Editorial

COMBINED THERAPY IN PRINCIPLE AND PRACTICE FOR THE CONTROL OF DAPSONE RESISTANCE

The widespread emergence of dapsone-resistant strains of *Mycobacterium leprae* in patients with lepromatous leprosy who had been treated with dapsone as monotherapy, is now fully accepted (WHO, 1977). This growing problem of acquired (secondary) dapsone resistance among the large pool of dapsone-treated lepromatous patients at risk, could be still more foreboding with the recent reports of new and previously untreated lepromatous patients presenting *de novo* with dapsone resistance (primary resistance) in known areas with secondary dapsone resistance (Pearson *et al.*, 1977; Jacobson, 1977). While it was anticipated that cases of primary dapsone resistance would be likely to occur eventually, once secondary resistance was proven, these reports substantiate our worst fears and establish that dapsone-resistant strains of *M. leprae* are infectious for man. Although the cases reported had lepromatous type leprosy all types will present with primary dapsone-resistant infections. In fact a proven case of primary dapsone resistance presenting as tuberculoid leprosy is reported by Waters and colleagues on page 127. Therefore alternative drugs are already required for treatment of the very substantial numbers of relapsed lepromatous patients with acquired dapsone resistance; if primary dapsone resistance should reach serious proportions, alternative drugs will also be required for tuberculoid as well as lepromatous patients and then the future use of dapsone would be uncertain. Any significant spread of primary dapsone resistance would be catastrophic for the control of leprosy, because there is no sure way of distinguishing in the field a dapsone-sensitive infection from a dapsone-resistant one, and therefore all new cases would require additional alternative drugs even if dapsone was given in combination. (While only the mouse footpad technique can identify a dapsone-resistant strain of *M. leprae*, the test takes at least 6 months, and can only be applied to bacilliferous patients. Hence the technique would not be universally applicable for identifying primary dapsone, since no tuberculoid patients could be tested and treatment of the lepromatous patients would have had to be initiated long before results were available from the mouse test.)

Now the existence of secondary
seriousness appreciated, it is clear from the discussions above that unless therapeutic regimens are rapidly introduced to diminish the emergence of secondary resistance and the chances of the spread of primary resistance, the whole future of the control and treatment of leprosy by chemotherapy, including dapsone, will be in jeopardy.

Therefore it is essential to introduce urgently other available anti-leprosy

0305–7518/0601–0097 $01.00/0

drugs and therapeutic regimens for the prevention of dapsone resistance before the problem becomes still more serious. There are two aspects of the problem, one is to prevent the emergence of dapsone resistance and the other is to treat dapsone resistance once established. For the prevention of drug resistance in general, combined therapy has proved to be highly successful, particularly in tuberculosis. For leprosy this would involve every new lepromatous patient being treated at onset with dapsone at full dosage with at least one companion drug. For the treatment of leprosy patients with established dapsone resistance alternative drugs would be required, as combined therapy for lepromatous patients and as monotherapy for tuberculoid leprosy.

For tackling these aspects of the problem, the essential requirement is for other currently available anti-leprosy drugs. Although the choice is small, it is important to select drugs with maximum potency and tolerance. The two papers by Colston and his colleagues in this number of *Leprosy Review* are highly relevant since they present a very detailed assessment of the anti-*M. leprae* and relevant pharmacological features of all the drugs available. Although most of the data is from their own studies, they have brought together data from the world liturature in presenting their final assessment of the best drugs to be selected for combined therapy.

Since *M. leprae* cannot be cultured *in vitro*, the mouse footpad model had to be adapted for assessing anti-leprosy drugs. In the last decade this model has played a major role in clarifying the bacteriological and pharmacological properties of anti-leprosy drugs and in rationalizing the chemotherapy of leprosy. Applying all the available and now highly sophisticated mouse models and pharmacological methods, Colston and colleagues have evaluated and compared the bacteriological and pharmacological activities of companion anti-leprosy drugs, or group of drugs, that are available for combined therapy. The drugs studied included two diphenyl thioureas-thiambutosine (Ciba, 1906) and thiocarlide (Isoxyl); thiacetazone (TBI); a long-acting sulphonamide—sulphamethoxypyridazine (Lederkyne, Medicel) and two thioamides—ethionamide and the propyl analogue, prothionamide. Each drug was tested against several strains of *M. leprae* and the minimum effective dose (MED) fed to mice that prevented bacterial multiplication in the footpad, was determined. The minimal inhibitory concentration (MIC) of each drug in the mouse was determined by estimating the serum concentration corresponding to the MED.These estimations involved the development of the very sensitive radiochemical and novel gas–liquid chromatographic procedures described in their current papers. Furthermore, the model was adapted to determine whether the drug was bacteriostatic or bactericidal against *M. leprae,* using the kinetic or proportional bactericidal tests. Finally, for each drug the ratio of its peak serum concentration in man, from an acceptable and non-toxic dose, to its MIC in the mouse was calculated, as was the duration in which the serum concentration exceeded the MIC.

From these extensive and comparative studies on the 6 companion drugs, only the two thioamides—ethionamide and prothionamide—are bactericidal and also have the highest peak serum/MIC ratios. The other companion drugs are only bacteriostatic, and with the exception of thiacetazone have poor peak serum/MIC ratios.

Using the same principles for determining the therapeutic activities of these 6 anti-leprosy drugs, Colston and colleagues review data for dapsone and the diacetetyl derivative, acedapsone (DADDS), clofazimine (B663) and rifampicin. By these criteria rifampicin, dapsone and clofazimine were bactericidal drugs, the former being the most powerful bactericidal anti-leprosy drug so far studied and in this respect clofazimine falling between the two. Regarding peak serum/MIC ratios, these were high for rifampicin and dapsone and in the case of dapsone significantly higher and more sustained than for any other anti-leprosy drug. Acedapsone giving by injection is dependent for its activity on the slow release of dapsone, and in the dose used sustains serum levels of dapsone approximately 15-fold above the MIC for some 200 days. The other criteria for assessing the anti-leprosy activities of drugs cannot be applied for clofazimine, because it is accumulated in the tissues.

From these basic principles on which the activities of anti-leprosy drugs can be defined using the mouse models, it remains to determine their practical application to the problems of the control and treatment of dapsone resistance.

In practice one of the principle criteria—that drugs with a low peak serum/MIC ratio have no value, excludes the use of thiambutosine (which also is no longer manufactured), isoxyl and any of the long-acting sulphonamides. Therefore, the two former drugs must never be used as companion drugs in combined therapy, and the long-acting sulphonamides must never be used as alternatives to dapsone. This leaves for consideration two sulphones, dapsone and acedapsone, rifampicin, clofazamine, thiacetazone and two thioamides, ethionamide and prothionamide. From the practical side however, there are other criteria, including drug toxicity and acceptability, likely cross-resistance between drugs and cost/effectiveness. By all these criteria dapsone is the first drug of choice and therefore it is *essential* that it retains this position. To prevent the emergence of dapsone resistance, all new lepromatous patients must be initiated on combined therapy i.e. dapsone and a companion drug. Because of the practical difficulties of ensuring unsupervised daily dapsone therapy, it is strongly recommended that dapsone intake should be boosted by also giving intramuscular injections of acedapsone every 3 months. The choice of the companion drugs rest between rifampicin, clofazimine, thiatezone and ethionamide/prothionamide. On the basis of cross-resistance, all are acceptable, since none of these companion drugs would show cross-resistance with dapsone. On the basis of drug toxicity and patient acceptability, the following criteria must be considered: (1) Thiacetazone toxicity appears to be regionally distributed, and in general is intolerable in countries east of India. (2) The intensity of skin pigmentation resulting from clofazimine therapy is intolerable for all paler skinned patients. (3) Of the two thioamides, prothionamide causes less gastric intolerance than ethionamide, and since this intolerance appears to be directly dose dependent, the new data of Colston and his colleagues indicating that a daily dose of 250 mg would still be bactericidal, strongly favours the use of prothionamide. Finally the cost of these companion drugs must be considered; on the basis of a standard daily dose the costs would be: rifampicin 95p, thiacetazone 1p, clofazimine 5.5p and prothionamide 13p.

For the treatment of lepromatous patients who have developed dapsone resistance, their subsequent therapy must depend entirely on "companion" drugs and also as combined therapy, to prevent the emergence of resistance to these drugs. The choice of drugs must take into account the criteria referred to above, but also the potential cross-resistance between the companion drugs. Unfortunately this does limit the number of companion drugs available for dual therapy because of cross-resistance between thiacetazone, ethionamide and prothionamide (Rees and Waters, unpublished data, 1978). Therefore, while these three drugs cannot be given together, they can be used in dual therapy with rifampicin or clofazimine, with no risk of cross-resistance. Of the three drugs, the choice would be between prothionamide as the least toxic and bactericidal, and thiacetazone as being only a bacteriostatic, but a very cheap, drug. Although the very high cost of rifampicin would rule its use out for most countries, its very high bactericidal activity must be considered. This exceptional activity should enable rifampicin to be administered for a much shorter time in combination. Where funds are limited the following extremes are suggested: a single initial 3-fold the standard dose, or initial courses using the standard dose for 1, 2, 3 or 4 weeks, all in combination with another drug. Thus, while the principles for treating and controlling dapsone resistance are reasonably well worked out, it is clear that to put these into practice each country will have to very carefully consider which approach would be feasible and financially possible. It is also clear that whichever one is chosen, it must go in parallel with a greatly upgraded leprosy control service, which can only be achieved by extensive retraining of all personnel at all levels (guidelines on these requirements are set out in the "*Heathrow Report*", copies of which can be obtained from LEPRA).

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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

Received for publication 22 February, 1978.

0305–7518/78/0601–0101 $02.00/0

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.

Acknowledgements

This work was supported in part by Grant No. G.976/766 of the Overseas Development Administration, London, and by the World Health Organization as part of the THELEP programme.

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Lepr. Rev. (1978) **49**, 115–126

Drugs for Combined Therapy: Experimental Studies on the Antileprosy Activity of Ethionamide and Prothionamide, and a General Review

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The activity of ethionamide and prothionamide against *Mycobacterium leprae* has been evaluated using the mouse footpad model. The minimum effective doses of both drugs were found to be approximately 0.01%, and their minimal inhibitory concentrations were estimated to be about 0.05 μg/ml. Both compounds were found to be bactericidal against *M. leprae* at dietary concentrations of 0.1%. These findings indicate the importance of studies to evaluate the potential role of ethionamide and prothionamide in clinical practice. The available experimental evidence concerning the relative antileprosy activities of drugs that might be used in the combined treatment of lepromatous patients is reviewed.

Introduction

The importance of finding new antileprosy drugs for use in combined treatment to combat the growing problem posed by the widespread emergence of dapsone-resistant strains of *Mycobacterium leprae* was discussed in the previous paper (Colston *et al.*, 1978*b*). In the current paper we report the determination of the minimum effective doses (MEDs) of ethionamide and prothionamide against several strains of *M. leprae* using the mouse footpad technique. Estimations of the minimal inhibitory concentrations (MICs) of these 2 drugs against *M. leprae* are also described, together with studies to assess the extent of their bactericidal activity. Finally we review current knowledge concerning the relative activities of the presently available antileprosy drugs.

The antituberculosis drug ethionamide (α-ethyl thioisonicotinamide) has been used to a limited extent in the treatment of leprosy, but has not gained

Received for publication 9 March 1978.

0305–7518/78/0601–0115 $02.00/0

widespread acceptance because of the relatively high incidence of gastric intolerance when it is given at dose levels normally used for the treatment of tuberculosis (500–750 mg per day). The most extensive study of ethionamide in the treatment of leprosy was made by Rollier and Rollier (1972), who treated 102 lepromatous patients with an initial dosage of 1 g per day. However, because of side effects, they subsequently reduced the daily dosage to 500 mg for adults and 250 mg for children. They concluded that the lower dosage of ethionamide was well tolerated, and after 4 years treatment most of the patients had a Bacterial Index of zero. The acceptability of lower doses of ethionamide accords with the excellent tolerance of the drug encountered when it was given at a dosage of 250 mg per day in the earlier study of Floch, Rist and Jacobi (1966).

Prothionamide (α-propyl thioisonicotinamide), the propyl analogue of ethionamide, has largely replaced ethionamide in the treatment of tuberculosis, since its activity against *M. tuberculosis* is equal or slightly superior to that of ethionamide (Grumbach *et al.*, 1956; Rist, 1960; Noufflard-Guy-Loé and Berteaux, 1962), and because patients have generally been found to tolerate prothionamide slightly better than ethionamide (Bruet, Chevallier and Névot, 1962; Molina *et al.*, 1964; Chambatte *et al.*, 1965; Martin-Lalande *et al.*, 1966; British Tuberculosis Association, 1968; Co-operative Study Unit on Chemotherapy of Tuberculosis of the National Sanatoria in Japan, 1968; Fox *et al.*, 1969). Freerksen (1975) has advocated the use of prothionamide in combination treatment, but it is difficult to assess from published reports the precise dosage of prothionamide employed by his collaborators.

In the mouse footpad, ethionamide has been found to be inactive against *M. leprae* when fed at 0.001% in the diet, bacteriostatic at 0.01%, and bactericidal at 0.1% or 0.2% (Shepard, 1969*a*,*b*, 1976). Prothionamide has not been tested previously for antileprosy activity in the mouse footpad.

Methods

DETERMINATION OF THE MINIMUM EFFECTIVE DOSES

The minimum effective doses (MEDs) of ethionamide and prothionamide were determined using the methods described in the accompanying paper (Colston *et al.*, 1978*b*). Three strains of *M. leprae* were used (SBL 16220, SBL 15337 and TG), and the 2 drugs were administered at dietary concentrations of 0.001%, 0.003%, 0.01% and 0.03% commencing on the day of inoculation and continuing throughout the experiment.

ASSESSMENT OF BACTERICIDAL ACTIVITY

Ethionamide and prothionamide were administered to mice infected with *M. leprae* strain TG at dietary concentrations of 0.03%, 0.1% and 0.2%, starting on the day of inoculation and continuing for 60 days. Monitoring of bacillary growth and assessment of bactericidal activity were carried out as described previously (Colston *et al.*, 1978*b*).

DETERMINATION OF MOUSE SERUM CONCENTRATIONS OF ETHIONAMIDE AND PROTHIONAMIDE

Because of the insensitivity of currently available ultraviolet methods for determining ethionamide and prothionamide in the serum (see Jenner and Ellard, 1978), and the small amounts of serum obtainable from mice, a radiochemical method was devised for measuring mouse serum ethionamide and prothionamide concentrations using the same basic approach as that described for the determination of thiacetazone (Colston *et al.*, 1978*b*). A novel gas–liquid chromatographic procedure, capable of measuring down to about 0.2 µg ethionamide or prothionamide in 1 ml serum, was also devised and used to analyse sera from mice fed with the highest dietary concentrations of the 2 drugs (0.2%).

(a) *Radiochemical method*

Batches of ^{14}C-labelled ethionamide or prothionamide, with specific activities ranging from about 0.05–1 mCi/mM were prepared for feeding to mice at dietary concentrations of 0.2%–0.01%, respectively, by mixing methanolic solutions containing 1 mg/ml of ethionamide or prothionamide labelled in the thiocarbamyl group, and unlabelled ("cold") drug, and evaporating to dryness under reduced pressure.

Groups of 10 mice were fed for 24 h on diet containing 0.01%, 0.03%, 0.1% and 0.2% ^{14}C-labelled ethionamide or prothionamide, and the concentrations of the drugs determined in pooled serum. In order to ascertain whether prolonged administration might induce the metabolism of these drugs, other groups of mice were fed 0.1% unlabelled ethionamide or prothionamide for 14 days prior to feeding 0.1% of the ^{14}C-labelled formulations in the diet for 24 h and determining the serum concentrations of the 2 drugs.

A 1 ml aliquot of serum from mice fed for 24 h on diet containing ^{14}C-labelled ethionamide or prothionamide was pipetted into a small centrifuge tube together with 0.1 ml of a methanolic solution containing 1 mg/ml "cold" ethionamide or prothionamide to act as a "marker" to estimate the recovery of the drugs in the separation system, and ethionamide/prothionamide extracted by shaking with 2 ml ether. This solvent was chosen to minimize any potential contribution from sulphoxide metabolites of the drugs to the assay (Kane, 1962). After centrifugation the upper organic phase was decanted using a Pasteur pipette and then extracted by shaking with 0.5 ml 0.1 N hydrochloric acid. The lower acid extract was then transferred to another centrifuge tube, 0.1 ml M dipotassium hydrogen phosphate added and the ethionamide/prothionamide extracted by shaking with 0.1 ml chloroform.

The chloroform extract was dried onto a silica gel thin layer chromatography plate (Merck) and then developed with acetone/methanol (1:1 v/v) for 50 min. After drying and examining under ultraviolet light, the u.v.-absorbing spot was scraped off and eluted by shaking with 2 ml methanol in a small centrifuge tube. This was centrifuged and the overall recovery of ethionamide/prothionamide in the combined separation procedure was determined by measuring the extinction of the methanol extract at its absorption maximum (approximately 290 nm). A 1.5 ml aliquot of the methanol extract was then dried down in a scintillation vial, 5 ml of 0.6% butyl-PBD in toluene added and counted in a scintillation counter. The concentration of ethionamide/prothionamide in the original mouse serum could then be estimated by calculating the ratio of the extract's radioactivity [(counts per min) absorbance] and comparing it with the ratio for standards prepared from the appropriate ^{14}C-labelled thioamide preparation in blank serum, taken through the same procedure.

(b) *Gas–liquid chromatographic method*

A 1 ml aliquot of serum was pipetted into a small centrifuge tube together with 0.1 ml of a methanolic solution containing 10 µg/ml ethionamide/prothionamide (prothionamide for the determination of ethionamide; ethionamide for the determination of prothionamide) to act as internal standard and extracted by shaking with 4 ml ether. After centrifugation, the ether extract was extracted with 1 ml 0.1 N hydrochloric acid. The acid extract was then transferred to

another centrifuge tube and the thioamides extracted into 1 ml chloroform after the addition of 0.2 ml M dipotassium hydrogen phosphate. The chloroform extract was transferred to a small tapered centrifuge tube, evaporated to dryness at 50°C under a stream of nitrogen and the residue redissolved in 20 µl chloroform, 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 (7 ft long × 2 mm i.d.) silanized with dimethyldichlorosilane and packed with 3% OV-225 on Gas Chrom Q (100–120 mesh) using a Pye 104 instrument equipped with a flame-ionization detector. The injector and detector were maintained at 170° and 250°, respectively, and the flow-rates were carrier gas (nitrogen) 25, air 600 and hydrogen 50 ml/min. Under these conditions the approximate retention times of ethionamide and prothionamide were 1.6 and 2.2 min, respectively, and a linear relationship was obtained between the ratios of the peak heights of test drug to internal standard for initial ethionamide/prothionamide concentrations of 0.2 to 5 µg/ml.

