LEPROSY REVIEW

Volume 54, Number 4, December 1983

Published Quarterly for the
British Leprosy Relief Association

ISSN 0305-7518

Lepr Rev (1983) **54,** 261–270

Editorial

SIDE-EFFECTS OF ANTILEPROSY DRUGS IN COMMON USE

Introduction

The drugs which I shall describe are dapsone, clofazimine, rifampicin, ethionamide and thiacetazone. I have chosen ethionamide rather than prothionamide, as the latter is no longer marketed in Britain although still available in some countries. This choice matters little, for these two closely related drugs are equally effective in leprosy, and the reported reduced incidence of gastric side-effects from prothionamide (which my own experience does not confirm) is offset by the lower cost of ethionamide. Before describing side-effects I must draw attention to two points. First, the difficulty in deciding which drug is responsible when multidrug therapy is being administered, especially as certain combinations of drugs are known to act synergistically, and this fact should be given consideration when side-effects of rifampicin, ethionamide and thiacetazone are discussed. Secondly, although reactions (reactional states) can be precipitated by chemotherapy, I propose to exclude them in this paper as the relationship is indirect and, furthermore, considerations of space are involved.

I describe side-effects under two headings in order to separate those which are mild from those of greater significance. Where I use the term 'gastro-intestinal symptoms' I include any or all of the following: anorexia, nausea, vomiting, abdominal discomfort and disturbed bowel function, and the term 'transient rash' implies an irritating erythematous rash, often localized to face and neck, but which may be generalized. All these symptoms are usually self-limiting. As regards serious side-effects, I shall describe in these introductory pages the clinical and laboratory features of those which are produced by more than one of these 5 drugs, and thus avoid repetition; details of those which occur with only one of them will be described in the appropriate sections. It should be noted that recovery is the general rule if the offending drug is promptly withdrawn.

A EFFECTS ON SKIN

The following skin complications may occur as part of a hypersensitivity

0305-7518/83/054261+10 $01.00

syndrome which includes fever, eosinophilia, lymphadenopathy and hepatitis, or may occur on their own as an incomplete form of the syndrome.

1 *Exanthematous skin eruption*

This is a generalized skin eruption, generally symmetrical but often the face is spared. It may simulate scarlatina or measles, or may consist of small erythematous papules or urticarial weals. Exfoliative dermatitis may supervene.

2 *Exfoliative dermatitis*

The patient feels cold and shivery, and temperature is raised. The entire skin, including that of the scalp, flakes off as scales and leaves a pronounced redness.

3 *Toxic epidermal necrolysis (Lyell's syndrome; the scalded skin syndrome)*

This is ushered in by malaise and fever. The skin feels tender and erythema begins in axillae and groins, extending over the entire skin surface excepting the scalp. The erythema may become papular and may irritate. Large flaccid blisters, filled with clear fluid, appear on face, trunk and limbs, and large areas of skin become detached as if scalded. A few days later the detached skin peels off in large sheets, revealing pink, oozing, painful areas. Death has been recorded as a rare event if the drug has not been withdrawn.

4 *Stevens–Johnson syndrome (erythema multiforme bullosum)*

This is a severe bullous form of erythema multiforme in which bullae of skin and oral mucosa are associated with fever and prostration. Bullae of mouth ulcerate and become crusted, while eyes may be involved in the form of conjunctivitis and corneal ulceration. The illness is likely to last for 2 or 3 weeks and carries a mortality of about 5–15%.

B EFFECT ON BLOOD

1 *Haemolytic anaemia*

A severe haemolysis will be suspected by pallor and slight jaundice. Laboratory findings include a marked reduction in haemoglobin and in haptoglobulin concentration, red blood cells are normochromic and show anisocytosis, poikilocytosis, and may contain Heinz bodies. Their lifespan is reduced. There is an increase in reticulocytes, and also in serum bilirubin and urinary urobilinogen.

2 *Agranulocytosis*

This should be suspected in a patient who develops fever, fatigue, headache and sore throat. The pharynx becomes ulcerated and there is a severe shortage of granulocytes in the blood.

