R.).
† Number of footpads positive/number of footpads harvested.

TABLE 2

Estimation of the minimum effective dose (MED) of thiocarlide against M. leprae

M. leprae strain	Percentage thiocarlide in the diet				MED
	0.003%	0.01%	0.03%	0.1%	
SBL 16325[a]*	6/6	6/6	0/5	0/6	0.03%
SBL 16220[a]	6/6	6/6	1/6	0/6	0.03%
SBL 15337[a]	6/6	5/5	3/6	0/6	0.1%
TG[a]	6/6	6/6	0/6	0/6	0.03%
9593[a]	6/6	5/6	3/6	0/6	0.1%

* Laboratory, see footnote to Table 1.
† Number of footpads positive/number of footpads harvested.

TABLE 3

Estimation of the minimum effective dose (MED) of thiacetazone against M. leprae

M. leprae strain	Percentage thiacetazone in the diet							MED
	0.003	0.008	0.01	0.03	0.04	0.1	0.2	
SBL 16325[a]*	4/6†	—	1/5	0/6	—	0/6	—	0.01%
SBL 16220[a]	5/6	—	0/6	0/5	—	0/6	—	0.01%
SBL 15337[a]	6/6	—	3/5	0/6	—	0/6	—	0.03%
TG[a]	6/6	—	3/6	0/6	—	0/6	—	0.03%
9593[a]	5/5	—	3/5	0/6	—	0/6	—	0.03%
SBL 16509[b]	—	12/12	—	—	6/12	—	1/12	>0.04%

* Laboratory, see footnote to Table 1.
† Number of footpads positive/number of footpads harvested.

0.05%. The results obtained with thiocarlide (Table 2) were very similar to those found with thiambutosine, with individual MEDs ranging from 0.03% to 0.1% and a representative value of 0.05%. The strains also appeared to show different degrees of susceptibility to thiacetazone (Table 3), the MEDs being 0.01% for 2 strains, 0.03% for 3 strains and greater than 0.04% for 1 strain. A representative value was considered to be 0.03%. The results obtained with sulphamethoxypyridazine against a single strain of *M. leprae* (Table 4) confirm the more extensive findings obtained by Pattyn *et al.* (1975). In his studies 5 strains of dapsone-sensitive *M. leprae* were partially inhibited by feeding 0.01% in the diet and totally inhibited by 0.05%, indicating that its MED lies between 0.01 and 0.05%.

The concentrations of thiambutosine, thiacetazone and sulphamethoxypyridazine in the serum of mice fed various dosages of the drugs are summarized in Table 5. For each drug, serum concentrations were approximately proportional to the percentage of drug fed in the diet. The minimal inhibitory concentrations (MICs) of the drugs were defined as the serum concentrations present in mice fed with the MEDs of the drugs, and were calculated by interpolation from the straight lines relating serum concentrations to dietary dosages. The estimated MICs (Table 6) were 0.5 μg/ml for thiambutosine, 0.2 μg/ml for thiacetazone and between 10 and 50 μg/ml for sulphamethoxypyridazine. We were however unable to determine the MIC

TABLE 4

Sensitivity of M. leprae *to inhibition by sulphamethoxypyridazine*

M. leprae strain	Percentage sulphamethoxypyridazine in the diet		
	0.001	0.01	0.1
SBL 16509[b]*	12/12†	6/12	0/12

* Laboratory, see footnote to Table 1.
† Number of footpads positive/number of footpads harvested.

TABLE 5

Mouse serum concentrations of thiambutosine, thiacetazone and sulphamethoxypyridazine

Percentage drug in the diet	Serum concentration (μg/ml)		
	Thiambutosine	Thiacetazone	Sulphamethoxypyridazine
0.001			1.0[b]
0.01			10.6±2.6[b]†
0.03	0.20[a]*	0.26[a]	
0.04		0.25[b]	
0.06	0.72[a]		
0.1	1.05[a]	0.56[a]	92.4±14.0[b]†
0.2	2.03[a]	1.36[b]	

* Laboratory, see footnote to Table 1.
† Results from individual mice; mean ± S.D. individual results.

of thiocarlide against *M. leprae* because of the lack of a suitable analytical method. Although it is impossible to make precise comparisons between the activity of compounds against *M. leprae* in the mouse footpad system and their activity against *M. tuberculosis in vitro* or *in vivo* in the mouse, consideration of the pertinent literature (Eisman *et al.*, 1954; Barnett *et al.*, 1963; Trnka, Urbančik and Polenská, 1963) suggests that the susceptibility of *M. leprae* to inhibition by thiambutosine, thiocarlide, or thiacetazone may be somewhat similar to that of *M. tuberculosis.*