Results

The results of the studies to determine the minimum effective doses of ethionamide and prothionamide are summarized in Table 1. The growth of the 3 strains of *M. leprae* was partially suppressed by feeding 0.003% ethionamide in the diet, and totally suppressed by 0.01%, confirming the results previously obtained by Shepard (1969*a*). The multiplication of strains SBL 15337 and TG was completely prevented by feeding 0.01% prothionamide, although 0.03% was required to entirely suppress the growth of strain SBL 16220. It was therefore concluded that the MEDs of both drugs were approximately 0.01%.

TABLE 1

Estimation of the minimum effective dose (MED) of ethionamide and prothionamide against M. leprae

M. leprae strain	Percentage drug in the diet 0.001	0.003	0.01	0.03	MED
		Ethionamide			
SBL 16220	—	5/6†	0/6	0/6	0.01%
SBL 15337	—	4/6	0/6	0/6	0.01%
TG	6/6	5/6	0/6	—	0.01%
		Prothionamide			
SBL 16220	—	6/6	2/6	0/6	0.03%
SBL 15337	—	6/6	0/6	0/6	0.01%
TG	6/6	4/6	0/6	0/6	0.01%

* Not tested.

† Number of footpads positive/number of footpads harvested.

The concentrations of ethionamide and prothionamide in the serum of mice fed various dosages of the 2 drugs determined using the radiochemical method are illustrated in Fig. 1. The results obtained when mice were fed 0.2% of the drugs in the diet were confirmed using the gas–liquid chromatographic method. Serum concentrations of both drugs were approximately proportional to the percentages fed in the diet, and, since there was no significant difference between the results obtained for ethionamide and prothionamide, a single regression line was plotted. The serum concentrations of ethionamide and pro-

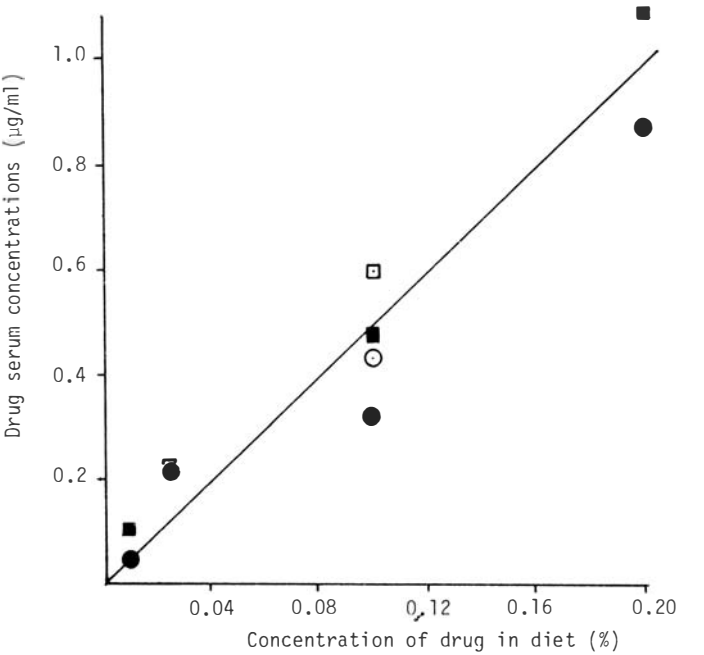

Fig. 1. Serum concentrations of ethionamide (●) and prothionamide (■) of mice fed for 24 h on diet containing graded doses of ^{14}C-labelled drug. Open symbols (⊙, ⊡) represent the values obtained in mice fed for 14 days with unlabelled drug, followed by labelled drug for 24 h.

thionamide in mice that had been fed with the drugs for 14 days were no lower than those that had only been fed on drug-containing diets for 24 h, indicating that the metabolism of neither drug had been induced.

The minimal inhibitory concentrations (MICs) of both ethionamide and prothionamide against *M. leprae,* defined as the serum concentrations present in mice fed with the MEDs of the 2 drugs (0.01%), were estimated from the data illustrated in Fig. 1 as being about 0.05 μg/ml. Thus, as had been previously demonstrated with *M. tuberculosis*, both drugs appear to have rather similar antimycobacterial activity.

The results of the kinetic experiment to assess the potential bactericidal activity of the 2 drugs are summarized in Table 2. Neither ethionamide nor prothionamide showed any bactericidal activity when administered for 60 days at 0.03% as indicated by excess growth delays of only 16 days. However, when the dietary concentration was increased to 0.1%, multiplication of bacilli could not be detected for periods of up to 442 days *post inoculum* when the final harvest was made. The excess growth delay, calculated by drawing a line through the final point parallel to the growth curve for the untreated controls, was 215 days for ethionamide and 225 days for prothionamide. The excess growth delay engendered by feeding 0.2% ethionamide for 60 days was 209 days, while in the single mouse fed on 0.2% prothionamide and sacrificed at day 442 after inoculation, there was no evidence of multiplication of *M. leprae.*

TABLE 2

Assessment of antibacterial activity by the kinetic technique: the effect of feeding of graded doses of ethionamide and prothionamide in the diet on the growth of M. leprae *in mouse footpads*

Drug	Dose*	Growth delay (days)	Excess growth delay† (days)
Ethionamide	0.03%	76	16
	0.1%	275	215‡
	0.2%	269	209‡
Prothionamide	0.03%	75	16
	0.1%	285	225‡
	0.2%	>332	>272

* Drug administered for 60 days, from day 0.
† Growth delay less period of drug administration.
‡ Estimate based on 1 positive footpad count.

These results indicate that feeding mice with 0.1% or 0.2% ethionamide or prothionamide resulted in significant killing of *M. leprae*, confirming the findings previously reported by Shepard for ethionamide (Shepard, 1969*a,b,* 1976). The similarity in the bactericidal activities displayed by ethionamide and prothionamide is also in accord with the results obtained using the more rigorous proportional bactericidal test method (Colston, Hilson and Banerjee, 1978). The bactericidal activity of ethionamide against *M. leprae* parallels that previously demonstrated against *M. tuberculosis* both *in vitro* and *in vivo* in the mouse (Dickinson and Mitchison, 1966; Rist *et al.*, 1958).

Discussion

ANTILEPROSY ACTIVITY OF ETHIONAMIDE AND PROTHIONAMIDE

The results obtained in this investigation indicate that these 2 drugs deserve serious consideration as candidates for use in the combined treatment of lepromatous leprosy. The minimal inhibitory concentrations of ethionamide and prothionamide against *M. leprae* determined in this study using the mouse footpad model were about 0.05 μg/ml, or about a tenth of the MICs originally reported against *M. tuberculosis* using conventional *in vitro* techniques (Grumbach *et al.*, 1956; Rist, 1960; Noufflard-Guy-Loé and Berteaux, 1962). It would however be unwise to conclude that *M. leprae* is necessarily much more sensitive than *M. tuberculosis* to inhibition by the thioamides, since it is possible that the sulphoxide metabolites of the 2 drugs that are formed in man and in the mouse (Kane, 1962; Johnson, Kane and Kibby, 1967; Rossi and Rübsammen, 1977) and have significant antituberculosis activity (Libermann, Rist and Grumbach, 1963; J. Peters and T. Welch, pers. comm., 1978) might also contribute to their antileprosy activity *in vivo.*

Peak serum concentrations of ethionamide and prothionamide are approximately proportional to dose, with concentrations of about 3 μg/ml being achieved after giving 500 mg of either drug (Riddell, 1960; Gray, Hamilton

and Eidus, 1962; Hughes, Smith and Kane, 1962; D. F. Muggleton, pers. comm., 1965; Jenner and Ellard, 1978). This suggests that, at this dose, peak serum concentrations of the drugs would exceed their MICs against *M. leprae* by a factor of approximately 60-fold. Thereafter serum concentrations fall with a half-life of about 2 h and by 24 h are of the same order as the MIC against *M. leprae* (Jenner and Ellard, 1978). Since significant bactericidal activity against *M. leprae* was demonstrated when ethionamide or prothionamide were fed to mice at 0.1%, and since such dietary concentrations gave rise to serum concentrations of about 0.5 μg/ml, there would seem to be reasonable grounds for hoping that bactericidal activity might be achieved in clinical practice if daily doses of 250–500 mg were given. Furthermore, in view of the established dose dependence of gastric intolerance of the 2 drugs and the appreciably better acceptability of prothionamide, it is likely that daily dosage with 250 mg prothionamide would be excellently tolerated by patients.

REVIEW OF EXPERIMENTAL EVIDENCE CONCERNING THE RELATIVE ACTIVITIES OF ANTILEPROSY DRUGS

The experimental data currently available concerning the relative activities of antileprosy drugs are summarized in Table 3. This Table shows the MICs of the drugs against *M. leprae* determined in the mouse footpad system, the ratios of peak serum concentrations to MIC for doses of the drugs that are well tolerated by patients, the durations for which serum concentrations exceed the MIC after such doses and the degree of bactericidal activity assessed using the proportional bactericidal test method (Colston *et al.*, 1978*a*).

TABLE 3

Minimal inhibitory concentrations against M. leprae *(MICs), peak serum concentrations, durations of coverage and bactericidal activities of antileprosy drugs*

Drug	MIC (μg/ml)	Dosage (mg)	Ratio peak serum MIC	Duration for which serum concs exceed MIC (days)	Bactericidal§ activity
Dapsone	0.003	100	500	10	+
Acedapsone	0.003*	225	15	200	N.T.†
Rifampicin	0.3	600	30	1	+++
Sulphadimethoxine	20	1500	7	4	N.T.
Sulphadoxine	35	1500	4	14	N.T.
Sulphamethoxypyridazine	30	1000	3	3	N.T.
Thiambutosine	0.5	1500	1	<1	—
Thiacetazone	0.2	150	8	1	—
Ethionamide‡	0.05	500	60	1	++
Prothionamide‡	0.05	500	60	1	++

* Acedapsone is inactive against *M. leprae* but is converted to dapsone – the figures for MIC and peak serum concentration refer to the values for dapsone.

† Not tested.

‡ Results obtained in the present study.

§ –, Purely bacteriostatic; +, ++, +++, relative degrees of bactericidal activity.

A considerable body of evidence is available concerning the activity and pharmacology of the clinically established drugs dapsone, acedapsone (DADDS), and rifampicin. The MIC of dapsone was shown by Peters *et al.* (1975*b*) and Levy and Peters (1976) to be about 0.003 μg/ml. Since peak serum levels in man following a dose of 100 mg dapsone are approximately 1.5 μg/ml (Glazko *et al.,* 1968; Peters, Gordon and Karat, 1975; Committee on Experimental Chemotherapy, 1976), the ratio of peak serum concentration to MIC is approximately 500. The serum half-life of dapsone has now been determined in over 200 patients, and found to vary from 13–53 h (Peters *et al.*, 1972; Ellard, Gammon and Harris, 1974; Peters *et al.*, 1974; Gelber and Rees, 1975; Peters *et al.*, 1975*a*, 1976, 1977). Assuming a representative value of 27 h, one can calculate that a 100 mg dose of dapsone will maintain serum concentrations in excess of the MIC for about 10 days.

Dapsone serum levels in excess of the MIC against *M. leprae* can be maintained for much longer periods by intramuscular injection of acedapsone (Glazko *et al.*, 1968; Ozawa *et al.*, 1971) A dose of 225 mg acedapsone produces mean peak dapsone plasma concentrations of 0.046 μg/ml, approximately 15 times the MIC, and it may be calculated from the data published by Peters *et al.*, (1977) that plasma concentrations would be maintain above the MIC for about 200 days.

The MIC of rifampicin has been estimated as 0.3 μg/ml (Holmes and Hilson, 1972; Holmes, 1974), and since serum plasma concentrations after dosage with 600 mg are approximately 10 μg/ml, with a half-life of 2–3 h (Furesz *et al.*, 1967; Dans *et al.*, 1970; Acocella *et al.*, 1971, 1972; Jeanes, Jessamine and Eidus, 1972; Boman, 1974; Garnham *et al.*, 1976; Männistö, 1977), the ratio of peak serum concentration to MIC is about 30-fold with inhibitory serum levels being maintained for about 24 h. It should however be noted that more recent estimates of the MED of rifampicin against *M. leprae* (Waters *et al.*, 1978) are approximately 4 times that originally determined by Holmes and Hilson (1972).

The pertinent data for sulphadimethoxine and sulphadoxine were reported earlier (Ellard, Gammon and Rees, 1970), while the studies from which the values for sulphamethoxypyridazine, thiambutosine and thiacetazone were derived are described in the accompanying paper (Colston *et al.*, 1978*b*). The relative bactericidal activities shown in the last column of Table 3 were determined using the proportional bactericidal test method (Colston *et al.*, 1978*a*). The doses of drugs tested in that investigation probably gave serum concentrations in mice that are similar to those achieved in patients under treatment and the relative bactericidal potencies determined experimentally in this way correlated closely with those realized in clinical practice. Corresponding data are not available for clofazimine since its accumulation in reticulo-endothelial cells makes it impossible to estimate its MIC against *M. leprae* (Banerjee *et al.*, 1974; Levy, 1974), although it is likely that after an extended period of treatment effective concentrations would be maintained for many weeks. Its bactericidal activity assessed using the proportional bactericidal test method was intermediate between that of dapsone and rifampicin (Colston *et al.*, 1978*a*).

It must be emphasized that the data assembled in Table 3 are very imprecise since the MIC determinations only have the precision with which the MEDs of the drugs have been estimated and because it is usually only practicable to determine MEDs using dietary doses differing by factors of at least 3-fold. Nevertheless the markedly superior ratios of peak serum concentrations to MIC, durations of coverage and estimates of the bactericidal activity displayed by the 2 most clinically potent antileprosy drugs, dapsone and rifampicin, indicate the potential relevance of such properties in attempting to assess the prospects of other medicaments that have yet to be evaluated using properly monitored controlled clinical trials. Thus the substantially lower peak serum/MIC ratios of sulphadimethoxine, sulphadoxine and sulphamethoxypyridazine suggest that the use of these long-acting sulphonamides should be discontinued.

The cross-resistance known to exist with *M. tuberculosis* between the diphenylthioureas, thiacetazone and thioamides (Rist *et al.*, 1959; Bartmann, 1960; Grosset and Benhassine, 1970) indicates that a similar phenomenon might occur for *M. leprae* and that these drugs should therefore be regarded as belonging to the same general class. The evidence summarized in Table 3 and discussed in detail in the previous paper (Colston *et al.*, 1978*b*) suggests that thiacetazone is a much better candidate for use in combined therapy than thiambutosine. However the results of the present study indicate that ethionamide and prothionamide are even more promising and strongly encourage the initiation of detailed investigations into the clinical and bacteriological efficacy of the 2 drugs.

Acknowledgements

This work was supported in part by Grant No. G. 976/766 of the Overseas Development Administration, London, and by the World Health Organization as part of the THELEP programme. We should also like to thank Dr M. Davis of May and Baker and Dr P. Frechet, formerly of Theraplix, for their help in obtaining samples of ^{14}C-labelled ethionamide and prothionamide.

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Lepr. Rev. (1978) **49**, 127–130

Proven Primary Dapsone Resistance in Leprosy—A Case Report

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A patient is described who at the age of 10 years developed tuberculoid leprosy; during the next 2 years while on oral dapsone therapy, his condition deteriorated and he became lepromatous. Both clinically, and experimentally by drug sensitivity testing in mice, his strain of *Mycobacterium leprae* was found to be fully resistant to dapsone. His father was a known case of secondary (acquired) dapsone resistance. The potential medical and economic importance of primary sulphone resistance is discussed.

Introduction

Clinical and experimental proof of secondary dapsone resistance (DR) in lepromatous leprosy was first obtained in 1964 by Pettit and Rees, more than 20 years after the introduction of the sulphone drugs. In their definitive account, Pettit *et al.* (1966) predicted that sooner or later primary cases of sulphone resistance would arise. Despite this warning, there is an apparent lack of anticipation of the condition. Therefore we now report our first proven primary-resistant patient, the son of one of the original resistant cases.

Case Report

The patient, ZA, was born in 1964. Both his parents were receiving treatment for lepromatous leprosy. His father (case number 9 of Pettit *et al.*, 1966) had been admitted to Sungei Buloh Leprosarium in 1950, but by 1964 he had relapsed with active leprosy. As his strain of *Mycobacterium leprae* was found to be sensitive to 0.025% dapsone in the mouse diet (but was not tested against lower concentrations of drug), and as he showed initial clinical improvement on dapsone 300 mg twice weekly by injection, he was reported as not suffering from DR. However, after a year he began slowly once again to relapse; his strain of *M. leprae* obtained in 1968 was shown to be resistant to 0.01% dapsone in the mouse diet (equivalent to a dosage of 100 mg dapsone daily in a full-sized adult); and his treatment was changed to clofazimine in May, 1969.

Received for publication 26 January, 1978.

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0305–7518/78/0601–0127 $01.00/0

His son, ZA, presented in 1970, with 2 hypopigmented macules. A diagnosis of indeterminate leprosy was tentatively made; however, no treatment was given, but the patient was placed under observation. In May 1974, he was found to have developed an anaesthetic, hypopigmented patch on his back, and the left great auricular nerve was thickened. Tuberculoid leprosy was diagnosed, and treatment was commenced with dapsone 100 mg twice weekly by mouth. His supply of dapsone was collected regularly every 3 months. However, in September 1975, his lesions became red and swollen, new lesions appeared and the left ulnar nerve was noticed to be enlarged and tender. A "reversal" or "upgrading" reaction, due to increase in cell-mediated immunity under effective treatment (Ridley, 1969; Waters *et al.*, 1971), was diagnosed, and treated with a short course of prednisolone. The reaction recurred in June, 1976; treatment with prednisolone was recommenced and he was admitted to the leprosarium, where the dosage was increased to 200 mg twice weekly and given by injection.

When first seen in the Leprosy Research Unit, in July 1976, ZA was clinically suffering from active borderline-lepromatous (BL) (Ridley and Jopling, 1966) leprosy in mild reaction, with multiple vague hypopigmented macules, erythematous moist plaques and papules, and widespread moderate nerve enlargement with some nerve tenderness. Slit skin-smears gave an average bacterial index of 3.7, and a morphological index of 2. Skin and superficial radial nerve were biopsied; histological examination of the skin revealed active sub-polar lepromatous leprosy (LLs) (Ridley, 1974), with minimal reaction (Dr D. S. Ridley) and that of the nerve showed early lepromatous leprosy developing from borderline leprosy (Dr A. C. McDougall).

As, over the course of 2 years, ZA had "downgraded" from presumed borderline-tuberculoid (BT) to lepromatous (BL/LLs) leprosy, it was considered that either he had failed to take the prescribed dapsone, or else he had suffered from the first from (primary) DR. Treatment was continued for 3.5 months with dapsone twice weekly by intramuscular injection but without any therapeutic response. His skin lesions remained active, and neuritis, especially of the left median and ulnar nerves, persisted. Nerve function began to deteriorate. Therefore treatment was changed in October to rifampicin and clofazimine. Rapid clinical improvement ensued, although the neuritis temporarily relapsed due to a reversal reaction. After 6 months on this alternative, effective treatment the skin lesions were all inactive. The skin was once again biopsied, and histological examination revealed quiescent mainly BT leprosy (but with some lepromatous foci), acid-fast bacilli were present in nerves only, and it was considered that marked upgrading towards tuberculoid leprosy had occurred (Dr D. S. Ridley).