3 *Thrombocytopenia*

There is an increased bleeding tendency in the form of petechiae or purpura, or less commonly of haemorrhagic bullae of oral mucosa or of bleeding from bowel or urinary tract. Thrombocytes (platelets) are much reduced.

C EFFECTS ON NERVES

Peripheral neuropathy

Classical findings are weakness in muscles, impaired sensation in limbs, and weak or absent tendon reflexes. These signs are bilateral and may be confined to upper or to lower limbs, or may affect both. Early manifestations are clumsiness in walking or in using the hands, and bilateral footdrop is a common sequel.

D EFFECTS ON BRAIN

Psychosis

Symptoms include insomnia, irritability, agitation, violence, delusions, disorientation, visual and auditory hallucinations, and speech which is slow and incoherent.

E EFFECTS ON LIVER

Hepatitis

This may occur as part of a hypersensitivity syndrome of fever, eosinophilia, lymphadenopathy, and any one of the hypersensitivity skin manifestations which have already been described, or it may occur on its own as an incomplete form of the syndrome. Hepatitis is heralded by malaise, anorexia, nausea, discomfort in muscles and joints, darkening of urine, and a faint yellowness of conjunctivae. Later developments are jaundice, dark urine, tender liver enlargement, bilirubinaemia, and abnormal liver function tests.

F EFFECTS ON KIDNEYS

Renal failure

Prodromal symptoms may occur soon after ingestion of the drug, disappearing within a few hours, and may be repeated on several occasions before oliguria is noticed. These are malaise, chilliness, nausea, vomiting, mild pyrexia, and lumbar discomfort. Protein is found in urine, plasma urea and creatinine are raised, and oliguria after a few days is replaced by anuria.

Five antileprosy drugs and their side-effects (Table 1)

1 DAPSONE (DDS; 4 :4′DIAMINODIPHENYL SULPHONE)

(a) *Mild side-effects*

These include gastro-intestinal symptoms, transient rash, headache, dizziness, and a sensation of 'woolly-headedness'—an inability to think clearly for a few hours after taking the tablet. Methaemoglobinaemia (causing a faint cyanosis) and a mild haemolysis commonly occur and are harmless.

(b) *Serious side-effects*

Because dapsone is effective in a number of dermatological disorders and often has to be used in dosage greater than 100 mg/day, serious side-effects are almost exclusively encountered by dermatologists. These are as follows:

(1) *Haemolytic anaemia*. This may occur when dosage is 200 mg/day or more; smaller dosage may be responsible in persons who are deficient in the enzyme glucose-6-phosphate dehydrogenase (G-6-PD). But deficiency of this enzyme is not the only factor, for a toxic dapsone derivative (DDS-NHOH) has been implicated. It is noteworthy that a mild haemolytic anaemia has been induced in an infant by dapsone transmitted in breast milk; the infant had significant serum concentrations of dapsone.

(2) *Agranulocytosis*. This is a manifestation of marrow depression, and although a few cases have been reported by dermatologists using dapsone monotherapy, in most of the reported cases dapsone has been used in combination with other drugs in the prophylaxis of malaria.

(3) *Hypersensitivity*. This is confined to the first 6 weeks of treatment, and manifestations include some or all of the following: fever, eosinophilia, mononucleosis, lymphadenopathy, hepatitis, and an exanthematous skin eruption which may progress to one of the dermatological emergencies which I have already described (exfoliative dermatitis, toxic epidermal necrolysis, and the Stevens–Johnson syndrome). A fatal hypersensitivity reaction known as 'DDS