The results of the first series of kinetic experiments are summarized in Table 7. When thiambutosine was fed for 60 days at a dietary concentration of 0.03% it failed to completely suppress the growth of either of the 2 strains tested despite their inhibition by this dose when given continuously. The difference between the results obtained by the continuous and kinetic methods probably indicates that such a dietary concentration permitted slight growth of *M. leprae* in the mouse footpad (Colston, 1977). When the dosage was increased to 0.1% or 0.2% only small excess growth delays were produced indicating that its activity was essentially bacteriostatic. Thiocarlide was also essentially bacteriostatic when fed for 30 or 60 days at concentrations of

TABLE 6

Minimum effective doses (MEDs) and minimum inhibitory serum concentrations (MICs) of thiambutosine, thiocarlide, thiacetazone and sulphamethoxypyridazine against M. leprae

Drug	MED (Percentage in the diet)	MIC (μg/ml)
Thiambutosine	0.05	0.5
Thiocarlide	0.05	—*
Thiacetazone	0.03	0.2
Sulphamethoxypyridazine	0.01–0.05†	10–50

* Not determined.
† Based primarily on the data of Pattyn *et al.* (1975).

TABLE 7

Assessment of antibacterial activity by the kinetic method: the effect of administration of graded doses of thiambutosine, thiocarlide and thiacetazone on the growth of M. leprae *in mouse footpads*

Drug	Dose*	Growth delay (days)			Excess growth delays‖ (days)		
		(1)†	(2)†	(3)†	(1)†	(2)†	(3)†
Thiambutosine	0.03%	20	23	—‡	Nil	Nil	—
	0.1%	75	91	—	15	31	—
	0.2%	103	88	—	43	28	—
Thiocarlide	0.01%	59	—	11§	Nil	—	Nil§
	0.03%	84	39§	18§	24	9§	Nil§
	0.1%	—	76§	38§	—	46§	8§
Thiacetazone	0.03%	80	70	—	20	10	—
	0.1%	138	143	—	78	83	—
	0.2%	128	149	—	68	89	—

* Normally administered for 60 days.
† Strain: (1) SBL 16220, (2) TG, (3) SBL 15337.
‡ Not tested.
§ Administered for only 30 days.
‖ Growth delay less period of drug administration.

0.01%, 0.03% or 0.1% to mice infected with 3 different strains of *M. leprae*. When fed at 0.03% in the diet the activity of thiacetazone was also purely bacteriostatic. However when the dosage of thiacetazone was increased to 0.1%, excess growth delays of 78 and 83 days were engendered in the 2 strains tested, suggesting the possibility of bactericidal activity or the induction of prolonged bacteriostasis, although there was no further increase in growth delays when the dietary concentration was increased to 0.2%.

The effect of varying periods of administration of 0.1% thiambutosine, thiocarlide and thiacetazone are summarized in Table 8. Again thiambutosine and thiocarlide showed no bactericidal activity since the excess growth delays encountered did not increase with increasing periods of treatment. Increasing duration of administration of thiacetazone however did result in increasing excess growth delays, although the similarity in the results obtained after giving the drug for 56 or 85 days suggests that the excess growth delay was

TABLE 8

Excess growth delays engendered by feeding 0.1% thiambutosine, thiocarlide and thiacetazone in the diet for varying periods of time

Drug	Duration of drug administration							
	7 days	14 days	21 days	28 days	42 days	56 days	70 days	85 days
Thiambutosine	—*	28	—	21	—	19	13	2
Thiocarlide	11	32	—	28	28	20	—	—
Thiacetazone	20	—	40	—	59	74	—	80

* Not tested.

due primarily to prolonged bacteriostasis rather than to killing of *M. leprae*. These results resemble those obtained when *M. tuberculosis* was exposed *in vitro* to pulses of thiocarlide and thiacetazone (Dickinson and Mitchison, 1966). Neither drug displayed any bactericidal activity and multiplication of the tubercle bacilli began immediately after removing the drugs, although the rate of growth of cultures after exposure to thiacetazone was slower than that in the controls. . The essential bacteriostatic nature of these drugs against *M. leprae* has also been demonstrated using the more rigorous proportional bactericidal test method (Colston, Hilson and Banerjee, 1978).