Experimental Methods and Results

M. leprae obtained from a fresh tissue skin biopsy in July 1976, and inoculated into mouse footpads (Rees, 1967), multiplied in mice receiving 0.01% dapsone in their diet.

An aliquot of the superficial radial nerve, biopsied in August 1976, was sent

as fresh tissue on wet ice to the National Institute for Medical Research, where a suspension of *M. leprae* was prepared from it, and inoculated into mouse footpads. This strain was also found to be fully resistant to 0.01% dapsone in the mouse diet.

Discussion

The history of our patient is a salutory warning to all engaged in leprosy control schemes. Although secondary dapsone resistance (DR) has only been reported in leprotamous (LL and BL) leprosy (Pearson *et al.*, 1975), it was anticipated that primary resistance would occur in any type of leprosy, with grave economic implications for developing countries (WHO 5th Expert Committee on Leprosy, 1977).

Our patient was suspected of suffering from indeterminate leprosy at the age of 6 years. Four years later, tuberculoid leprosy was diagnosed, and dapsone therapy was commenced. It is academic to argue whether or not he took his treatment regularly or at all, as either way the evidence confirms primary drug resistance. Relapse due to the development of secondary DR has not been detected in Malaysia in less than 5 years of treatment (Pearson *et al.*, 1975) and in all cases there was an initial response to therapy. This is in marked contrast to ZA. The whole progression of his disease, throughout his 2.4 years on dapsone, is in keeping with that of a patient receiving no effective treatment, with the development and spread of new skin lesions, the increasing involvement of the nerves of predilection, and the loss of cell-mediated immunity associated with a shift from BT to LLs leprosy. Therefore he underwent a mild "downgrading", not a "reversal" reaction. We have observed a similar downgrading from borderline to LLs leprosy in a patient undergoing investigation for secondary thiambutosine resistance (Pearson and Waters, unpublished data).

DR was proved, first clinically by his failure to respond while receiving 3.5 months of parenteral sulphone therapy in hospital, and then experimentally by dapsone-sensitivity studies in mice of his strain of *M. leprae* obtained from both skin and nerve (performed in Malaysia and London respectively). Both tests showed that his strain was fully resistant to dapsone, at the same level as the strain obtained from his father in 1968. During the first 5 years of his life, there was ample opportunity for ZA to become infected with the latter strain of *M. leprae*, as it was not until 1969 that his father's treatment was changed.

Primary DR is now a very real risk wherever secondary DR has occurred. *Prime facie* evidence of primary resistance has been reported from Columbia (Londono, 1977), and from Micronesia, where 2 child contacts of a known resistant patient developed tuberculoid leprosy (Russell *et al.,* 1976). Pearson *et al.* (1977b), in the first organized survey for primary DR, found that 5 of 8 strains of *M. leprae* from newly diagnosed cases of lepromatous leprosy in Addis Ababa, where there is a very high incidence of secondary DR (Pearson *et al.,* 1977*a*) were resistant to dapsone; although the levels of drug sensitivity were not fully titrated at least 2 strains were resistant to 0.001% dapsone in the mouse diet. Jacobson (1977) has also briefly reported the detection of 4 cases of primary DR, at the 0.0001% (but sensitive at the 0.001%) level in the diet of

the mouse. As usual, prevention is to be preferred to cure; this in turn implies the full-scale application of those measures recommended by the WHO 5th Expert Committee on Leprosy (1977) designed to keep the incidence of secondary DR to a minimum, and to recognize it early and to treat it effectively whenever it occurs.

Our patient is important, not because he is perhaps the first case of primary DR to be both clinically and experimentally proven, but rather because his history illustrates the risks associated with unrecognized primary resistance occurring in tuberculoid leprosy. There is a great need for an increased awareness of the condition. This will involve better training of both doctors and para-medical workers involved in leprosy control schemes. It also implies better communication between those treating diagnosed cases of DR and those involved in the tracing and supervision of contacts, although the majority of primary DR patients may well have no history of household or familial leprosy.

Acknowledgements

We should like to thank Dr D. S. Ridley (Hospital for Tropical Diseases, London) and Dr A. C. McDougall (Slade Hospital, Oxford) for their histological reports, and Dr J. M. H. Pearson for his helpful comments. The Leprosy Research Unit is jointly sponsored by the Malaysian Ministry of Health and the British Medical Research Council.

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Lepr. Rev. (1978) **49**, 131–140

The Action of Dapsone on a Susceptible Strain of *Mycobacterium kansasii*

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Because studies of the action of dapsone on *Mycobacterium leprae* have been obstructed by the need to conduct the studies in infected animals, a study of the action of the drug has been carried out on a strain of *M. kansasii* shown to be inhibited by 0.3 μg dapsone per ml, a concentration 100 times larger than the minimal inhibitory concentration of the drug for *M. leprae*. In stationary broth cultures, dapsone was bactericidal in concentrations of 1.0 μg per ml or larger; populations of *M. kansasii* as small as 1.7×10^6 organisms appeared to contain individuals resistant to 1.0 and 10 μg dapsone per ml. The organisms were shown to bind the drug against a concentration gradient. The action of the drug was antagonized by 4-aminobenzoic acid (PABA) in a mole ratio (PABA: dapsone) of 2:1. PABA itself, in a concentration of 10 μg per ml, inhibited multiplication of this strain of *M. kansasii*.

Introduction

Dapsone (4,4′diaminodiphenylsulfone, DDS) is widely used in the treatment of leprosy, and *Mycobacterium leprae* has been shown to be exquisitely susceptible to the drug (Peters *et al.*, 1975). However, because *M. leprae* has not yet been cultivated in a cell-free medium, many aspects of the action of DDS on the organism have been little studied. Earlier workers studied the susceptibility to DDS of a variety of cultivable mycobacterial species in a search for chemotherapeutic agents active in various mycobacterial diseases, and also in an effort to find a cultivable mycobacterial species that could serve as a surrogate for *M. leprae* in studies of drug action. The description by Pattyn and van Ermengem (1968) of strains of *M. kansasii* more susceptible to dapsone than any cultivable mycobacterial strain heretofore described stimulated us to attempt to characterize the antimycobacterial action of this drug.

In the work to be described, we have measured the minimal inhibitory concentration (MIC) of dapsone for one rather suceptible strain of *M. kansasii* both on solid and in liquid media, and demonstrated that dapsone is

Received for publication 12 January, 1978.

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0305–7510/78/0601–0131 $01.00/0

bactericidal in concentrations not much greater than the MIC. In addition, we have adduced evidence that the drug is bound by the organisms. Finally, we have studied the combined effects of dapsone and 4-aminobenzoic acid (PABA) on this strain of *M. kansasii*.

Materials and Methods

Dapsone was purchased from K & K Laboratories, Hollywood, California; PABA was purchased from General Biochemicals, Chagrin Falls, Ohio. The strain of *M. kansasii* was supplied by S. R. Pattyn, Prince Leopold Institute for Tropical Medicine, Antwerp, Belgium (Pattyn and van Ermengem, 1968).

Cultures of *M. kansasii* were performed in Dubos broth supplemented with albumin (Difco Laboratories, Detroit, Michigan), Dubos agar to which oleic acid-albumin supplement had been added (Difco), or glycerol-urea agar (Tsukamura, 1967). To prepare inocula, the surface growth of a culture of the organism was carefully scraped from a Lowenstein-Jensen slant and weighed in a tared vial. A measured quantity of Dubos broth was added and the bacterial mass suspended with the aid of a variable-speed mixer. Single-cell inocula of *M. kansasii* were prepared by filtering portions of the bacterial suspension through a membrane filter with a mean pore size of 5 μm.

Direct counts of the organisms were performed on Reich counting slides (Bellco Glass, Vineland, New Jersey) according to the method of Shepard and McRae (1968). A typical preparation was found to contain 86% of the organisms as individuals and 14% in clumps of 2 organisms; no larger clumps were encountered. The plating efficiency of these suspensions was 92% on the average.

All cultures were incubated at 37°C. Growth of the organisms in broth was measured as the absorbance at 580 nm in a Coleman Sr. spectrophotometer. A comparison of direct counts with turbidimetric measurements of suspensions of *M. kansasii* in Dubos broth demonstrated that the concentration of organisms was linearly related to the absorbance of the suspension according to the regression equation: number of organisms $\times 10^{-7}$ per ml $= 7.40 + 23.6$ (absorbance $- 0.282$).

The concentration of dapsone was measured by fluorescence assay (Peters *et al.*, 1970). The drug was found to be stable in Dubos broth incubated at 37°C for 2 months.

Results

The first studies of the inhibition by dapsone of the multiplication of *M. kansasii* were performed on solid media. Glycerol-urea and Dubos agar plates containing no drug or 0.1, 0.3, 0.5, or 1.0 μg dapsone per ml were inoculated with about 60 organisms per plate from a suitably diluted single-cell suspension and incubated: colonies were enumerated at intervals for 2 months. The results of this experiment, presented in Table 1, show that colonies of *M. kansasii* generally appeared earlier on Dubos than on glycerol-urea agar. Dapsone in a concentration of 0.1 μg per ml slowed the appearance of colonies in relation to the time of appearance of colonies on drug-free medium. No

colonies appeared on glycerol-urea agar containing 0.5 μg dapsone per ml or on either medium containing 1.0 μg dapsone per ml. Only about three-quarters of the expected number of colonies appeared on the plates containing 0.3 μg dapsone per ml. Thus, slowing of the rate of appearance of colonies—i.e. of the rate of growth of the organisms—appears to be characteristic of the inhibition by dapsone of the growth of *M. kansasii* on solid media. The MIC of dapsone for this strain of *M. kansasii*, measured on solid media, appeared to lie in the range 0.3–0.5 μg per ml.

TABLE 1

Rate of appearance of colonies of M. kansasii *on minimal and complex solid media in presence of dapsone*

Duration of incubation (days)	Number of CFU per plate								
	Dapsone concentration (μg/ml)								
	0		0.1		0.3		0.5	1.0	
	GU*	Du†	GU	Du	GU	Du	GU	GU	Du
13	0	62 (46–78)‡	0	0	0	0	0	0	0
17	48 (34–62)	64 (48–80)	0	52 (38–66)	0	0	0	0	0
20	52 (38–66)	64 (48–80)	0	60 (45–75)	0	0	0	0	0
29	58 (43–73)	64 (48–80)	86 (68–104)	60 (45–75)	0	0	0	0	0
44	58 (43–73)	64 (48–80)	86 (68–104)	60 (45–75)	39 (27–51)	53 (39–67)	0	0	0

* Glycerol-urea agar
† Dubos agar
‡ 95% confidence limits.

Next, the effect of inoculum size on dapsone inhibition in broth cultures of *M. kansasii* was examined. Replicate tubes of Dubos broth containing a variety of concentrations of dapsone were inoculated with serial 10-fold dilutions of a suspension of *M. kansasii*, and the number of days of incubation before the appearance of turbidity was recorded (Table 2).

The MIC of dapsone for this strain of *M. kansasii*, measured in broth, appeared to lie in the range 0.1–1.0 μg per ml. The turbidity of the cultures inoculated with 1.7×10^7 organisms per ml, which had been rendered turbid by the inoculum, increased markedly after incubation for 1 day. All of the tubes inoculated with 1.7×10^6 organisms per ml were turbid after 2 days of incubation. Those cultures receiving smaller inocula became turbid later. The appearance of turbidity was further delayed in the tubes containing 0.1 μg dapsone per ml.

Turbidity appeared in 3 sets of cultures only after incubation for 22 days;

aliquots from these cultures were plated on dapsone-containing Dubos agar. The cultures inoculated with 1.7×10^5 *M. kansasii* per ml yielded growth on agar containing 1.0 or 10 μg dapsone per ml. Those inoculated with 1.7×10^4 organisms per ml yielded growth on agar containing 1.0 μg dapsone per ml, but not on agar containing 10 μg dapsone per ml. Thus, populations of 1.7×10^5 *M. kansasii* (10 ml, 1.7×10^4 organisms per ml) did not include individual organisms resistant to 10 μg dapsone per ml.

TABLE 2

Effect of inoculum size on inhibition of M. kansasii *by dapsone*

Number of *M. kansasii* inoculated per ml	Number of days from inoculation to appearance of turbidity				
	Dapsone concentration (μg/ml)				
	0	0.1	1.0	10	100
1.7×10^7	1	1	1	1	1
1.7×10^6	2	2	2	2	2
1.7×10^5	4	6	22	22	>24
1.7×10^4	7	8	22	>24	>24

The cultures containing 100 μg dapsone per ml that had been inoculated with 1.7×10^4 *M. kansasii* per ml, all of which failed to become turbid, were filtered onto membrane filters with a mean pore size of 0.15 μm. The filters were washed with sterile Dubos broth, transferred to the surfaces of drug-free Dubos agar plates, and incubated. No colonies appeared on the filters after incubation for 6 weeks, whereas colonies appeared after incubation for only $2\frac{1}{2}$ weeks on filters that had been used to filter drug-free cultures, demonstrating that dapsone in a concentration of 100 μg per ml had been bactericidal.

To obtain further evidence of the bactericidal activity of dapsone on *M. kansasii*, replicate sets of tubes of Dubos broth into which dapsone had been incorporated in concentrations of 1, 2, 4 or 8 μg per ml were inoculated with 2×10^3 *M. kansasii* per ml and incubated. After 1, 4 and 8 days of incubation, 0.1 ml aliquots were removed from each culture and spread on the surface of drug-free Dubos agar plates, which were incubated and observed at intervals during 8 weeks for the appearance of colonies. All 4 concentrations of dapsone were found to be bactericidal to *M. kansasii*. After incubation for 8 days, the mean number of colonies per ml of the cultures containing 1 μg dapsone per ml had been reduced by 80%, and no colonies were seen on the plates inoculated with the aliquots from the cultures containing 4 and 8 μg dapsone per ml. As shown in Fig. 1, the plots of the common logarithm of the mean number of CFU per ml of culture as a function of time of incubation are fairly linear, and give half-times of killing of *M. kansasii* of 2.8 days for 1 μg dapsone per ml, 1.4 days for 2 μg dapsone per ml, and 0.73 days for 4 and 8 μg dapsone per ml. Only the slopes of the regression lines corresponding to 1 and 8 μg dapsone

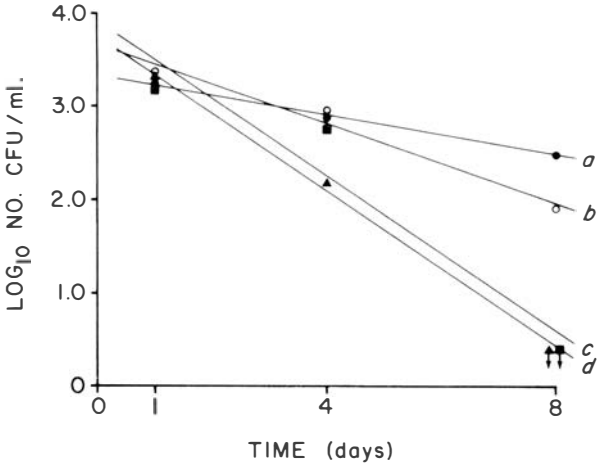

Fig. 1. Regression of the $\log_{10}$ number of CFU of *M. kansasii* per ml culture on the duration of incubation in the presence of several concentrations of dapsone; line *a* (●—●), 1 μg dapsone per ml; line *b* (○—○), 2 μg dapsone per ml; line *c* (■—■), 4 μg dapsone per ml; line *d* (▲—▲), 8 μg dapsone per ml.

per ml are significantly different from 0; they are also significantly different from each other (Goldstein, 1964).

To examine the possibility of binding of dapsone by *M. kansasii*, replicate sets of tubes of Dubos broth containing 0, 0.1, 1.0, 10 and 100 μg dapsone per ml were inoculated with 2.5×10^7 organisms per ml and incubated. Aliquots were removed for filtration on membrane filters with a mean pore size of 0.15 μm at intervals during 8 days. The concentration of dapsone was determined in both the culture filtrates and the filtered organisms (see Table 3). No dapsone was detected in the organisms filtered from the cultures containing

TABLE 3

Binding of dapsone by M. kansasii

Time of incubation (h)	Concentration of dapsone in medium (μg/ml)					
	0.1	1.0	10		100	
	DDS_C* (μg/mg)	DDS_C (μg/mg)	DDS_C (μg/mg)	Ratio†	DDS_C (μg/mg)	Ratio
54	<0.01	<0.01	0.08	8.1	0.71	7.1
198	<0.01	<0.01	0.14	14.0	1.5	15.0

* Dapsone concentration of filtered *M. kansasii*. Bacterial mass calculated from absorbance measured immediately before filtration, according to the experimentally-derived relationship: Absorbance = 0.282 + 0.627 (bacterial mass in mg − 0.5).

† $DDS_C \times 1000$/ concentration of dapsone in medium.

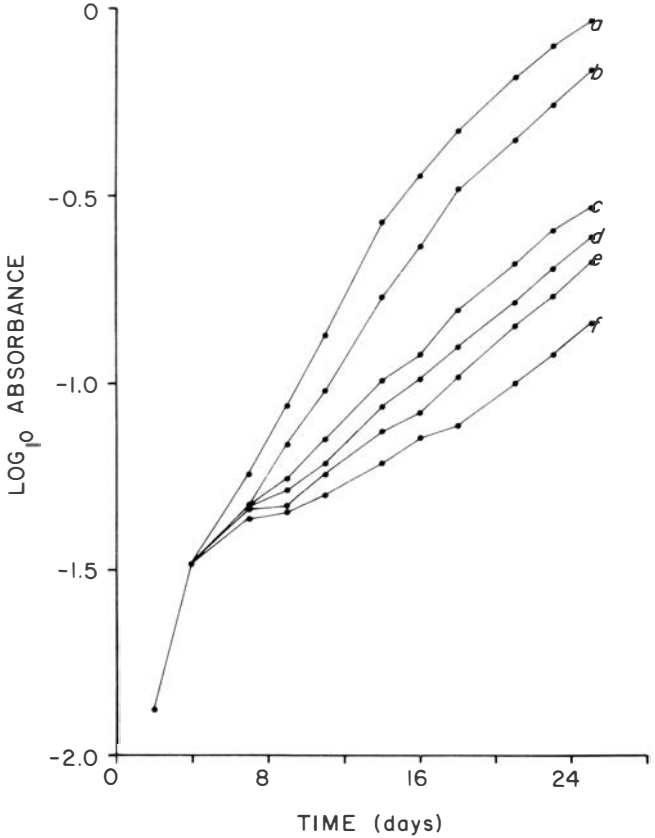

Fig. 2. Log_{10} absorbance of broth cultures of *M. kansasii* as a function of duration of incubation. Curve *a* represents multiplication of the organisms in drug-free broth, in broth containing 0.01, 0.1 or 1.0 μg PABA per ml, and in broth containing 0.1 μg dapsone *plus* 0.1 μg PABA or 0.8 μg dapsone *plus* 1.0 μg PABA per ml. Curve *b* represents the growth curve of *M. kansasii* in medium containing 10 μg PABA per ml or 0.8 μg dapsone *plus* 0.1 μg PABA per ml. Curves *c, e,* and *f* are respectively the growth curves of *M. kansasii* in the presence of 0.1, 0.4, and 0.8 μg dapsone per ml. Curve *d* represents the multiplication of the organisms in broth containing 0.2 μg dapsone per ml or 0.8 μg dapsone *plus* 0.01 μg PABA per ml.