Table 1. Significant side-effects of five antileprosy drugs

	Side-effect	DDS	B663	RFP	ETH	TBI
Adrenals	Adrenal crisis			Yes		
Blood	Agranulocytosis	Yes				Yes
	Haemolytic anaemia	Yes		Yes		Yes
	Thrombocytopenia			Yes		Yes
Bones	Osteomalacia			Yes		
Bowel	Eosinophilic enteritis		Yes			
	'Small bowel syndrome'		Yes			
Brain	Psychosis	Yes		Yes		
	Pellagra-like encephalopathy				Yes	
Kidneys	Nephrotic syndrome	Yes				
	Renal failure			Yes		
	Renal papillary necrosis	Yes				
Liver	Hepatitis	Yes		Yes	Yes	Yes
Nerves	Peripheral neuropathy	Yes			Yes	
Skin	Exanthematous eruption	Yes				Yes
	Exfoliative dermatitis	Yes				Yes
	Fixed drug eruption	Yes				
	Ichthyosis		Yes			
	Pemphigus vulgaris			Yes		
	Porphyria cutanea tarda			Yes		
	Stevens–Johnson syndrome	Yes		Yes		Yes
	Toxic epidermal necrolysis	Yes				Yes
Thyroid	Hypothyroidism				Yes	
Miscellaneous	'DDS syndrome'	Yes				
	'Flu' syndrome			Yes		
	Gynaecomastia				Yes	
	Haemorrhagic disease of newborn			Yes		
	Hypoalbuminaemia	Yes				
	Hypoglycaemia				Yes	

syndrome' was described by leprologists in the early years of the drug's use. It disappeared during the decades when low dosage was in vogue, only to reappear as a rare phenomenon in recent years. One such case has been reported in a patient taking 100 mg/day. After 3 weeks he complained of malaise, myalgia, and a rash on abdomen. A few days later he developed fever, slight jaundice, sore throat, and an erythematous maculopapular rash covering the entire body. Clinical examination revealed exudative pharyngotonsilitis, generalized tender lymphadenopathy, hepatosplenomegaly, and epigastric tenderness. The number of white blood cells steadily increased to 72,800 mm^3 with 28% eosinophils. Skin biopsy was consistent with lepromatous leprosy and erythema multiforme. Death was ascribed to an extreme hypersensitivity reaction to dapsone.

(4) *Fixed drug eruption*. This takes the form of one or more sharply marginated round or oval erythematous macules which become violet, brown or black, and persist for a long time.

(5) *Peripheral neuropathy*. This has been reported by dermatologists, and clearly could pose a diagnostic problem in leprosy. However, the diagnosis could be suspected if there were no tender nerves on palpation and if tendon reflexes were depressed or absent.

(6) *Psychosis*. To attribute this complication to dapsone could be difficult when treatment is administered in a leprosarium, for the incidence of psychosis in segregated patients is about 10%. However, the role of dapsone in causing psychosis has been established as a rare occurrence in out-patients.

(7) *Hypoalbuminaemia*. This is a very rare side-effect which has never been encountered in leprosy. The few reports have been by dermatologists in patients who have received dapsone over many years for dermatitis herpetiformis. Symptoms include dyspnoea, pleural effusion, peripheral oedema, polyuria, and ascites, and tests show hypoalbuminaemia. The syndrome is attributed to great increase in intravascular albumin catabolism and a modest decrease in synthesis. There has been one death in the 5 cases described to date.

(8) *Nephrotic syndrome*. The case has been reported of a patient suffering from a 'pruritic skin eruption' receiving dapsone 100 mg/day for 3 weeks and developing massive proteinuria, hypoproteinaemia, oedema and lipidaemia. Recovery was uneventful.

(9) *Renal papillary necrosis*. A patient suffering from dermatitis herpetiformis who had received fairly large daily doses of dapsone for 15 years began having attacks of renal colic, and 5 years later was found to have bilateral renal papillary necrosis. A blood count showed a moderate haemolytic anaemia and reticulocytosis.

2 CLOFAZIMINE (B663; LAMPRENE)

A bright red iminophenazine dye.