Discussion

Because of the cross-resistance shown by *M. leprae* between dapsone and the long-acting sulphonamides sulphadimethoxine and sulphadoxine (sulphormethoxine) (Rees, 1967*a,b*), sulphamethoxypyridazine cannot be considered as a potential drug for use in combined treatment with dapsone. The published data concerning the serum concentrations achieved in man after dosage with sulphamethoxypyridazine (Nicolas and Finland, 1957; Sheth, Kulkarni and Karmath, 1958), indicate that treating patients with 500 mg of the drug daily or 750 mg every other day would probably result in peak serum concentrations of between 100 and 150 μg/ml falling between doses to trough levels of about 100 or 50 μg/ml, respectively. If the MIC of sulphamethoxypyridazine against *M. leprae* is assumed to be about 30 μg/ml, peak serum concentrations would only exceed the MIC by a factor of about 4 in contrast to a factor of about 500 when daily doses of 100 mg dapsone are employed (see Colston, Ellard and Gammon, 1978). It must therefore be concluded that, as with the other long-acting sulphonamides sulphadimethoxine and sulphadoxine (Ellard *et al.*, 1970), there is no place for sulphamethoxypyridazine in the treatment of leprosy.

For an antileprosy drug to be therapeutically effective in man, well-tolerated doses must produce tissue concentrations that exceed its MIC against *M. leprae*. If a drug has powerful bactericidal activity like rifampicin (see Waters *et al.*, 1978), highly effective therapy can be achieved without continuously maintaining tissue concentrations above its MIC against *M. leprae*. However this investigation has shown that thiambutosine, thiocarlide and thiacetazone are primarily bacteriostatic compounds. It is therefore likely that their efficacy when combined with dapsone in preventing the emergence of dapsone-resistant strains of *M. leprae* will depend on tissue concentrations being maintained above their MICs. Assuming that the concentrations of these drugs in the tissues parallel those in the serum, and that their relative tissue penetration is similar in mouse and man, one may attempt to compare their relative potentialities for use in combined treatment by assessing the ease with which their serum concentrations in man can be maintained above their MICs against *M. leprae* determined in the mouse footpad system.

The MIC of thiambutosine against *M. leprae* was found to be approximately 0.5 μg/ml. Peak serum concentrations achieved in man after oral dosage with 1500 mg of the drug are very similar to this value and by 24 h

have fallen to about a third of the MIC (Waters, Pearson and Rees, 1978). In view of these findings, the weak clinical activity of thiambutosine monotherapy, revealed by its inactivity in exceptionally poor absorbers of the drug (Garrod and Ellard, 1968; Waters *et al.*, 1976; Waters, Pearson and Rees, 1978), is readily understood. These findings argue strongly against the suggestion that treatment with thiambutosine should be initiated with substantially lower doses of the drug (Browne, 1975). There is however the possibility that part of the antileprosy activity of thiambutosine in man could be due to one of its major metabolites, the so-called "CIBA propoxy acid" (Ba-22,330), formed by ω-oxidation of thiambutosine's butoxy group (Ellard, 1964), which prevented the multiplication of *M. leprae* in the mouse footpad when fed at a dietary concentration of 0.1% (Rees, 1967*a*).

Because of the absence of a suitable analytical method for determining thiocarlide, the serum concentrations achieved during treatment with the drug are not known, and we were unable to determine its MIC against *M. leprae*. However the similarity of its MED to that of thiambutosine, and the evidence generated by the kinetic experiments described in this paper that its activity against *M. leprae* is purely bacteriostatic, suggest that its potential value in combination treatment is likely to be as limited as that of thiambutosine.

The MIC of thiacetazone against *M. leprae* was found to be approximately 0.2 μg/ml. Thiacetazone has been extensively used at a dosage of 150 mg a day as a component of regimens for the treatment of pulmonary tuberculosis. Such doses result in peak serum concentrations of about 1.7 μg/ml (i.e. some 8 times thiacetazone's MIC against *M. leprae*) falling to approximately 0.6 μg/ml at 24 h (Ellard *et al.*, 1974). Thus, if used at this dosage it should be possible to maintain concentrations in excess of those required to prevent the multiplication of *M. leprae*, although it would be clearly unwise to initiate antileprosy treatment with substantially lower doses of thiacetazone as has been recommended by Dharmendra (1977).

We would therefore conclude that of the 4 antileprosy drugs investigated in this study, the one that holds most promise for use in combination therapy is thiacetazone.

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