0.1 and 1.0 μg dapsone per ml. Analysis of the filtrates from the cultures containing 1 and 10 μg dapsone per ml revealed that there had been no loss of dapsone nor production of monoacetyldapsone during the incubation. The organisms filtered from the cultures containing 10 and 100 μg dapsone per ml were found to contain dapsone in concentrations 7- to 8-fold larger than that of the medium after a little more than 2 days' incubation, and 14- to 15-fold that of the medium after incubation for a little more than 8 days.

To measure the effect of PABA on inhibition by dapsone of the multiplication of *M. kansasii* in liquid medium, replicate sets of tubes containing drug-free Dubos broth or broth into which had been incorporated dapsone, PABA,

or both in a variety of concentrations were inoculated with a single cell suspension of *M. kansasii* providing a concentration of 6.7×10^5 organisms per ml and incubated. The turbidity of the cultures was measured 3 times weekly. The results of this experiment are shown in Fig. 2 as plots of the common logarithm of the absorbance as a function of the duration of incubation. For the sake of clarity, similar growth curves of *M. kansasii* have been combined. Taken together, the curves suggest that the absorbance increases logarithmically with time over the absorbance range 0.050–0.300, whereas the increase is arithmetic above an absorbance value of 0.400. During the period of logarithmic multiplication in the drug-free cultures (curve *a*), the organisms doubled at the rate of once every 3.3 days. All 4 concentrations of dapsone exerted an inhibitory effect on multiplication of *M. kansasii* (curves *c–f*). The absorbance of the dapsone-containing cultures increased at the same rate as that of the drug-free cultures until the fourth day of incubation, by which time the number of organisms had increased about 22-fold (about 4.5 doublings). Thereafter, the organisms multiplied more slowly in the dapsone-containing media, with generation times calculated from the slopes of the regression lines as follows: 6.7 days in the cultures containing 0.1 μg dapsone per ml; 7.3 days in those containing 0.2 μg dapsone per ml; 7.1 days in the presence of 0.4 μg dapsone per ml; and 8.6 days in the presence of 0.8 μg dapsone per ml. The generation times of 6.7 and 7.3 days are not significantly different from each other, although they are significantly longer than those in drug-free cultures, and significantly shorter than the generation time of 8.6 days for growth in the presence of the largest concentration of dapsone (Goldstein, 1964).

The growth of *M. kansasii* in the presence of PABA was like that in the control cultures, except for the cultures containing 10 μg PABA per ml. This largest concentration of PABA inhibited bacterial multiplication somewhat (curve *b*); the doubling time of the organisms in the cultures containing 10 μg PABA per ml was 3.9 days, a value significantly larger than that for the control cultures. The inclusion of PABA in the dapsone-containing cultures blocked the inhibitory effects of dapsone. PABA in a concentration of 1 μg per ml completely antagonized the inhibition produced by 0.8 μg dapsone per ml; and 0.1 μg PABA per ml completely antagonized the inhibitory effect of 0.1 μg dapsone per ml. The effects of 0.8 μg dapsone per ml were incompletely blocked by 0.1 μg PABA per ml.

Discussion

The purpose of this research was to study some characteristics of the action of the antileprosy agent, dapsone, on a susceptible cultivable *Mycobacterium*. *M. leprae* is inhibited from multiplying in mice or rats by administration of dapsone to the animals in dosages yielding plasma concentrations in the range 1–5 ng per ml (Ellard *et al.*, 1971; Ozawa *et al.*, 1971; Peters *et al.*, 1975). However, because these organisms multiply only in the living host, it is impossible to study directly the actions of drugs on the organism.

It is apparent that treatment of patients with lepromatous leprosy with dapsone in effective dosage is accompanied by death of *M. leprae* (Shepard *et*

al., 1968), but it is impossible to state with certainty whether bacterial killing results from a bactericidal action of the drug, or whether, on the other hand, the drug exerts only a bacteriostatic action, and the organisms are killed by the host defense mechanisms, known to be deficient in patients with lepromatous leprosy (Shepard, 1968). Dapsone treatment of mice infected with *M. leprae* followed by withdrawal of the drug results in delay of resumption of bacterial multiplication longer than can be explained by the persistence of effective levels of the drug in the mouse (Levy *et al.,* 1972). But one cannot readily distinguish between the killing of a fraction of the population of *M. leprae* followed by multiplication of the survivors on the one hand and survival of the entire population in a state of prolonged bacteriostatis on the other (Levy, 1970, 1972).

A cultivable *Mycobacterium* that is susceptible to dapsone might represent a model of *M. leprae* suitable for studies of the action of the drug. Pattyn demonstrated that the MIC of dapsone for several strains of *M. kansasii* was 0.3 μg per ml (Pattyn and van Ermengem, 1968), 100 times that for *M. leprae.* Morrison (1968) has studied extensively a strain of *M. smegmatis* 607 as a model of *M. leprae*, but the MIC of dapsone for this organism is 2 μg per ml, 1000 times that for *M. leprae.* It appeared to us that the more susceptible organism might be more suitable as a model of *M. leprae.*

Our studies showed that dapsone was equally effective in inhibiting multiplication of *M. kansasii* on solid media, whether the medium was simple (glycerol-urea) or complex (Dubos), and confirmed Pattyn's estimate of the MIC, measured on Lowenstein-Jensen medium, a complex solid medium (Pattyn and van Ermengem, 1968). The number of colonies appearing on media containing 0.3 μg dapsone per ml was smaller than that appearing on drug-free medium, and no colonies appeared on media containing 0.5 or 1.0 μg dapsone per ml. Moreover, colonies appeared more slowly on dapsone-containing than on drug-free media.

Studies in stationary broth culture demonstrated slowing of multiplication of *M. kansasii* by dapsone in a concentration of 0.1 μg per ml, and suggested that the drug was bactericidal for smaller inocula in 10-fold greater concentration. Populations of 1.7×10^5 and 1.7×10^6 *M. kansasii* were found to contain individuals resistant to 1 and 10 μg dapsone per ml. In an additional study, the drug was found to be bactericidal for *M. kansasii* in concentrations of 1–8 μg per ml; the rate of killing appeared to increase with increasing dapsone concentration. Thus, dapsone is bactericidal at least for this strain of *M. kansasii* in concentrations only slightly larger than the MIC.

The experiments described in this report also suggest a basis for the prolonged bacteriostatic action of dapsone. In the course of incubation in stationary broth cultures containing dapsone, *M. kansasii* was found to accumulate the drug against a concentration gradient. In the presence of 10 and 100 μg dapsone per ml, the organisms were shown to accumulate dapsone to a concentration 7–15 times that of the medium. It is not possible to conclude whether the binding resulted from an active or a passive process. The apparent increase of binding during the period between 54 and 198 h favours an active process, because one would expect a passive binding process to reach

equilibrium well within 54 h (Riley and Levy, 1973). On the other hand, the demonstration of binding of dapsone *in vivo* and *in vitro* by the plasma proteins of a variety of mamalian species (Biggs *et al.*, 1971), and the demonstration here that these concentrations of dapsone are bactericidal suggest that the process was passive.

In additional studies in stationary broth culture, the activity of dapsone in the concentration range 0.1–0.8 μg per ml was quantitatively antagonized by PABA in a mole ratio (PABA: dapsone) of 2:1, a result similar to that reported by Pattyn and van Ermengem, 1968). Shepard reported (Shepard, 1967) that PABA only partially antagonizes the action of dapsone on *M. leprae* when the compounds were administered to mice in a mole ratio (PABA:dapsone) of about 200:1. The observation that PABA itself inhibited multiplication of *M. kansasii* is of some interest. The inhibition of a mycobacterium by PABA was first reported by Steenken and Heise (1943), who described inhibition of multiplication of *M. tuberculosis* H37Rv and H37Ra by PABA. Nitti subsequently reported (1951) inhibition of a strain of *M. smegmatis* by PABA in a concentration of 500 μg per ml. The Trudeau Mycobacterial Culture Collection (1972) lists 5 strains of *M. kansasii*, of which 3 do not grow in the presence of 2 mg PABA per ml. Thus, although other investigators have described inhibition of mycobacterial multiplication by PABA, we could find no report of a mycobacterial strain inhibited, as was our strain of *M. kansasii*, by PABA in a concentration as small as 10 μg per ml.

Although the results described in this present report may help to elucidate the action of dapsone against *M. leprae*, the 100-fold greater MIC of dapsone for *M. kansasii* than for *M. leprae* suggests that the actions of the drug on the 2 organisms may differ qualitatively as well as quantitatively.

Acknowledgements

This work was supported in part by the U.S. Leprosy Panel of the U.S.–Japan Cooperative Medical Science Program administered by the Geographic Medicine Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland (Grant R22 AI 07801). The generous advice of Sally Hageman, Ph.D., and Lawrence G. Wayne, Ph.D., and the dedicated assistance of Mr Kenneth E. Schilling are gratefully acknowledged.

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Lepr. Rev. (1978) **49**, 141–147

The Dermatological Clinic in a Leprosy Control Scheme: 10 Years' Experience in Malawi

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A few years after starting a leprosy control scheme in the southern part of Malawi, it became clear that increasing numbers of patients with general dermatological conditions were being referred to the leprologist for diagnosis and treatment. Weekly clinics were therefore established in the 2 urban centres of the control area, and between 1968 and 1975 over 9000 new patients were seen. This paper describes the main conditions diagnosed during these years, listing those of one recent year (1975) in detail. Many curable conditions were seen and the clinics were also a valuable source of new cases of leprosy. The role of the general skin clinic within a leprosy control programme (and in a country with nearly 5 million people and no dermatologist) is discussed, and it is concluded that it may be of considerable diagnostic, therapeutic and research value.

Introduction

From the start of the LEPRA control scheme in the Southern Region of Malawi in 1965, many patients attended the main centre in Blantyre to seek advice and treatment for a wide variety of skin diseases. Initially, most of these patients were referred by medical officers of the adjacent Queen Elizabeth Central Hospital or other hospitals in the vicinity of Blantyre, usually to rule out leprosy. In other instances, patients came from afar, often on their own initiative, and the number of patients attending the centre soon reached such proportions as to warrant the commencement of special dermatological clinics.

From 1968 onwards, skin clinics were held once a week in Blantyre and once a month in Zomba. Leprosy patients, if they came from within the project area, were charted; all clinical and bacteriological particulars were recorded on their cards. They were then issued with the first supply of dapsone and referred to the mobile treatment unit nearest to their own village. Patients with leprosy from outside the project area were given a letter to the medical assistant of the nearest health centre and advice was given as to the type of leprosy and the dosage and length of treatment.

Dermatological cases were diagnosed and treated accordingly. Routine investigations, including skin biopsies, scrapings for fungus infections, urine examination (Wood's light) for porphyria, serological tests for syphilis and

Received for publication 12 January, 1978.

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0305–7518/78/0601–0141 $01.00/0

skin snips for onchocerciasis were performed during the out-patient attendance and interpreted as rapidly as possible in order to minimize repeated visits.

Reviewing a total of 1508 patients attending the skin clinics in a 12-month period 1970–1971, Worth (1972) listed the main dermatological diagnoses, and Rampen (1976) published a more detailed account of 2664 diagnoses made at the same clinics. Moreover, Rampen (1976) drew particular attention to the frequency of onchocerciasis and its geographical distribution in Malawi.

Findings

From 1968–1975 over 9000 new skin patients were seen at the dermatological clinics in Blantyre and Zomba. The male : female ratio was 1.7 : 1. The male preponderance is readily explained by the population distribution of the industrial city of Blantyre-Limbe. Most of our skin patients come from this urban area with a population which is chiefly composed of unattached adult males. In the relatively large number of patients who were requested to report back, the response was poor; the average number of attendances per patient over a sustained period of 12 months was 2.3. About 50% of those needing regular supervision attended only once. Patients with chronic dermatoses like acne vulgaris, atopic eczema and psoriasis also had a poor follow-up, ranging from 2.7 to 3.2 attendances over a 1-year period.

Among the dermatoses most commonly encountered were impetigo, tinea versicolor and other superficial fungus infections, and scabies. The Thyolo district constitutes part of a large onchocerciasis endemic focus and many cases were seen, not only at the skin clinics but also attending the mobile teams.

The drawbacks of a modern society are clearly illustrated by the high incidence of fixed drug eruptions caused by phenolphthalein-containing laxatives, and by the frequent occurrence of vaseline dermatitis, a pustular folliculitis with atrophy, predominantly involving the shins, which is caused by the repeated applications of vaseline creams for cosmetic purposes. At the end of the project it was also noticed that many patients presented with eczematous rashes confined to the exposed parts of the skin. Among other things, the use of soap was incriminated for this phenomenon since Malawians often apply soap after the bath, leaving it on the skin as a cosmetic. Apart from provoking irritant contact dermatitis, some of the locally available soaps were clearly causing a photosensitivity reaction, possibly because they contained halogenated salicylanilides.

A list of skin diseases seen at the clinics during 1975 is shown in Table 1.

Figure 1 shows the numbers of leprosy cases which were registered during the project, and the new dermatological patients seen at the skin clinics from 1968–1975. Whereas the numbers of leprosy patients showed a clear decline, the attendance of skin patients rose considerably. The total numbers of skin attendances (new cases and return visits) averaged 3300 per year over the last 3 years of the project.

Of the leprosy patients seen at the skin clinics (1399 cases), 65% came from

TABLE 1

Analysis of skin cases seen during 1975

Infections and infestations		*Cutaneovascular disorders*	
Leprosy	125	Urticaria	36
"Observation"	19	Fixed drug eruptions	29
Impetigo, sycosis	33	Photosensitivity reactions	28
Ulcers	6	Other erythemas and drug rashes	19
Tuberculosis cutis	6		
Verrucae	16		
Other virus infections	16	*Miscellaneous*	
Tinea	283	Lupus erythematosus	18
Candidiasis	5	Granuloma annulare	2
Athlete's foot	50	Pityriasis rosea	31
Scabies	41	Pellagra	39
Insect bites	17	Other avitaminoses	14
Onchocerciasis	53	Pemphigus	3
Other infections	9	Alopecia areata	11
		Vitiligo	34
Dermatitis		Pigment disturbances	6
Atopic eczema	74	Acne vulgaris	24
Seborrhoic eczema	48	Miliaria	9
Nonspecific eczema	41	Nail disorders, chronic paronychia	10
Neurodermatitis	15	Naevi	13
Contact dermatitis	20	Haem/lymphangiomata	5
Pityriasis alba	14	Kaposi's sarcoma	7
Vaseline dermatitis	34	Other malignant tumours	4
Skinlightener dermatitis	5	Syphilis	5
Other eczemas	20	Herpes genitalis	7
		Condylomata acuminata	4
Papulosquamous eruptions		Other ano-genital conditions	15
Psoriasis	23	Pruritus	19
Lichen planus	44	Other skin diseases	41
Palmoplantar keratoses	21	No diagnosis made	100
Follicular keratoses	20		
Other keratoses	17	TOTAL	1608

within the project area. Over the 8 years of the study this figure ranged from 53%–76% without showing a definite trend.

Figure 2 represents the downward trend of all leprosy cases, registered in the project area from 1968–1975, against the number of leprosy cases, who were recorded at the dermatological clinics, i.e. patients who came from the project area only. Whereas the annual totals of leprosy cases in the whole area show a progressive decline, the numbers registered at the skin clinics are more or less stable. The relative importance of the skin clinics for the diagnosis of leprosy is demonstrated in Fig. 3: the percentage of leprosy patients diagnosed at the clinics as compared to the cases registered in the whole area increased from 6.7% in 1968 to 26.1% in 1975.

The annual totals of new skin patients increased steadily since 1968, whereas the numbers of all leprosy patients diagnosed at the clinics—from within and outside the project—remained stable (Fig. 4). Consequently the

percentages of leprosy cases in proportion to the total numbers of skin patients dropped from 26.0% in 1968 to 10.6% in 1975 (Fig. 5).

Discussion

Since the total attendances at our skin clinics increased substantially from 1968–1975, the work load increased correspondingly and at the end of the LEPRA project it was estimated that 15–20% of the working hours of the senior medical staff was spent on dermatology. Although the ratio of leprosy

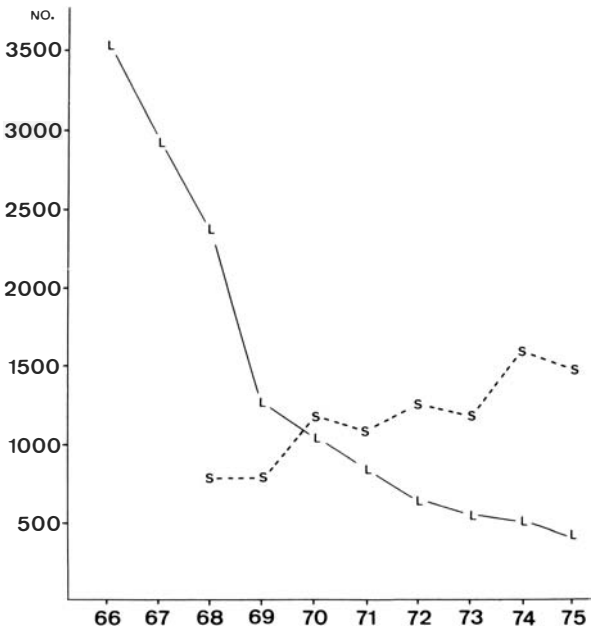

Fig. 1. Leprosy cases (L) registered during the 10 years of the project, compared with new skin patients (S) attending the dermatological clinics from 1968–1975.

cases diagnosed at the clinics dropped in relation to the total number of skin attendances, the relative importance of the skin clinics in proportion to the total of leprosy patients registered in the whole area gradually increased over the years of the study. From this point of view, the skin clinics, though time consuming, served their purpose.

The dermatoses constitute a high proportion of the total morbidity in tropical countries. However, the mortality rate is negligible. Nevertheless, conditions like tuberculosis of the skin, syphilis, erythroderma, pemphigus, malignant melanoma, Kapsi's sarcoma, etc., require early recognition and adequate management. It is also clear that the collection of these data is potentially important to research on infective, parasitic and malignant disease

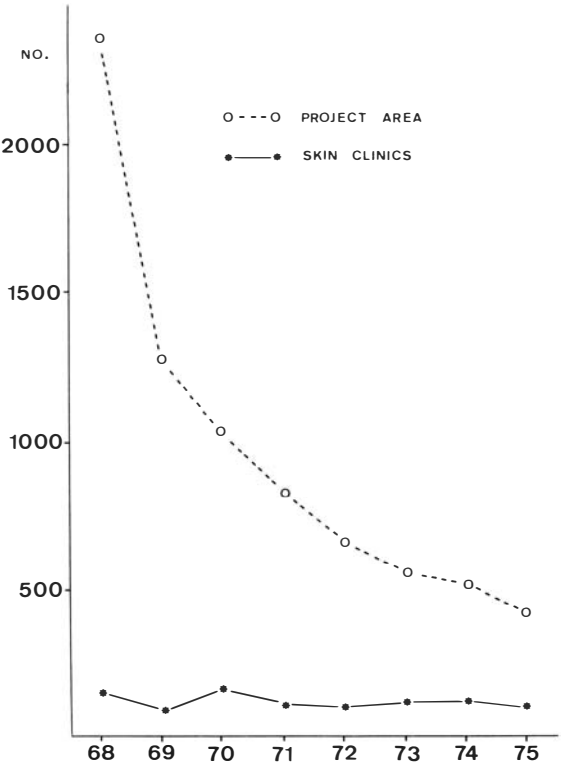

Fig. 2. Leprosy cases registered in the whole project area, compared with the cases first diagnosed at the skin clinics (1968–1975).