(a) *Mild side-effects*

These are dose-related and reversible, the commonest being a red-brown colouration of skin and mauve or brownish-black pigmentation of skin lesions. These changes are scarcely noticeable in dark skins, but light-skinned persons find them unacceptable, and some of my patients have complained that dark marks on face and limbs have embarrassed them for 5 years after stopping the drug. Conjunctivae show varying degrees of red-brown pigmentation, and redness may be seen in urine, faeces, sputum, sweat and tears during treatment. Less common side-effects are gastro-intestinal symptoms and a general dryness of the skin which may progress to ichthyosis of limbs. Less common still are other

dermal manifestations such as transient rash, acne, itching or burning discomfort in skin lesions, and phototoxicity (increased reactivity of skin to light). There is one report of a reversible ocular side-effect in the form of brownish pigmentation in cornea and macula.

(b) *Serious side-effects*

These are confined to the small bowel and are dose-related. *An early syndrome* commences within a few days or weeks of instituting treatment and consists of gastro-intestinal symptoms which can be relieved by reducing dosage. *A late syndrome* is of more serious significance. It begins after months or years of high dosage of clofazimine, and a good name for it would be 'small bowel syndrome'. The three cardinal symptoms are persistent diarrhoea, abdominal pain, and weight loss. Localized areas of narrowing and dilatation of the ileum are seen on X-ray, and histology of a thickened portion of ileal wall shows a non-specific granuloma characterized by foreign-body giant cells and lymphocytes, together with clofazimine crystals. Crystals are also deposited in mesenteric lymph nodes. This 'small bowel syndrome' has terminated fatally in a small number of cases in which high dosage has been prolonged in order to control chronic or recurrent ENL reaction, and the lesson is that it would be preferable to reserve clofazimine for the treatment of leprosy in the modern dosage of 300–350 mg/week and to use much more effective drugs, such as prednisone or thalidomide when control of ENL is necessary. Eosinophilic enteritis has been reported in a female Samoan who was treated with 600 mg daily for 3 years. She presented with abdominal pain and was found to have an eosinophilia (3600 mm^3). At laparotomy there were about 20 nodular areas in the wall of upper ileum, and a biopsy showed a dense cellular infiltrate of eosinophils and histiocytes together with clofazimine crystals.

3 RIFAMPICIN (RFP; RMP; RIFAMPIN; RIFADIN; RIMACTANE)

A semi-synthetic derivative of rifamycin B, one of a group of antibiotic compounds produced by *Streptomyces mediterranei.*

A *Side-effects of daily and intermittent administration*

(a) *Mild side-effects*

The commonest is red colouration of urine. Other side-effects are uncommon and include transient rash, gastro-intestinal symptoms, drowsiness, weakness and dizziness.

(b) *Serious side-effects*

An uncommon one is hepatitis, and rarer ones are thrombocytopenia, psychosis

and osteomalacia. Osteomalacia has been reported as a complication of daily therapy and is heralded by a fall in plasma 25-hydroxycole calciferol (25-OHD), and if treatment is continued the patient complains of generalized bone pain and tenderness. X-ray shows generalized demineralization, and bone biopsy confirms the diagnosis. Serious sensitivity reactions, characterized by bullous skin lesions, with or without involvement of oral mucosa, have been reported as rare events and have been diagnosed as Stevens–Johnson syndrome, porphyria cutanea tarda, and pemphigus vulgaris respectively. Long-term antibiotic therapy may deplete the body's supply of vitamin K through depression of synthesis of this vitamin in the intestinal tract. This can occur when rifampicin is given daily over a long period of time, and if the patient is a pregnant woman she may give birth to an infant with haemorrhagic disease of the newborn. This complication has been reported from Germany where a woman suffering from lepromatous leprosy was treated throughout her pregnancy with multidrug therapy containing rifampicin.

B *Side-effects confined to intermittent administration*

These have occurred in the treatment of tuberculosis when rifampicin has been administered once-weekly or twice-weekly, but are very unlikely with once-monthly treatment of leprosy as recommended by the 1982 WHO Study Group. These include: (1) *'Flu' syndrome*. Episodes of fever and malaise, sometimes associated with headache, dizziness and pains in limbs, begin 1–2 hours after each treatment and last up to 8 hours. The syndrome is unlikely during the first 3 months of therapy, but thereafter may occur in up to 20% of patients given once-weekly dosage. (2) Shock, dyspnoea, haemolytic anaemia and renal failure may rarely complicate intermittent treatment with rifampicin; they may also occur when treatment has been resumed in full dosage after a long interval.

Here I must add a note on the reduced effectiveness of steroid when given concurrently with rifampicin. This is due to rifampicin's ability to stimulate the production of hepatic microsomal enzymes which increase the metabolic degradation of steroid. Similarly, it can impair the effectiveness of oral contraceptives, and this could lead to an undesired pregnancy in a lepromatous woman; worse still, if the woman is given thalidomide to control a severe ENL reaction on the strength that the pill will prevent pregnancy, the consequences could be disastrous. Associated with rifampicin's capacity to counteract the effect of prednisone during ENL reaction is its counteracting effect on endogenous cortisol. This causes no problem when cortisol production is normal, but in patients with adrenal cortical dysfunction it can be dangerous to give rifampicin. This risk has been highlighted by a recent report of acute adrenal crisis in 2 patients with adrenal insufficiency due to tuberculosis who were treated with rifampicin.

4 ETHIONAMIDE (2-ETHYLPYRIDINE-4-CARBOTHIONAMIDE)

(a) *Mild side-effects*

These include gastro-intestinal symptoms, ptyalism (excessive salivation), a metallic taste, stomatitis, transient rash, acne, headache and dizziness. All are uncommon apart from gastric symptoms.

(b) *Serious side-effects*

These are uncommon. (1) Peripheral neuropathy. (2) Hepatitis. Although clinical jaundice is rare, abnormal liver function tests have been described in 15%, and a rise in transaminase is an indication to keep progress under review while continuing treatment. (3) Hypothyroidism. A diffuse enlargement of the thyroid gland is an early sign. (4) Hypoglycaemia. A connection between sleepiness and hypoglycaemia has been observed, indicating that blood sugar levels should be tested in a patient who becomes drowsy. (5) Psychosis. A pellagra-like encephalopathy has been described, with depression, slowed cerebration, personality changes, and difficulty in walking. Tendon reflexes are brisk and plantar responses may be extensor. Response to nicotinamide is dramatic. This syndrome is more likely to occur if ethionamide is given together with isoniazid (INH) because INH interferes with pyridoxine metabolism, and pyridoxine is a co-factor in the synthesis of nicotinamide from tryptophane. (6) Alopecia. A very rare association between alopecia and ethionamide has been reported, but the mechanism is not understood. (7) Endocrine disturbance. Gynaecomastia has been attributed to ethionamide.

5 THIACETAZONE (TBI; AMITHIOZONE; 4-ACETAMINO BENZALDEHYDE THIOSEMICARBAZONE)

(a) *Mild side-effects*

These include gastro-intestinal symptoms, transient rash, dizziness, headache and drowsiness.

(b) *Serious side-effects*

(1) Skin. Exanthematous skin eruption, exfoliative dermatitis, toxic epidermal necrolysis, and the Stevens–Johnson syndrome have all been encountered, and skin lesions similar to those of lichen planus have been attributed to thiacetazone. (2) Blood. Haemolytic anaemia, agranulocytosis and thrombocytopenia have been reported. (3) Liver. Hepatitis may develop as part of a hypersensitivity syndrome or may occur on its own. It is not uncommon for thiacetazone to cause

abnormal liver function tests without clinical jaundice, and because of its hepatotoxicity it is inadvisable to give the drug to those with preexisting liver impairment. (4) Ototoxicity. It has been found that streptomycin's capacity to cause disturbance of hearing and balance is enhanced if given with thiacetazone.

A unique quality of thiacetazone is that the frequency and severity of its side-effects are influenced by racial and geographical factors. For example, in some areas of India the drug is well tolerated whereas in others there is a high incidence of serious dermatological complications.

Finally, thiacetazone and ethionamide should not be combined in the treatment of leprosy or tuberculosis because of cross-resistance.