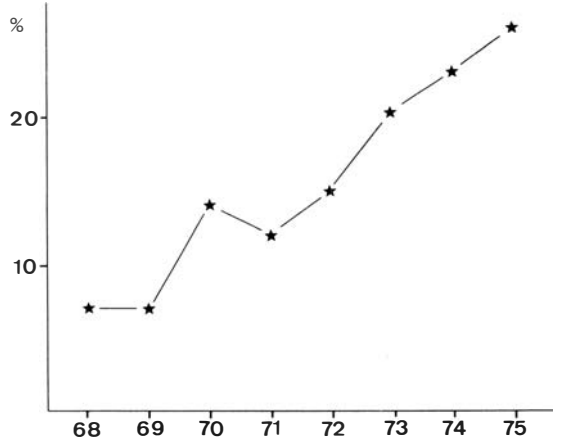

Fig. 3. Percentages of leprosy instances, coming from within the project area and first diagnosed at the skin clinics, proportional to the total number of leprosy patients registered.

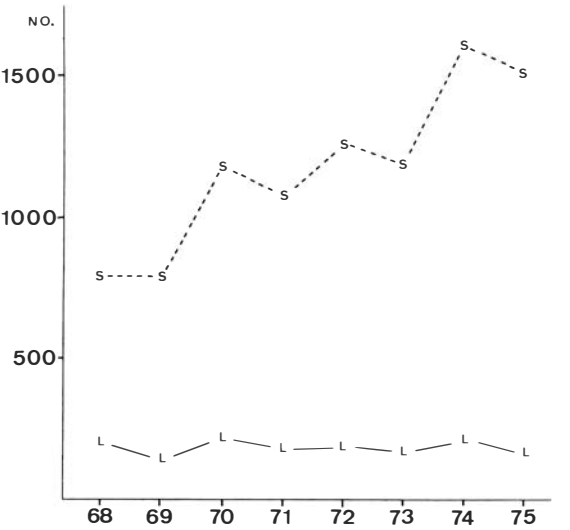

Fig. 4. Annual totals of new skin patients (S), compared with all leprosy cases (L) attending the dermatological clinics.

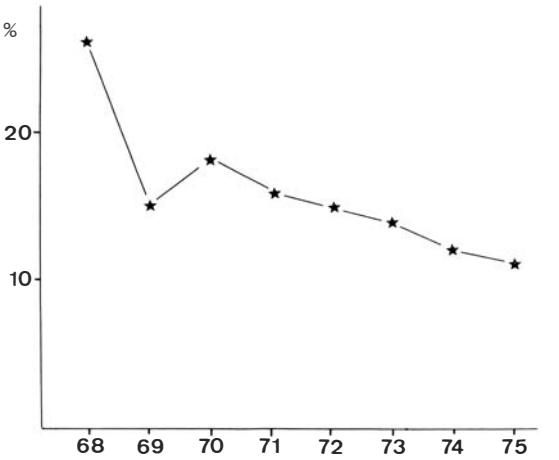

Fig. 5. Percentages of leprosy cases (in and outside area combined) seen at the skin clinics, proportional to the annual totals of new skin attendances.

in Africa. Dermatology, for obvious reasons, is not a priority of the health ministries of developing countries; in the whole of tropical Africa, there are fewer dermatologists than in cities like Glasgow or Amsterdam. The duties of the leprologist in an endemic area of Africa (and many other countries) entail a knowledge of clinical medicine, neurology, administration and practical psychology, but he can hardly avoid acquiring a vast experience in the field of dermatology also. The establishment of skin clinics, at the urban centres of this leprosy control scheme in Malawi, seems to have been of considerable diagnostic and therapeutic benefit to a large number of people, and has in addition attracted a significant number of new patients with leprosy.

Acknowledgement

I am grateful to Dr B. D. Molesworth, Director of LEPRA, Malawi, for permission to publish.

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Lepr. Rev. (1978) **49**, 149–151

Superficial Peroneal Nerve Thickening as an Early Diagnostic Sign in Leprosy

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Clinical examination of 1020 persons in a region of India in which leprosy is endemic revealed thickening of *one or both* superficial peroneal nerves in 54 persons, 19 having previously received treatment for leprosy, whether full or incomplete (old cases), and 35 never having been treated (new cases). Biopsies of thickened superficial peroneal nerves showed that of 33 symptom-free new patients, 18 had diagnostic histological changes (54.5%), and a plea is made that screening examinations for leprosy should always include palpation of superficial peroneal nerves, for subsequent nerve biopsies are likely to provide early diagnostic evidence in more than 50% of cases.

Introduction

This study was carried out in 1969 at Hemerijckx Leprosy centre Polambakkam, India, a centre which covers a total population of 658,311, of which 537,349 have been examined. The total number of registered leprosy patients was 26,743, and at the time of this study, 15,882 were under treatment. Kandaswami (1968) has reported from this centre that of 10,868 discharged patients, 462 relapsed (4.3%). During the first 9 months of 1969 377 new cases were registered, of which 103 were from contact houses and the remaining 274 gave no history of contact with leprosy.

TABLE 1

New cases registered between 1.1.1969 and 30.9.1969

Type of leprosy	Number of cases
Lepromatous	19
Non-lepromatous	325
Unclassified	33
Total	377

All patients were examined at roadside clinics in bright sunlight and when thickening of one or both superficial peroneal nerves was palpated, the patient was transferred to the centre for nerve biopsy. Six skin smears (slit smear) were made in addition, the sites being ear lobes, forehead, cheeks, and back. The patients who were examined included new cases, persons who had previously been treated for leprosy and had been discharged, and those who

Received for publication 13 October, 1977.
0305–7518/0601–0149 $01.00/0

had defaulted on treatment. Of the new cases, some presented with dermatoses such as scabies and ringworm, some had skin lesions of leprosy and/or skin anaesthesia and some had no symptoms. Of a total of 1020 persons examined 54 (5.3%) were found to have thickening of superficial peroneal nerves, and these patients were selected for our study.

Investigations

Of the 54 patients (35 new cases and 19 old cases) 5 had symptoms related to skin or nerves and 49 were symptom free. Table 2 shows how these findings differed as between new and old cases.

TABLE 2

Presence or absence of symptoms

Cases	With symptoms	Without symptoms	Total
New cases	2	33	35
Old cases	3	16	19
Total	5	49	54

Biopsy of one superficial peroneal nerve was carried out in all 54 patients. This nerve is purely sensory and therefore can safely be biopsied. Our method was: through a small incision of about 2 cm long the nerve was identified at the lower third of the leg on the lateral aspect and a complete segment of about 1 cm of the nerve was taken for biopsy.

Histological examination revealed cellular infiltration in 9, cellular infiltration plus acid-fast bacilli in 20, and normal histology in 25. Table 3 shows how these findings differed as between new and old cases.

TABLE 3

Histological studies of superficial peroneal nerves

Cases	Cellular infiltration without A.F.B.	Cellular infiltration and A.F.B.	N.A.D.	Total
New cases without symptoms	4	14	15	33
New cases with symptoms	2	—	—	2
Old cases without symptoms	2	4	10	16
Old cases with symptoms	1	2	—	3
Total	9	20	25	54

Discussion

It can be seen from the above table that of 49 patients without symptoms (33 new and 16 old cases), 24 showed diagnostic changes in the superficial peroneal nerves (48.9%) and of 33 new patients without symptoms, 18 showed diagnostic changes (54.5%). Eight of 15 patients who had no symptoms were followed-up for 2 years and were unchanged at the end of that time, and it is probable that the palpable nerve thickening could have been related to trauma as the location of this nerve makes it liable to repeated trauma. Of the 33 symptom-free new patients, 18 of whom had leprous changes within nerves, none developed symptoms (skin lesions and/or anaesthesia) over the next 2 years. Skin smears of the 35 new patients were negative for acid-fast bacili, and only one of the 35 was from a contact house.

It is our conviction that screening examinations for leprosy should always include palpation of superficial peroneal nerves. Subsequent nerve biopsies are likely to give irrefutable evidence of leprosy in over 50% of cases.

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Lepr. Rev. (1978) **49**, 153–157

The Time Interval Between the Start of Anti-Leprosy Treatment and the Development of Reactions in Borderline Patients

B. NAAFS AND H. W. WHEATE

All Africa Leprosy and Rehabilitation Training Centre, P.O. Box 165, Addis Ababa, Ethiopia

One hundred patients who developed a reversal reaction were analysed with respect to the time lapse between the start of treatment and the start of the reaction.

It was found that in nearly all cases BT and BB patients developed reaction in the first half year of treatment. For BL patients such an obvious relationship could not be found.

The authors express the opinion that the reversal reaction is a natural occurrence in the course of untreated borderline leprosy and that although sulphone treatment may accelerate the reaction it certainly does not cause it.

Introduction

The mechanism of reaction in borderline leprosy—known as "reversal reaction"—is not yet completely understood; but it is thought to be related to an enhanced cell mediated immunity (CMI) against *M. leprae* antigen (Godal *et al.*, 1973). Plasma factors may be involved (Bjune and Barnetson, 1976). However it is generally accepted that early and adequate treatment can prevent nerve damage in many cases. Some authors hold sulphone treatment responsible for this nerve damage and therefore favour leprosy treatment with low doses of sulphone (Jopling, 1974; Leiker, 1976). When a reaction occurs discontinuance of the sulphones is considered (Sixth International Congress of Leprosy, Madrid, 1953; Wheate, 1962).

Early papers concerning sulphone treatment and the occurrence of reactions are very confusing. Some authors claim that reactions are caused by sulphones (Cochrane, 1949); others, however, noticed subsiding of the reaction after starting sulphone treatment (De Souza Lima, 1948). Although many of the authors were good clinicians and keen observers the issue continued to be controversial for many years.

Recently however some authors have shown that DDS on its own can reverse nerve damage when no reaction occurs (Naafs *et al.*, 1976). Prasad (1971) showed in a controlled study that tuberculoid patients on 200 mg DDS 6 days a week from the start of treatment did better than a group of patients receiving more conventional doses of DDS (starting with 25 mg twice weekly,

Received for publication 13 October, 1977.
0305–7518/78/0601–0153 $01.00/0

increasing to 300 mg twice weekly after 4 months). Barnetson *et al.* (1976) showed that in a group of borderline patients receiving DDS 50 mg daily there were significantly less reactions than in a group of borderline patients receiving only 5 mg daily.

In this article we analyse the data of 100 borderline patients who developed a reaction, with particular reference to the time interval between the start of treatment and the start of reaction.

Patients and Methods

The patients in this study attended the Out-patient Department of the Addis Ababa Leprosy Hospital. The majority were self-reporting. Clinical classification was according to the Ridley-Jopling 5-point scale (Ridley and Jopling, 1966) and in the majority of cases the clinical classification was confirmed histologically. Patients were considered to be in reaction when prednisolone was needed to prevent further nerve damage. Nerve damage was assessed by means of voluntary muscle testing, sensory testing and motor conduction velocity measurements. In many cases the presence of reaction was confirmed histologically. In those patients in whom a lymphocyte transformation test (LTT) was done (± 30%) the LTT was markedly raised indicating an enhanced CMI.

Results

Table 1 shows the distribution according to classification of the 100 patients analysed (50 BT, 13 BB and 37 BL patients) and the time lapse between the start of treatment and the moment of established reaction.

TABLE 1

	Total number	Before treatment	< 1 month	< 3 months	< 6 months	< 1 year	< 2 years	> 2 years
BT	50	30	5	10	4	–	1	–
BB	13	9	2	1	–	–	1	–
BL	37	12	2	2	1	8	6	6

Distribution of borderline patients who developed a reaction according to time lapse between start of treatment and start of reversal reaction.

It will be seen that many of the patients in the series presented themselves at the clinic already in reaction: 60% of the BT patients and 70% of the BB patients. The remaining patients in these 2 classifications developed reactions during the first half year of treatment, except 2 who developed their reaction after 2 years. In these patients, biopsy of the dorsal cutaneous branch of the radial nerve still showed fragments of bacilli, although both the initial skin smear and the skin biopsy had been negative for acid-fast bacilli.

BL patients developed reactions over a much longer period but, even so,

30% of them reported themselves for treatment already in reaction. In about 30% of the patients in our study urinary estimation of DDS was carried out before starting treatment, with negative results in all. This test therefore provided no evidence of previous treatment.

Discussion

Many leprologists believe that a reversal reaction is produced by antileprosy treatment (Jopling, 1974; Leiker, 1976; Carayon, 1976) and when a patient presents himself in reaction claiming to have received no previous treatment, some go so far as to suspect that he has bought DDS on the black market. However, our series indicates that reversal reaction is a natural occurrence in the course of borderline leprosy. More than 50% of our cases had already developed reaction before starting antileprosy treatment. Further evidence for the natural occurrence of reversal reaction in untreated cases is the great number of new patients who report themselves for treatment with already established nerve damage (Schneider, 1975).

In a clinic in which new cases are usually self reporting, as in ours, many present with early signs of reaction such as hyperactive oedematous skin patches, rheumatic pains, tingling paraesthesiae or recent loss of sensation. It is, in fact these reactional signs which bring these patients to ask for treatment.

After starting dapsone treatment (50 mg daily) we observed that in the majority of cases these signs settled within 3–6 months. This is in accordance with earlier observations of Lowe (1950) and Cochrane (1964). De Souza Lima (1949) and Lowe (1950) reported that patients who go into reaction usually do so within the first months of dapsone treatment. Our series shows the same trend.

Barnetson *et al.* (1976) showed that a daily dosage of DDS of 50 mg settles reactional signs in the majority of cases, and Haile Gebre Selassie and Pearson (1977) showed that a reaction could effectively be treated with DDS in a dose of 200 mg daily; in many cases no prednisolone was needed. This may be due to a supposed immunosuppressive effect of dapsone (Beiguelman and Pisani, 1974; Barnetson *et al.*, 1976).

That a reaction can happen in untreated cases is understandable, when we assume that multiplication of *M. leprae* leads to an increase of *M. leprae* antigens, thus bringing the antigen level above the "threshold" needed to give a full blown reversal reaction.

However, dapsone treatment which prevents the multiplication of the bacilli does not prevent a reaction in all cases. This may be explained by assuming that the antigens responsible for reversal reaction are released from dead and dying bacilli and not, or to a lesser extent, from live bacilli. Some workers are at present investigating this hypothesis (Harboe, 1977). During the first half year of treatment, expecially in "active" cases, the number of dead bacilli may increase and the total antigen load may reach the threshold level and a reaction occurs. The observation that Rifampicin increases the number of reversal reactions (Steenbergen and Pfaltzgraff, 1975) can be explained by the fact that Rifampicin is a rapid killer, thus increasing the number of dead and dying bacilli so quickly that the immunosuppressive effect is inadequate.

In the BL group it appears more difficult to find a time relationship between the start of treatment and the reaction. In this group there is an enormous amount of antigens; it takes the body a long time (years) to dispose of them and the risk of a reversal reaction is prolonged.

This small paper may contribute to the following conclusions:

(1) Reversal reaction belongs to the normal sequence in untreated BT and BB patients. DDS treatment is not directly responsible for this phenomenon. It may accelerate the process but certainly does not cause it.

(2) Borderline patients should be under good supervision especially during the first half year of treatment. For BT and BB patients the risk of reaction after this period is negligible and if it does occur care should be taken to check the initial classification and to exclude DDS resistance.

Acknowledgements

Our thanks are due to Dr Barnetson, Dr Pearson and Dr Ridley, who did the histology, the staff of the Armauer Hansen Research Institute who did the LTT's and to our colleagues the physicians of ALERT, who were always willing to help and to discuss findings and theories.

Resume

Une groupe de cente malades lèpreux en réaction d'inversion a été suivée pour voir si le début de la réaction a un rapport quelconque avec le début du traitement.

Les malades BT et BB ont presque tous été en réaction d'inversion dans les premiers six mois du traitement. Une relation aussi étroite n'a pu être établie pour les cas BL. Les auteurs sont d'avis que la réaction d'inversion est un phenomène normal dans la lèpre borderline non traitée. Ils pensent que, si les sulphones peuvent précipiter cette réaction, ils n'en sont pas la cause.

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Lepr. Rev. (1978) **49**, 159–160

Obituary

RAOUL FOLLEREAU
1903–1977

Raoul Follereau is no more. He died in Paris on 6 December, 1977 in his seventy-fifth year. And the world of leprosy will never be the same again.

He was a colourful figure, larger than life. The sober scientist and the detached research worker in leprosy might try to ignore him, to dismiss him as a theatrical interloper into the domain of their serious unemotional activities. But Raoul Follereau had to be heard; he made himself heard, like a barrister pleading a case—or a cause.

He was master of the magnificent gesture and the sonorous phrase. At the International Leprosy Congress in Tokyo in 1958, he soundly berated the participants, in that eloquent rounded French of his, for their preoccupation with the experimentally infected mice to the apparent neglect of the actually infected man. Even those who could not follow the flow of his untranslatable Gallic oratory could not but be impressed by the sincerity of his convictions.

This was the real Follereau, trained in philosophy and law, a born journalist with a real literary flair, a man genuinely moved by compassion for the underdog, the sufferer from leprosy.

Having seen for himself the deformed victims of neglected leprosy, he resolved fifty years ago to devote himself to their well-being. Adzopé, on the Ivory Coast, was his brain-child. The Order of Charity he founded in 1948. World Leprosy Day, now observed in no fewer than 137 countries, was another of his realized dreams. ELEP, the Federation of European Anti-leprosy Associations, was formed in 1966 largely owing to his vision and persuasive advocacy. Now ILEP (the "I" representing an international component) brings together voluntary organizations that are responsible for the medical care of a third of those of the world's leprosy sufferers who are getting treatment. Two years later, the Fondation Follereau was founded, now with branches in several countries.

It was Follereau who roused the conscience of French-speaking peoples in both metropolitan France and beyond the seas to the plight of neglected and ostracized leprosy sufferers, and it was he who in the course of 32 round-the-world journeys and visits to 102 countries goaded governments into action and spurred individuals to do something for leprosy sufferers. He was more than a thorn-in-the flesh to reluctant officials, more than an exposer of bumbledom and bureacratic procrastination. He saw that decisions must be taken at the highest level if attitudes were to be changed. At his instigation the French National Assembly unanimously passed, on 25 May, 1954, a Resolution calling on the United Nations to adopt a veritable Charter for leprosy sufferers the world over. That they did so is a tribute to his prestige and pertinacity.

Sometimes, it must be admitted, his flowery and highly-charged French phrases grated on less emotional Anglo-Saxon ears, and his equating of

deformity with leprosy did not exactly please his more scientific listeners. But Follereau never claimed to be a scientist. He was a man with a heart, a large heart, and his sympathy and love overflowed to those who were—and, unfortunately, still are in some situations—despised and ostracized. He was in his own inimitable and highly personal way trying to remove the stigma and the segregation, the ignorance and the inertia, that kept leprosy sufferers from being accepted as men, like other men.

The Apostle of Charity, this travelling Vagabond, this St Francis of the twentieth century, is no more. Untiring in his travels, eloquent in speech, indeflectable in his advocacy of the rights of leprosy sufferers, he rode roughshod over bureaucratic red tape and scientific pretensions, and with a worthy impatience confronted the world of leprosy.

We shall not see his like again. When he was needed, with his special gifts and unique experience, he was there. And there to help.

Raoul Follereau is gone, but he lives on in those he has inspired to follow his ideals, and he lives too in those whose lives have been made richer and fuller by his presence and his touch.

S. G. BROWNE

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Leprosy and the Community

REPORT OF THE SECOND WEST AFRICA LEPROSY CONFERENCE, HELD AT THE UNIVERSITY OF CAPE COAST, GHANA, JULY 1977

This report comes from the West Africa Leprosy Secretariat, P.O. Box 673, Freetown, Sierra Leone, following a Conference in July 1977 at which the participating countries were Ghana, Nigeria, the Gambia, Sierra Leone, Liberia and Togo. Workshop groups consisting of about 12 members, dealt with the following subjects—"Treatment and Control", "Health Education and Rehabilitation", "Training", and "The Role of Surgery", and the Report shows that they did this in a commendably practical way, giving every evidence of people talking about a problem, and about countries, which they know extremely well at first-hand. Under the first of these headings, it is heartening to see a section devoted to "The Follow-up of Patients Crossing Borders", with emphasis on the importance of a Transfer Certificate and the need to make treatment freely and easily available by International Agreement in the 6 countries concerned. Another heading which catches the eye is "How to Attract Indigenous Doctors to Work in Leprosy" and this is followed up, in the Section on Training, with a detailed consideration of the "Future for Leprosy Workers", concluding that "recruitment of special staff for Leprosy Control, if required, should be at the same level as that required for parallel cadres in the general health services" and that more attention should be focused on a career structure for leprosy staff.

In the light of current concern about dapsone resistance, perhaps one of the most interesting paragraphs is on page 3, under "Suggestions for Combined Treatment". It is advised that multi-bacillary leprosy should be treated with combined treatment, dapsone being given in full doses in all cases, together with another drug, but, unfortunately, as set out here, it is not clear if this should be rifampicin *or* clofazimine, or both at the same time. The next sentence is remarkable: "Drug resistance is not a major problem but is present in West Africa—in a relatively small percentage of patients". The participants included a number of experienced observers, and the statement contrasts strangely with the concern that is currently being expressed by workers in, for instance, Ethiopia, and by WHO.

This interesting report should be read in conjunction with—
LEPROSY SURVEY, Sierra Leone, August 1976–August 1977, by Dr E. S. Johnson.
This also comes from the West Africa Leprosy Secretariat, P.O. Box 673, Freetown, Sierra Leone, W.A., and the basic reasons for carrying out the survey are worth quoting in full (from p. 2):

"On account of this rapid expression of leprosy control activity and the resulting extensive coverage of the population achieved over the past 3 years since the National Programme was launched, a serious discussion among leprosy workers in the country began to surface, that probably most of the leprosy cases existing were now under treatment. Moreover, there was general suspicion that the level of endemicity of the disease may not be as high as previously estimated. Accordingly, it was decided to conduct a prevalence study in 1976, with the following objectives:

(1) To determine as accurately as possible, the true leprosy prevalence rate in Sierra Leone and therefore determine the exact number of patients in the country yet to be detected and put under treatment.
(2) To identify what parts of the country the disease was more prevalent.
(3) To evaluate the progress of the 3-year-old national leprosy programme.
(4) To study the epidemiological pattern of the disease.
(5) To collect data that will be used to plan the next 3–5 years of the leprosy programme."

Dr Johnson's survey was based on the "Enumeration Areas" which were established in the 1974 Nation-Wide Population Census and 6 of these were eventually selected in each district, each containing about 6000 people, as giving a statistically satisfactory sample. The survey was carried out by experienced leprosy supervisors and the leprologist later saw and confirmed virtually all the cases detected. On pages 4–8, the author analyses the results in considerable detail, and later concludes: "The results of this survey strongly suggest that a highly successful National Leprosy Control Programme is well established in Sierra Leone". There was a prevalence rate of 0.9% (standard error $\pm 0.1\%$) in a population of 3 million and the estimated number of cases was about 27,000. The lepromatous rate was 7.3%. There was a close correlation between (1) the figure of 27,000, (2) the number of patients actually known to be registered in Sierra Leone, which is 15,120, and (3) the survey's estimate of the number of cases still to be diagnosed and brought under treatment, which was about 14,742. A disconcerting finding (p. 10) and one which needs careful confirmation, is that "40% of the cases yet to be found are actually living in localities where clinics are held and 80% are within 3 miles of existing clinics". Dr Johnson rightly concludes that serious thought should be given to this rather disturbing revelation.

A. C. McDOUGALL

HIND KUSHT NIVARAN SANGH ANDHRA PRADESH BRANCH SEMINAR ON LEPROSY

A Seminar on leprosy under the auspices of the Hind Kusht Nivaran Sangh Andhra Pradesh Branch was held on 28 and 29 November 1977 at the Osmania Medical College, Hyderabad. About 550 delegates, consisting of medical and para-medical workers, together with social workers from

Government and Voluntary Organizations, attended the Seminar, which was led by distinguished specialists and men in public life. The State of Andhra Pradesh has an enormous leprosy problem. In his report on the Leprosy Control Programme in the State, Dr Anand Raj, Zonal Leprosy Officer, Hyderabad stated that the official estimated number of persons with leprosy in Andhra Pradesh was 620,000, with a prevalence rate of 12.8 per thousand, rising to 17 per thousand in some very large densely populated Districts. The Government National Leprosy Control Programme has been operating in the State since its inception, working along well established lines, but it is clear that much remains to be done. In addition to discussing current problems the Seminar also had an important educative function. Much credit for its success must be given to Dr S. N. Mathur, the dynamic State Secretary of the Hind Kusht Nivaran Sangh. In a series of resolutions adopted by the Seminar emphasis was laid on the need for hospital care for patients with acute reaction at local rather than District level; the need for better leprosy education of local hospital and health staff; the need for refresher courses for senior medical staff; the need for references to leprosy on the mass media to be screened for accuracy; and in addition it was recommended that leprosy control services should not be integrated into the State general Health Service until the results of the present "attack phase" on the disease have been evaluated, and the whole medical profession have been mobilized to deal with the problem in its later, diluted form.

T. F. DAVEY

TENTH BIENNIAL CONFERENCE OF THE INDIAN ASSOCIATION OF LEPROLOGISTS AND XIVTH ALL INDIA LEPROSY WORKERS CONFERENCE, BARODA 10–14 APRIL 1976

(*Leprosy in India*, Vol. 48, No. 4, Supplement)

The Biennial Leprosy Conferences conducted by the Indian Association of Leprologists and the Hind Kusht Nivaran Sangh are always an important stimulant to research and the sharing of experience in a country with over 3 million sufferers from leprosy. The Baroda Conference in 1976 was no exception. Among the wide range of subjects covered by original articles and discussion, the following merit special note.

EPIDEMIOLOGY

"Epidemiological surveys in 3 areas of Maharashtra where leprosy control is well established, have shown", said P. V. Kapoor, "that there has been a definite fall in leprosy incidence in children in all 3 areas, with lepromatous rate and deformity rate virtually down to zero. Among adults progress after 15 years has become slower." S. K. Noordeen and P. N. Neelan found chemoprophylaxis with dapsone effective in preventing leprosy among household contacts below 15 years of age exposed to non-lepromatous leprosy, though the efficacy rate was only 35%. B. R. Chatterji, following up over several years clinically normal persons who harboured AFB in the ear lobes, failed to show any incidence of leprosy among them higher than in

controls. R. Ganapati, S. S. Naik and S. S. Pandya reported important studies in school children in Bombay [see *Lepr. Rev.* **47**(2), 133].

MICROBIOLOGY AND PATHOLOGY

K. V. Desikan reported experiments in which multiplication occurred in mouse footpads using an inoculum of AFB in which no normal staining rods were found, based on a count of 100 bacilli. E. J. Ambrose, N. H. Antia and S. R. Khanolkar with a view to developing a rapid *in vitro* assay for the viability of *M. leprae* combined radioactivity labelled metabolites with high resolution autoradiography and found a significant correlation between MI and labelling index. D. K. Dastur reported on the role of the perineureum in leprous neuritis. V. Sengupta, M. J. Worms and R. J. W. Rees presented evidence that *M. lepraemurium* can be transmitted mechanically by mosquitoes (*Aedes aegypti*).

THERAPY

One full session was devoted to *clofazimine therapy,* and well exposed the established facts with this drug. L. M. Hogerzeil reported that long term steroid therapy had no adverse effect on the bacteriological decline in lepromatous patients provided they were treated with clofazimine at the same time. In the Session on *Immunology,* V. Mehra, S. N. S. Hanjan, Zera Kidwal, L. K. Bhutani and G. P. Talwar presented evidence of an alteration in the surface characteristics of lymphocytes derived from the peripheral blood of untreated lepromatous leprosy subjects. K. Saha reported dramatic improvement following the transplantation of human foetal thymus tissue into severe reactional cases of lepromatous leprosy. An important Session on Deformities and Rehabilitation concentrated on the long term results of surgical procedures in leprosy. The technical sessions of the Conference were succeeded by the very important Leprosy Workers Conference, concerned with many practical problems in the vast undertaking of leprosy control in India, and on this occasion, especially with assessing progress and evaluating control procedures.

T. F. DAVEY

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News and Notes

XI INTERNATIONAL CONGRESS OF LEPROSY, MEXICO CITY, MEXICO 11–18 NOVEMBER 1978

The attention of all our readers is drawn to this important Congress.

Registrations, hotel reservations, social events, tours and in general all administrative matters concerning this Congress will be handled by the Local National Committee. Please address to: *XI International Congress of Leprosy, Associacon Mexicana de Accion Contra La Lepra A.C., Dr Vertiz 464, Mexico 7. D. F. MEXICO.*

Scientific Programme

WORKSHOPS

(1) Chairmen and members have been named to participate in workshops on the following themes: experimental leprosy; microbiology; immunology; experimental chemotherapy; epidemiology and control (including field therapy) and social aspects. The respective chairmen will inform members of their workshop on which day they will meet (9, 10 and 11 November) before the opening day of the Congress. Reports of these workshops will be presented to participants when they register.

ORGANIZATION OF CONGRESS SESSIONS

(2) After the first plenary session (on epidemiology and control), simultaneous scientific sessions will be organized under the following themes: experimental leprosy; clinical aspects; microbiology; immunology; social aspects; experimental chemotherapy; therapy; rehabilitation and clinicopathological aspects (including nerve damage).

The main presentations at each session will be made by participants who have already been informed by the chairmen of the session at which their paper will be presented.

FREE COMMUNICATIONS

(3) A very limited number of papers (of 10 min duration) on aforementioned themes will be accepted for reading at each of the scientific sessions.

POSTER PRESENTATIONS

(4) It will be an innovation at the Congress.

CLINICAL SESSION

(5) Patients with Lucio Leprosy will be shown at the Pascua Dermatological Centre.

For information about the Scientific Programme, presentation of free papers and so on, please write to: *Dr Stanley G. Browne, Secretary General, International Leprosy Association, 57A, Wimpole Street, London W1M 7IF, England.*

4TH LEPROSY WORKSHOP IN BOMBAY

Leprosy research in Bombay continues to be stimulated by the periodical workshops organized by the Acworth Leprosy Hospital Society for Research, Rehabilitation and Education in Leprosy [see *Lepr. Rev.* **48**(2), 135]. The Proceedings of the 4th workshop included: Electrodermatological studies in leprosy, by V. V. Dongre and J. C. Sharaff; Recording of compound nerve action potentials in sensory nerves, by S. S. Pandya; A follow-up of private school detected leprosy cases in Bombay, by C. R. Revankar and S. S. Jha; Associated cases in the families of school children with leprosy, by R. Ganapati [see *Lepr. Rev.* **49**(1), 43–46]; Irregularity of dapsone intake in infectious leprosy patients attending an urban treatment centre, its magnitude and causes, by S. S. Naik, Honorary Secretary of the Society. His address is: *Acworth Leprosy Hospital, Wadala, Bombay 400 031, India.*

OLD ISSUES OF *LEPROSY REVIEW*

Requests are being received, especially from developing libraries in countries where leprosy is endemic, for old issues of *Leprosy Review,* especially Volumes 1 to 30. It will be appreciated if anyone able to spare any of these will contact *The Director, Lepra, Fairfax House, Causton Road, Colchester CO1 1PU.*

WORLD HEALTH ORGANIZATION

Special Programme for Research and Training in Tropical Diseases

THE SEARCH FOR SCIENTISTS TO SEARCH FOR THE TOOLS TO CONTROL THE TROPICAL DISEASES

The search for new tools to control disease in the tropical countries requires scientists of many disciplines. Molecular and cell biologists, biochemists, immunologists, parasitologists and entomologists are among those whose contributions are needed.

The research areas covered by the Special Programme for Research and Training in Tropical Diseases are:

— malaria, schistosomiasis, filariasis, trypanosomiasis, leishmaniasis and leprosy;
— epidemiology, biomedical sciences, biological control of vectors, and socio-economic research.

Specific fields within these research areas are developed by the Programme's Scientific Working Groups. Scientists from any country are welcome to submit proposals for research grants within the specific fields. All enquiries should be addressed to:

The Special Programme for Research and Training in Tropical Diseases
World Health Organization
1211 Geneva 27, Switzerland

For those persons seeking contact with Special Programme participants in the United Kingdom, a Special Programme Committee has been set up by a group of interested scientists there to provide such information. The Committee also has research proposal forms available. Correspondence should be addressed to:

Professor David Bradley, Chairman
UK National Committee of the TDR
c/o Mrs J. Beard
Medical Research Council
20 Park Crescent, London W1N 4AL, U.K.

Should scientists in other countries wish to organize such liaison Committees, we should be pleased to collaborate with them and to publicize their activities. Any suggestions in this regard should be addressed to the Director of the Special Programme.

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Letter to the Editor

Dermal Microfilariasis and Leprosy

The recent article entitled "Dermal Microfilariasis and Leprosy" by McDougall and Waudby (*Lepr. Rev.* **48,** 161–168) makes one very important point so far as differential diagnosis between these 2 diseases is concerned. This is that hypopigmented macules which occur in onchocerciasis may be visually identical with those of indeterminate leprosy. I have been noting these for a number of years, and have done biopsies on a few, but have never had any in which there was confirmation that the lesion was specifically related to the filarial infestation. Perhaps there are regional differences, so that this similarity is not so common elsewhere, but here we see many instances of it each year.

In an attempt to confirm that the etiology actually was onchocerciasis, I have tried treating a number of these patients intensively, and have found that the hypopigmented macules do begin to regain normal colour again, though I do not recall any in which there was complete return to normal in spite of several months of follow-up.

It is possible that some of the other skin manifestations of onchocerciasis, such as the pachydermis, and smaller skin nodules may be confused with leprosy by one completely inexperienced in tropical dermatology, but their diagnosis is not really difficult. However, great caution is needed to prevent confusion between these vague areas of hypopigmentation in onchocerciasis and those of the indeterminate stage of leprosy. We have been disappointed with the assistance we may expect from a biopsy, but I think careful investigation which insists on finding at least one other sign of leprosy will always confirm the diagnosis. Hence, one should always:

(1) Check for loss of sensation.
(2) Palpate for enlarged peripheral nerves.
(3) Look for *Mycobacterium leprae* in skin smears.

It should not be necessary to say that where there is a possibility of onchocercal infestation, even though 4 skin snips may be negative, one still needs to give the patient a tablet of diethylcarbamazine, and note the response.

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Lepr. Rev. (1978) **49**, 169–170

Book Reviews

Guide to Leprosy and Leprosy Control, 2nd Edition, by P. Kapoor, 1977. Published by Dr J. M. Mehta, Poona District Leprosy Committee, 593/2, Rasta Peth, Poona–411 011, India. Price (paperback) Rs. 8.50.

This is a booklet of 106 pages, inexpensively yet strongly produced, small enough to go in the pocket, yet remarkably comprehensive in its content. There are 28 chapters (but regrettably no index) and for the most part the headings are clear and easy to follow. This book has obviously been written for people who need to get on with the job and to learn the essential steps for handling leprosy in the field. Forthright headings include "How to test for the presence of anaesthesia", "How to take smears", "When to refer a case?", "Criteria for declaring a case cured", "Measures to get leprosy patients in early stages", and "How to communicate". There are 21 figures, nearly all of clinical lesions, and their quality—understandably at this price—is variable. Figure 8, however, is an extremely good example of diffuse, widespread lepromatous leprosy in an Indian female patient aged about 20, which shows absolutely nothing wrong (apart from slight loss of eyebrow hair, which would pass unnoticed except to an experienced observer). There are estimated to be 3.2 million patients in India with leprosy and if many of them look as normal as this, they must indeed present a formidable problem in the early diagnosis of lepromatous leprosy. Chapter 13 on the "Treatment and Management of Leprosy" is disappointing in that it mentions only one drug, dapsone. Chapter 11 on "Reaction in Leprosy" does not given a good account of the fundamentally different immunological processes at work in lepromatous, as opposed to non-lepromatous reactions, and Chapter 14 on the "Treatment and Management of Reactions in Leprosy" does not read well; after A, B, C, there suddenly appears D, "Alternative Drugs Useful in Leprosy", which includes INH, Thiosemicarbazone, Lamprene, Streptomycin injections and Rifampicin. This list is out of place; it should come under the treatment of the bacillary infection. Furthermore, the inclusion of Streptomycin, with its known toxicity and inconvenience, is surprising—and that of INH even more so. (There is no clear, confirmed evidence that isoniazid is active against *M. leprae* in man or in the experimental animal.) At the end of this same chapter (p. 54) there is another paragraph E, "Multitherapy" which similarly should not be considered under the heading of reactions, and its first sentence: "The present thinking is to use 3 or 4 anti-leprosy drugs simultaneously to avoid development of drug-resistant germs" is neither a universally accepted view, nor in accordance with recent WHO advice.

Although these are not minor criticisms, they do not detract from the overall excellence of this booklet; it is full of useful information. It should be of great value to doctors and field workers, in a country where there is clearly a leprosy problem of enormous extent.

A. C. McDOUGALL

Lignes Directrices de Lutte Contre La Lèpre (A l'usage des auxiliaries médicaux). All African Leprosy and Rehabilitation Training Centre, P.O. Box 165, Addis Ababa, Ethiopia. August, 1975.

This 86-page document, typed on A4 size paper and paperbacked, is essentially a French translation of "*A Simple Guide to Leprosy*" which was prepared at ALERT some years ago, mainly under the stimulus of Dr Felton Ross, the then Director of Training.

There are 6 main sections which include "all that medical and paramedical personnel should know in order to diagnose and treat most leprosy patients in rural areas".

The text contains a number of simple diagrams and drawings which admirably illustrate some of the more important points in the handling of patients, and these are particularly good on the subjects of deformity, disability and the care of the eyes, hands and feet.

Testing points in a guide of this kind are (1) the description of reactions and their treatment and (2) the recognition and treatment of dapsone resistance. The latter is well covered; there is a particularly important observation on page 68, under "How do you recognize dapsone resistance?" where attention is drawn to the appearance of "new nodules at unusual sites", amongst which are the abdomen, forearm and conjunctiva (the white of the eye). It should be more widely emphasized that these unusually distributed lesions, many of which have been illustrated in publications on histoid leprosy, may be good clinical indicators of dapsone resistance. The nature of reactions (as opposed to relapses) is well explained in Section 4, and the case for, and against antileprosy drugs (such as dapsone) being responsible for reversal reactions, is carefully presented. On page 64 it is recommended that all patients with acute neuritis during Type 1 (reversal) reaction should be referred to a dispensary or hospital as soon as possible. Under rural conditions it is advised that cortico-steroids should be used only "to help the patient en route to the hospital".

On the very last page, there is a "Post-Face" in which it is stated that it is envisaged that this translation should be used by those who have attended ALERT—a point which is so important that it should be at the beginning of the book, in capitals. In some ways, the whole business of leprosy control has become even more complex since these "Lignes Directrices" were written—specifically in the matter of the treatment of adverse reactions under simple out-patient conditions, hundreds of miles from the nearest referral centre, and in the detection and treatment of dapsone resistance. The testing point for all the information in this excellent document may still be ahead—its acceptance and practical application in the French-speaking areas of Africa.

A. C. McDOUGALL

Leprosy in England—Yesterday and Today, by S. G. Browne. The Leprosy Study Centre, 57a Wimpole Street, London W1M 7DF.

This is a 48-page booklet, available from the above address, price 25p, plus 7 or 9p for U.K. postage, or 20p for airmail postage abroad. Dr Browne begins by describing the history of leprosy in the world, as a background to the origin, development, extent and eventual decline of the disease in England (and Scotland). The section on mediaeval leprosy is particularly interesting, as is also that headed "The Leprosy Campaign—in England and Abroad". The latter part of the book is devoted to a detailed description of the Hospital and Homes of St Giles at East Hanningfield in Essex which, since 1968, has been the only hospital in this country for in-patients suffering from leprosy. This is a fascinatingly written, and well illustrated account of leprosy in this country which will undoubtedly have a wide appeal to both medical and lay readers.

A. C. McDOUGALL

Lepr. Rev. (1978) **49**, 171–181

Abstracts

14. WATERS, M. F. R., REES, R. J. W., PEARSON, J. M. H., LAING, A. B. G., HELMY, H. S. & GELBER, R. H. **Rifampicin for lepromatous leprosy: nine years' experience.** *Brit. Med. J.* 21 January 1978, 133–136.

This paper is a very important and authoritative contribution to the subject of alternative chemotherapy in leprosy. It presents the results of 9 years' experience of rifampicin in leprosy treatment by a distinguished group of clinicians and bacteriologists, based on 4 carefully designed clinical trials covering over 100 patients with lepromatous leprosy. It is noteworthy that rifampicin was first used for treating leprosy after it was found to inhibit the multiplication of *Mycobacterium leprae* in mice. In clinical trials the drug was used either alone or in combination usually at a dosage of 600 mg daily, occasionally 900 mg twice weekly.

The rapid bactericidal action of rifampicin on *M. leprae* was confirmed, with a fall in Morphological Index within 14 days and signs of clinical improvement detectable in from 10–21 days. The action was nevertheless incomplete, with persisting viable *M. leprae* detected even after 5 years of treatment with either rifampicin alone or rifampicin in combination with thiambutosine. Nevertheless, when combined with dapsone, fewer persisting viable bacilli were detected than are usual after the use of dapsone alone.

Although in the groups of patients studied ENL appeared to develop on average earlier than with dapsone alone, there was no evidence of sudden severe ENL.

In a long term trial, the rapid initial clinical and bacteriological progress on rifampicin was not continued after the first 3–4 months, subsequent progress being comparable with that under treatment with clofazimine.

Although rifampicin is considered to be more effective in leprosy treatment than dapsone, it is not considered likely that used by itself it can significantly shorten the period of treatment essential in lepromatous leprosy. The further investigation of intensive combined treatment from the start is advocated, using rifampicin combined with one or more other bactericidal drugs both in untreated leprosy and in patients with lepromatous leprosy resistant to dapsone.

This paper deserves to be read by all concerned with leprosy treatment.

T. F. Davey

15. PRABHAKARAN, K., HARRIS, E. B. & KIRCHHEIMER, W. F. **Hypopigmentation of skin lesions in leprosy and occurrence of *o*-diphenoloxidase in *Mycobacterium leprae*.** Pigment Cell, v. 3, 152–164 (Karger, Basel, 1976).

This article follows on Dr Prabhakaran's general thesis that *Mycobacterium leprae* is unique in utilizing DOPA. It deals mainly with certain distinguishing characteristics of the *o*-diphenyloxidase in *M. leprae,* together with experimental evidence suggesting a possible mechanism of pigment loss in the skin lesions of leprosy.

Although at least 2 of the figures, and some of the points made in the text are similar to those of, for instance, "The interaction of *Mycobacterium leprae* and melanocytes *in vitro*": Prabhakaran *et al.* (1971), *Cytobios,* **4,** 93–95, it is becoming difficult to keep up with the sequence of the numerous publications and letters on this subject by Dr Prabhakaran and his associates. Apart from its potential value in the identification of *M. leprae,* by no means universally accepted by other workers now, the point which has interested leprologists and dermatologists for a long time concerns the alteration of skin colouring in leprosy lesions, usually towards hypopigmentation. In tuberculoid leprosy, where bacilli are often difficult to find at all, hypopigmentation is of course marked, whereas in lepromatous leprosy, with as many as 7

billion (U.S.A. terminology) bacilli per g of tissue, hypopigmentation may be minimal or absent. The authors attribute this situation in lepromatous leprosy (p. 163) to the presence in the skin of "numerous mast cells loaded with catecholamines", which they have shown to be readily oxidizable by *M. leprae*. (In some instances of erythema nodosum leprosum, mast cells may be conspicuous, but apart from this, few experienced histologists would agree that they are "numerous" in lepromatous tissues.)

A. C. McDougall

16. HARBOE, M., CLOSS, O. & DEVERILL, J. **Production of monospecific antisera against antigenic components of *Mycobacterium bovis* (BCG).** *Scand. J. Immunol.*, v. 5, 861–866 (1976).

The authors describe a technique for the production of antisera specific for individual antigenic components of BCG, by immunizing rabbits with antigen–antibody precipitates cut out from agarose gels.

Culture supernatant from BCG grown in Sautons medium was concentrated by ultrafiltration, and preliminary fractionation was performed by dialysis against buffers of low ionic strength, at various pH values. The resulting precipitates were washed by centrifugation, dissolved in buffer, and then partially separated into individual components by electrophoresis in agarose gels on glass plates.

The plates were then rotated through 90° and re-electrophoresed so that these antigenic components ran into a gel containing a reference anti-BCG serum (crossed immuno-electrophoresis). There was sufficient separation between individual antigen–antibody precipitates formed in this way, to allow some of them to be cut out and used for immunization of rabbits. An electrophoresis system similar to that described above was used to prove that the antibody produced by these rabbits was monospecific.

The authors discuss the possible value of this elegant technique for taxonomy, and for the elucidation of the mechanisms underlying the immunological spectrum of leprosy. If the position of a leprosy patient in the clinical and immunological spectrum is determined by which antigenic components of the organism he can respond to, the technique will clearly be of immense value. If on the other hand, the patient's position in the spectrum is determined by other factors, the technique will be of less help.

G. A. W. Rook

17. FREERKSEN, E. & ROSENFELD, M. **Leprosy Eradication Project in Malta: first published report 5 years running.** *Chemotherapy,* v. 23, 356–386 (1977).

This article describes "the initial 5-year period of the eradication programme since its introduction in the Maltese Islands in the second half of 1972". There were originally 206 patients, including those on the closely adjacent island of Gozo. By the end of the 5-year period, 20 had died or emigrated; treatment had been discontinued in 180; thus 6 patients only remained under treatment. Professor Freerksen emphasizes that this is an eradication project based on chemotherapy and he gives great weight to the use of "almost bactericidal combined therapy". The majority of patients, irrespective of classification and severity of disease, were given rifampicin, isoniazid, prothionamide and dapsone. A combined tablet of isoniazid, prothionamide and dapsone was used, but "in rare cases when intolerance phenomena were observed or in refractory cases, we gave a suitable alternative fixed combination without DDS; prothionamide and ethambutol". Other patients began with the combined rifampicin, isoniazid, prothionamide and dapsone tablet and then switched to isoniazid, prothionamide and dapsone alone. "According to tolerance, or due to other reasons (e.g. temporary unavailability of the drug) rifampicin was also given in combination with other partners (for instance, DDS, sulfonamides, sulfonamide-trimethoprim combination or ethambutol) during shorter or longer periods".

The clinical and bacteriological progress of patients under treatment is described in detail and was judged, on the somewhat limited follow-up periods after stopping therapy, to be satisfactory.

Professor Freerksen lists 88 references in support of the policies he has advised for the eradication of leprosy in Malta, of which 17 are from his own publications, and largely concerned with multiple drug combination. He believes (Abstract) that "fixed combinations not only make treatment simple, but also guarantees a more reliable acceptance of the medication and the adherence to dosage". The Abstract continues: "For an eradication programme the classification into different leprosy types plays not a too important role" but—towards the end—records that 30,000 histological slides have been collected, "representing all stages of leprosy, i.e. from the period before, during and after treatment (about 5000 biopsies)". On page 375, the author gives his reasons for not using clofazimine which—like a great many other statements and opinions in this interesting article—are totally at variance with those of other observers.

A. C. McDougall

18. McDOUGALL, A. C. **The work of the Leprosy Study Centre in London: a review of over 13,000 biopsies.** *Proc. roy. Soc. Med.* 1977, v. 70, 731.

That leprosy has taken its rightful place in the main stream of medical research is due in no small measure to R. G. Cochrane, whose scientific approach is evident, not only in his writings but in the incomparable collection of histological material matched by clinical records at his consulting rooms in London. It was his dream that this should form the nucleus of an international focal point of leprosy study, and the Leprosy Study Centre is the fulfilment of that dream. Standards of excellence in patient care, in training and in histopathology have given the Centre a high reputation. Biopsy material has been sent from many parts of the world, and now in magnitude, range and in detailed records the histological collection is unique.

This Article, the result of much careful study of this great wealth of material, concentrates more on the results than on the techniques used, already well described by Harman [*Lepr. Rev.* **46,** 125 (1975)]. Unusual aspects of differential diagnosis are mentioned, as is the value of serial sections in indeterminate cases. There is particular reference to microfilariasis and the contribution of the Centre to elucidating exit routes of leprosy bacilli from the body.

T. F. Davey

19. EDITORIAL. **Relapse in leprosy.** *Brit. Med. J.* 8/10/77, 914.

This timely article states the present position regarding relapse in leprosy, succinctly, clearly and with authority. Inevitably the main stress is on drug resistance as a cause of relapse. The list of antileprosy drugs to which resistance can arise has now been extended by the addition of rifampicin, but by far the most important aspect of the subject is the widespread emergence of resistance to dapsone. "Each year this phenomenon may be expected to emerge in about 3% of patients with multibacillary leprosy who have been under dapsone alone for 8 years or longer." This particularly applies when treatment has been irregular.

The need for early recognition of relapse due to resistant organisms, and the great desirability of combined therapy from the start in multibacillary leprosy are rightly emphasized. Both these aspects pinpoint the need for higher standards of training and management, especially in field workers engaged in antileprosy programmes.

T. F. Davey

The Abstracts which follow are reprinted from Tropical Diseases Bulletin, *October and November 1977 and January 1978, through the courtesy of the Director, Bureau of Hygiene and Tropical Diseases. They are classified according to subject.*

I. MICROBIOLOGY

20. MATSUO, Y., TASAKA, H. & UTSUNOMIYA, S. **A culturable mycobacterium isolated from leproma of a leprosy-transmitted armadillo.** *Lepro,* 1976, v. 45, No. 2, 63–67.

Mycobacterium scrofulaceum was isolated, apparently, from a lesion of an armadillo infected with *M. leprae.* The culture medium was a monolayer growth of mouse footpad cells, which

produced a 3-fold increase in the number of inoculated bacilli on primary culture, followed by a heavy increase on secondary culture from which *Myco. scrofulaceum* was isolated. The authors think there is no doubt that this contaminant originated from the leprosy lesion of the armadillo. Organisms transmitted from the lesion to a mouse footpad were identified as *Myco. leprae.*

[It is of interest also that Pattyn and others are recently reported to have identified the Skinsnes organism as *Myco. scrofulaceum,* while Kato and Skinsnes suggest that *Myco. leprae* grown *in vitro* might be related to *Myco. scrofulaceum* (see *Int. J. Lepr.,* 1976, v. 44, 385 and 491)].

D. S. Ridley

21. KIRCHHEIMER, W. F. **Occurrence of *Mycobacterium leprae* in nature.** *Lepr. India,* 1977, v. 49, No. 1, 44–47.

The discovery of a leprosy-like disease in wild armadillos in southern Louisiana was reported in 1975 [*Trop. Dis. Vull.,* 1976, v. 73, abstr. 896], but other workers have not so far confirmed the natural occurrence of leprosy in these animals. Three hundred and nine armadillos from Louisiana, Florida and Texas were examined at Carville. A histopathological study of lymph nodes, spleens, livers and other organs was made on 164 of these; blood buffy coat and ear-clip examinations were done on 159, and in 14 both kinds of examination were performed. No evidence of "mycobacteriosis" was found. The negative results of other studies in Colombia and in Paraguay are reported. A mycobacterium cultured from an armadillo caught in Louisiana was typed as *Mycobacterium peregrinum.*

F. I. C. Apted

22. MATSUO, Y. & UTSUNOMIYA, S. **Viability of *Mycobacterium leprae* pretreated with rifampicin.** *Lepro,* 976, v. 45, No. 3, 174–176.

Suspensions of *Mycobacterium leprae* were incubated at 4°C or 30°C for 60 min with rifampicin at a concentration of 2 mg/ml. Before inoculation of mice, halves of the suspension were repeatedly washed with a balanced salt solution. The unwashed bacilli did not multiply in mouse footpads regardless of the exposure temperatures to the drug. The washed ones pretreated at 4°C multiplied normally. The organisms treated with the same procedure but at 30°C resulted in a significant growth delay.

23. OLITZKI, A. L. **Further potential sources of energy modifying the multiplication of *Mycobacterium leprae.*** *Boll. Ist. Sieroter. Milan,* 1977, v. 56, No. 4, 384–386.

The multiplication of *Mycobacterium leprae* was modified by graded dilutions of organic acids. 0.01%–0.05% gluconic acid inhibited its multiplication. 0.005% of it promoted the growth of 2 out of 6 strains. 0.2%–1.0% glucuronic acid promoted the multiplication of the majority of strains. 2.0% inhibited their multiplication, and 0.05% promoted the growth of one strain.

Galacturonic and pyruvic acids were active in 0.2–2.0% concentrations, while the activity of citric acid was mainly noted at 1.0 and 2.0% concentrations.

[See *Trop. Dis. Bull.,* 1977, v. 74, abstr. 54.]

24. PATTYN, S. R. **The effect on the multiplication of *Mycobacterium leprae* of irregular administration of dapsone to mice. Results of the total minimal inhibitory test.** *Ann. Soc. Belg. Méd. Trop.,* 1977, v. 57, No. 3, 175–179.

Dapsone in a 0.01% concentration in the food was administered to mice for 1–6 days a week every week and every 2, 3 and 4 weeks. It was further administered daily for periods ranging

from 4–28 weeks after infection. In all drug regimens dapsone was purely bacteriostatic, since multplication started in some of the animals sometime after stopping treatment. It is concluded that human paucibacillary leprosy should preferably be treated with a more bactericidal drug and multibacillary cases during an initial phase with drug combinations.

2. IMMUNOLOGY, PATHOLOGY

25. KAWAGUCHI, Y. & MATSUOKA, M. **Observation of host reactions to murine leprosy bacilli in spread subcutaneous tissue preparations of various strains of mice.** *Jap. J. Exp. Med.,* 1977, v. 47, No. 2, 71–79.

Male mice of 6 inbred strains (C3H, CF1, KK, BALB/C, DDD and C57BL/6) were inoculated subcutaneously in the back with 0.25 ml of a 1 : 1000 leproma suspension (Hawaiian strain). Growth patterns of murine leprosy bacilli in subcutaneous tissue at the inoculation site were examined on the spread tissue preparations.

No remarkable differences were observed among these mouse strains during the first 3 weeks after inoculation. An acute inflammatory reaction with accumulation of many polymorphonuclears disappeared in 1 week and elongation of the bacilli was evident in mononuclears without increase in number. The bacilli were about 2–3 times as long as the initial length. At 2 weeks the elongated bacilli were fairly abundant within the cells, but some were present extracellularly. At 3 weeks enlarged mononuclears, being crowded with long bacilli, could easily be demonstrable by low magnification. Four weeks after inoculation, however, significant differences in the growth patterns were seen among these mouse strains. In C3H and CF1 mice, an infiltrate consisting mainly of mononuclears was seen in the subcutaneous tissue at the inoculation site. Most of the mononuclears were heavily loaded with the long bacilli and were scattered or accumulated in the whole specimens. In contrast, lymphocytes and polymorphonuclears were predominant in the other 4 strains of mice, and they surrounded a smaller number of mononuclears containing the long bacilli. Such differences between mouse strains became more remarkable at 5 weeks because of more pronounced cellular reactions in these 4 strains. The difference between C3H and CF1 mice was manifested in 8–10 weeks by infiltration of lymphocytes, surrounding accumulated mononuclears loaded with the bacilli, which was seen only in CF1 mice.

The mouse strain differences as above in response to murine leprosy bacilli are discussed on the basis of cellular immunity in the hosts.

26. WESTERHOF, W. **A possible dysfunction of melanosome in leprosy: an electron-microscopic study.** *Acta Derm.-Vener.,* 1977, v. 57, No. 4, 297–304.

An E.M. study was carried out to investigate whether *Mycobacterium leprae* occur intracellularly in epidermal melanocytes. As this could not be confirmed, the selective killing of melanocytes by cytotoxic lymphocytes could not explain the hypopigmentation in types of leprosy with a relatively good immune response. There were indications that these hypopigmented lesions resulted from a disturbed transfer of melanosomes from melanocytes to keratinocytes. Further research in progress.

27. CHOGLE, J. B. & KHANOLKAR, S. R. **T & B lymphocytes in the spectrum of leprosy.** *Lepr. India,* 1977, v. 49, No. 1, 36–43.

The percentage of T and B lymphocytes were estimated in 52 leprosy patients by "E" and "EAC" rosette techniques. The mean percentage values for "T" lymphocytes were significantly lower in lepromatous group as compared with that of tuberculoid and borderline groups. Also, a significant difference was observed in the mean percentage values of T and B lymphocytes of the borderline and tuberculoid patients and of the normal control group. These findings were correlated with skin smears and lepromin testing.

28. SAHA, K. & GUPTA, I. **Immunologic aspects of leprosy with special reference to the circulating antispermatozoal antibodies.** *Int. J. Lepr.,* 1977, v. 45, No. 1, 28–37.

Macroscopic sperm agglutination in gelatin, sperm immobilization and tanned red cell hemagglutination tests could detect antispermatozoal antibodies respectively in 41%, 37% and 23% sera of 35 leprosy patients, including 5 female cases. Interestingly, all of the above tests were positive in one serum from a female patient with borderline leprosy. Sperm antibodies were detected in both lepromatous and tuberculoid forms of leprosy by the above three technics and no significant difference was observed in their incidences among the 2 groups of patients. A three-dimensional correlation was observed in 57% of 42 tests performed with 14 sera. Head-to-head type of agglutination was the predominant feature of spermagglutination observed in the sera of these patients. In the control group, only 1 of 50 normal fertile males showed a positive spermagglutination test. Not one in this group showed positive sperm immobilization and tanned red cell hemagglutination tests.

Antihuman globulin consumption test, presumably a very sensitive test, was also employed to demonstrate sperm-specific antibodies in the sera of these leprosy patients. These antibodies were adsorbed on the surface of the normal donors' spermatozoa when the latter were incubated with the patients' sera. Antispermatozoal antibody could be demonstrated by this sensitive technic in the sera of 2 female patients. Moreover, antihuman globulin was consumed more intensely by the antispermatozoal antibodies present in the sera in the lepromatous than in the tuberculoid and borderline leprosy groups.

29. BALAKRISHNAN, S. & RAMU, G. **Blood DDS levels and acetylation rates of sulphadimidine in leprosy patients.** *Lepr. India,* 1977, v. 49, No. 1, 59–64.

The plasma DDS clearance rates and the acetylation rates of Sulphadimidine were studied in a group of 30 leprosy patients comprising of 17 non-responders and 13 responders to DDS treatment. No differences in the acetylator type or in the plasma DDS clearance were seen between the responders and non-responders. Acetylation rate did not bear any relation to the plasma clearance of DDS in the non-responders. The findings indicate that the resistance to DDS therapy in these patients is not related to any abnormal metabolic disposition of DDS.

30. HARADA, K. **A modified allochrome procedure for demonstrating mycobacteria in tissue sections.** *Int. J. Lepr.,* 1977, v. 45, No. 1, 49–51.

A modified allochrome staining procedure is presented as being the most reliable and sensitive method for demonstrating mycobacteria in tissue sections. The technic is as follows: Deparaffinize formalin fixed sections, oxidize in 10% periodic acid for 24 h, differentiate in 1% HCl-70% ethanol, stain in Weigert's iron hematoxylin nuclear stain, and counterstain in picro-methyl blue. Mycobacteria stained brilliant red in contrast with the allochrome-stained background tissues, and apparently otherwise chromophobic bacilli are demonstrated.

31. NATH, I., CURTIS, J., SHARMA, A. K. & TALWAR, G. P. **Circulating T-cell numbers and their mitogenic potential in leprosy—correlation with mycobacterial load.** *Clin. Exp. Immunol.,* 1977, v. 29, No. 3, 393–400.

The effect of treatment and mycobacterial load on circulating T-cell numbers and their functional ability was investigated in 41 patients with leprosy. Both early binding T-cells and their responses to phytohaemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM) were profoundly and uniformly depressed in untreated, and partially treated, bacilliferous lepromatous leprosy (LL) patients as compared with normal subjects and tuberculoid patients. On elimination of mycobacteria, subsequent to chemotherapy, LL patients regain normality in T-cell numbers and their functions. On the other hand, the specific response

of lymphocytes to *M. leprae* did not alter with decrease in mycobacterial load. It appears that the decrease in T-cell numbers and the deficit in their mitogenic potential is a secondary consequence of disease and is related to the antigenic load in patients with lepromatous leprosy.

32. YOUNGCHAIYUD, U., CHANDANAYINGYONG, D. & VIBHATAVANIJA, T. **The incidence of HLA antigens in leprosy.** *Vox Sang.,* 1977, v. 32, No. 6, 342–345.

HLA antigens were studied in 36 patients with leprosy, 20 cases of lepromatous and 16 cases of tuberculoid type. Eleven out of 36 (30.55%) had BW40 as compared to 9.33% of 150 controls. The frequency of BW40 in tuberculoid patients (31.25%) was not different from that in lepromatous cases (30%).

33. KWAPINSKI, G., KWAPINSKI, E. & ALMEIDA, J. O. **Preliminary investigations on abnormal immunoglobulin(s) in leprosy.** *Int. J. Lepr.,* 1977, v. 45, No. 1, 24–27.

A new, abnormal immunoglobulin designated IgK has been discovered in leprous Rubino-negative sera. The IgK having a negative net charge and its own antigenic specificity appears to be partly related to the Fc fragment of IgA and to Fab fragments of known immunoglobulins, but its net charge is negative at pH 8.8. Its molecule seems to possess kappa but not lambda light polypeptide chains. Implications of this discovery are discussed.

34. POULTER, L. W. & LEFFORD, M. J. **Development of delayed-type hypersensitivity during *Mycobacterium lapraemurium* infection in mice.** *Infection & Immunity,* 1977, v. 17, No. 2, 439–446.

Various preparations of *Mycobacterium lepraemurium* were used to elicit delayed-type hypersensitivity in the footpad of mice infected with this organism. With a sonicated preparation of the mycobacterium, a significant increase in footpad swelling was elicited in mice infected with *M. lepraemurium* 5 weeks previously, but not in BCG-infected animals or uninfected controls. This footpad reaction was shown to peak at 24 h and to be associated with an infiltration of mononuclear cells. The kinetics of footpad swelling, its association with lympho-proliferation, and its dependence on T lymphocytes were each examined. The results support the hypothesis that this is a delayed-type hypersensitivity reaction. The ability to transfer this reactivity to normal mice with cells but not serum offers further confirmation that this hypersensitivity is dependent on cell-mediated immunological mechanisms rather than humoral antibody. The relevance of this to the study of the immunological response of mice to murine leprosy is discussed.

35. JACOB, W., PATTYN, S. R. & DOCKX, P. **Cytochemical evidence for aerobic pathways in *Mycobacterium lepraemurium.*** *Int. J. Lepr.,* 1977, v. 45, No. 1, 9–13.

Three enzymes of aerobic pathways (cytochrome *c* oxidase, peroxidase and catalase) and one key enzyme of the tricarboxylic acid cycle (succinate dehydrogenase) were investigated for their ultrastructural localization in *M. lepraemurium* in infected mouse liver and in cultures of *M. fortuitum* as a control. All 4 enzymes were localized in *M. fortuitum.*

To *M. lepraemurium* only cytochrome *c* oxidase and peroxidatic activity were detected. The localization of the latter enzyme activity was different compared with *M. fortuitum.* Succinate dehydrogenase was not detected in *M. lepraemurium* but rather surprisingly was found in the membrane of the phagosomes containing the bacteria.

It is concluded that *M. lepraemurium* can function aerobically and has either a glyoxalate pathway or is an obligate autotroph.

3. CLINICAL

36. QUINETE, S. S., MARQUES, A. S., RANGEL, E. R. & ROCHA, G. L. Lepra de Lucio [**Leprosy: Lucio type.**] *Anais Bras. Derm.,* 1977, v. 52, No. 1, 107–115.

The English summary appended to the paper is as follows:

"One case of lepromatous leprosy with Lucio phenomenon is presented. The conception of Lucio leprosy and of Lucio phenomenon is discussed; the vasculitis underlying the phenomenon in the case presently described is stressed."

37. SEHGAL, V. N., REGE, V. L. & SINGH, K. P. **The age of onset of leprosy.** *Int. J. Lepr.,* 1977, v. 45, No. 1, 52–55.

The age of onset was determined in 1053 consecutive patients having different types of leprosy. There were 675 males and 378 females. The majority had onset of the disease between ages 20 and 39, although all age groups were affected. The age of onset was significantly related to the type of leprosy; the mean was lowest in tuberculoid, highest in neuritic, while in borderline and lepromatous it was in between. The comparison of reports of the age of onset from India and elsewhere suggest that this varies in different regions within the country, and from country to country.

38. HARRELL, J. D. **Ocular leprosy in the Canal Zone.** *Int. J. Lepr.,* 1977, v. 45, No. 1, 56–60.

The results of a 2 year survey of eye problems among the patients at the Palo Seco Hospital in the Canal Zone are presented. Only 2 patients, one classified as having lepromatous leprosy and the other as having the tuberculoid form of the disease, failed to exhibit ocular complications. The high prevalence of leprotic ocular disease (96%) is most probably due to the advanced age of the patients, the lengthy duration of their illness, and the high percentage of patients afflicted by the lepromatous form of the disease.

4. THERAPY

39. PATTYN, S. R. & SAERENS, E. J. **Activity of three new rifamycin derivates on the experimental infection by *Mycobacterium leprae*.** *Ann. Soc. Belg. Méd. Trop.,* 1977, v. 57, No. 3, 169–173.

Three new rifamycin derivates characterized by longer lasting serum levels were tested against *M. leprae* in the mouse model. Their minimal effective dose is slightly to moderately lower than that of rifampicin. Intervals of administration can however not be increased over once every 2 weeks.

On a weight basis one of the drugs is 8 times more potent than rifampicin.

40. SHEPARD, C. C., VAN LANDINGHAM, R. & WALKER, L. L. **Effect of levamisole on *Mycobacterium leprae* in mice.** *Infection & Immunity,* 1977, v. 16, No. 2, 564–567.

Levamisole, an antihelminthic drug that is capable of enhancing immune responses in mice and in humans, was tested in experimental *Mycobacterium leprae* infections in mice by a number of schedules. Intermittent schedules were used, and administration of the drug was started (i) around the time of inoculation with *M. leprae,* (ii) when the *M. leprae* population was approaching the plateau level, (iii) after the onset of the plateau phase, or (iv) after BCG vaccination 28 days following the inoculation with *M. leprae.* No effect of drug could be discerned with any of the schedules.

41. SANTOS, I. Tetramisol em Hanseníase. I. Viragem lepromínica. [**Tetramisole in leprosy. I. Effect on lepromin reaction.**] *Anais Bras. Derm.,* 1977, v. 52, No. 2, 165–173.

The English summary appended to the paper is as follows:

"Tetramisole was administered to 30 lepromatous patients and 10 healthy lepromin-negative persons, in a daily dosage of 160 mg during 30 days. An activation of the Mitsuda reaction was obtained in 19 lepromatous patients and in 6 healthy people."

42. ALMEIDA NETO, E. & JORGE, M. D. Tratamento da lepra com a associação sulfamoxol e trimetoprin. Ensaio duplo cego com o DDS em 20 pacientes lepromatosos. [**Treatment of leprosy with a combination of sulphamoxole and trimethoprim. Double blind test with DDS in 20 patients.**] *Anais Bras. Derm.,* 1977, v. 52, No. 2, 153–164.

The English summary appended to the paper is as follows:

"Therapeutic effectiveness of the association sulfamoxole + trimethoprim as compared to DDS for the treatment of lepromatous patients is studied through a double-blind test, over a period of 12 months. The authors conclude that trimethoprim is devoid of therapeutic activity and that sulfamoxole is specifically active but less than DDS."

5. EPIDEMIOLOGY, PREVENTION, CONTROL

43. LECHAT, M. F., MISSON, C. B., BOUCKAERT, A. & VELLUT, C. **An epidemiometric model of leprosy: a computer simulation of various control methods with increasing coverage.** *Int. J. Lepr.,* 1977, v. 45, No. 1, 1–8.

An epidemiometric model of leprosy has been developed to predict and simulate trends of leprosy under various control conditions. This model, whose structure was previously described, is based on data collected in the Polambakkam leprosy control scheme in South India over a 16-year period, from 1954–1970. Incidence of leprosy has been computer simulated at 5, 10, 15 and 20 years. The following control measures, some of them still in the development stage, were considered: (a) unmodified leprosy control as carried out in the study population, based on early detection and regular treatment; (b) vaccination with a BCG-like type vaccine effective for preventing development of leprosy as the lepromatous type and converting potentially lepromatous cases into tuberculoid ones; (c) vaccination with a disease specific vaccine supposed to be 100% effective in preventing leprosy; (d) improvement over present conditions in case-holding; (e) isolation of lepromatous patients for 1 year after detection. The methods tested were simulated at different ranges of coverage, ranging from 10–100%, and compared to the results predicted with the present control strategy taken as base lines.

Under all circumstances, specific vaccination was the most effective method. A 100% coverage of the population with an effective vaccine will interrupt transmission after 10 years. A 90% incidence reduction is achieved at approximately 8 years with 100% coverage, 10.5 years with 90% coverage, and 18 years with 80% coverage. Compared to other methods, specific vaccination with 20% coverage is as effective for controlling the disease as isolation of all the lepromatous patients for 1 year after detection. This clearly stresses research in the development of a vaccine as the highest priority for leprosy control.

A computer program has also been designed which predicts annual prevalences and cumulative prevalences over time, since these parameters can be of particular interest to governments and funding agencies. These data provide the base lines for cost-effectiveness analysis of leprosy control.

[See Lechat *et al., Trop. Dis. Bull.,* 1976, v. 73, abstr. 1324.]

44. KOLONEL, L. N. & HIROHATA, T. **Leprosy and cancer: a retrospective cohort study in Hawaii.** *J. Natn. Cancer Inst.,* 1977, v. 58, No. 6, 1577–1581.

We used data collected on a retrospective cohort of 1123 leprosy patients living in Hawaii between 1940 and 1970, to test the hypothesis that patients with lepromatous leprosy, who have an impairment in their cellular immune response, would have an increased risk for cancer and that patients with tuberculoid leprosy, who are immunologically competent, would have a normal or even a reduced cancer risk from beneficial stimulation of their cellular immune system by exposure to the *Mycobacterium leprae* organisms. Based on a survival analysis method, the results of the study supported the predicted increase in cancer cases among the lepromatous leprosy patients (19 observed, 12.7 expected; risk ratio = 1.5) and the predicted decrease among the tuberculoid leprosy patients (14 observed, 17.8 expected; risk ratio = 0.8); in both groups, the findings were consistent across the 5 racial categories of the study. However, none of these differences between observed and expected cases was statistically significant at the 5% level. The study provided no support for the alternate hypothesis that chronic antigenic stimulation by the *M. leprae* organisms might lead to an increase in tumors of the lymphoreticular system.

6. REHABILITATION AND SOCIAL ASPECTS

45. SANKALE, M., NDIAYE, P. & BEYE, I. Enquête préliminaire sur l'opinion du noir sénégalais vis-à-vis de la lèpre. [**Preliminary enquiry into the opinions held by the Senegalese about leprosy.**] *Méd. Afr. Noire,* 1977, v. 24, Nos 8/9, 571–581.

By means of a verbal questionnaire distributed to health workers in contact with a cross-section of African opinion in Senegalese villages, the authors hoped to obtain information on currently held beliefs about leprosy. Out of 1310 forms distributed, only 532 were returned adequately completed from healthy people and 100 from patients with leprosy. This highly selective sample is analysed.

Most of the replies came from one ethnic group, and the background of the individuals composing this group is not given in detail, apart from age and sex structure and district of residence.

Among the healthy, most people seem to know what leprosy is, and fear it as a "great sickness": various equivalents in local dialects are given. The cause of the disease is commonly held to be explicable only in supernatural terms—it is a punishment or a curse (42%), but heredity (29%) or the taking of certain foods, e.g. goat meat, fish, milk (17%), may also be factors.

About two-thirds of the replies held that leprosy was an hereditary condition, and cited as proof its common appearance among the young in families where there was already a sufferer, but a high proportion (98%) thought that it was contagious, being transmitted by clothing, body secretions (such as sweat, saliva, sputum), sexual relations or other forms of physical contact.

The authors emphasize that public opinions and attitudes are based on beliefs. Thus the great majority of persons questioned considered that those suffering from leprosy should be segregated, although most of them admitted that the disease was curable—especially by doctors (rather than by medicine men).

To avoid catching leprosy, opinion seemed to advocate: no contact with sufferers from the disease, and maintain high standards of cleanliness and hygiene.

[Despite the relative sophistication of many of those responding to the questionnaire, the prevailing ignorance about leprosy is very obvious, as is the need for health education.]

S. G. Browne

46. FRIST, E. **A study of community attitudes and knowledge in relation to leprosy.** *Hansenologia Int.,* 1976, v. 1, No. 2, 184–190.

A study of community attitudes and knowledge in relation to leprosy was undertaken in the Bauru Region of the Brazilian State of São Paulo as preparation for an integration project in the

region. A representative sample of approximately 500 persons was interviewed in 7 municipalities by 15 psychology students. The results of the study showed that the level of knowledge about leprosy in the region is very low with the mean score on a basic knowledge test being 37.5% correct. While results showed the existence of a "leprosy stigma" in the region, they also demonstrated a considerable degree of acceptance on the part of the general population to maintaining close work and friendship relationships with patients under treatment. Other answers to questions in the study indicated that the roots of the "leprosy stigma" lie more in the fear of "contagion" and the disease's effect on social relationships than in the fear of physical problems such as pain and deformities. The author is left with a feeling of cautious optimism as to the success of integration efforts when these are accompanied by health education activities with those with whom the patient is to maintain close contacts.

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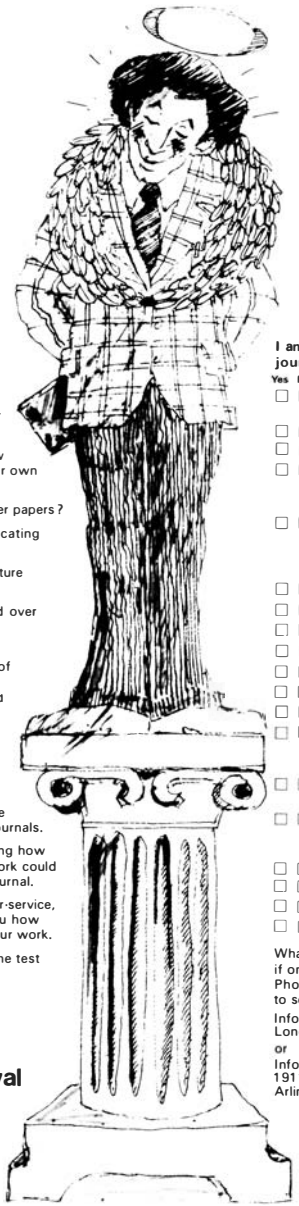

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