W H JOPLING

[The author has kindly offered to make available a list of references on this subject to anyone who cares to apply to the Editorial Office in Oxford. *EDITOR*]

Editorial Note: Ocular leprosy; theme for 1984

This number carries an article on leprosy and the eye, 'A computer form to aid in the collection of data on the ocular complications of leprosy' by Mr T J ffytche. At a recent meeting of the Editorial Board it was decided to publish further articles on the theme of ocular leprosy during 1984. Contributions on this subject, whether as original articles, case reports, or letters will be most welcome.

EDITOR

Lepr Rev (1983) **54,** 271–281

A computer form to aid in the collection of data on the ocular complications of leprosy

T J FFYTCHE
St Thomas' Hospital, London

Accepted for publication 23 February 1983

Summary. There is a need for data on the wide variety of ocular complications of leprosy that have been observed in patients in different parts of the world. Such data would be valuable in the planning of eye care and preventive measures. A computer form has been designed to collect information from groups of leprosy patients so that the results can be centralized and submitted for analysis. The form can be used by medical students as well as medical and para-medical workers, and guidelines on ophthalmic examination in leprosy and the recording of the findings are presented.

Introduction

Information on the ocular complications of leprosy remains incomplete and often anecdotal, and yet visual impairment is one of the major features of the disease. Vision plays an important role in the health and well-being of leprosy patients, its preservation may decrease the severity of the disabilities that typify the advanced condition and its loss imposes a harsh burden, isolating the individual and greatly diminishing the quality of life.

It is becoming increasingly apparent that many of the complications that affect the eyes in leprosy are potentially preventable if they are diagnosed and treated early enough, and health care directed towards these aspects would alleviate much suffering and incapacity. One of the main difficulties in assessing ocular damage from leprosy is the wide variation in the manifestations of eye disease in different parts of the world. Ocular involvement depends on a number of known factors such as the type of leprosy, its status, duration and therapy, and on several less-understood factors including the race and occupation of the patient and the locality and climate of the environment. Reports from different areas of the world emphasize the wide spectrum of ocular leprosy, high-lighting regional variations; and the few statistics to emerge are difficult to interpret. If preventive ophthalmic care is to be successful it is important to determine the

0305-7518/83/054271+11 $01.00

geographic and regional differences in ocular involvement so that leprosy populations at risk can be identified. A computer form has been designed in order to record a number of parameters in groups of patients so that variations of ocular manifestations can be analysed according to factors known or suspected to be influential. By centralizing this data, patterns of ocular disease can be determined and fitted into the mosaic of existing knowledge of the global distribution of leprosy.

Data collection

The ocular complications are usually confined to the anterior segment of the eye and are therefore visible to an examiner using basic equipment of direct illumination and some form of magnification. The slit-lamp and ophthalmoscope are, of course, valuable instruments and give more specialized information, but they require an observer skilled not only in their use but also in the interpretation of what they display. A survey has been set up which is designed to provide data on the gross ocular changes that occur in leprosy without the necessity for the examiner to have detailed ophthalmic knowledge. Observations are recorded on a proforma which can be used by informed para-medical workers and medical students as well as doctors, and it has already been shown by the Peace Corps Survey in South Korea that intelligent observers without formal medical training can provide valuable statistical information on ocular complications by this means.[1]

The proforma is demonstrated in Figure 1 and consists of a questionnaire recording 35 factors for each patient examined. The findings are coded numerically so that they can be transferred easily to a computer. The key to the proforma is tabulated (Figure 2). In any situation where a number of different individuals are assessing ocular findings there is bound to be much variation in the interpretation and recording of clinical signs. This depends not only on the skill and ophthalmic experience of the examiner, but on other factors such as the available equipment and the communication and cooperation of the patients. An attempt should be made to standardize the examination procedures and the following guidelines are suggested to assist in completing the proforma.

Guidelines

It should be possible to re-study patients at intervals and for this reason all patients should be given a reference number consisting of two letters to denote the country where the survey is being carried out (e.g. 'NE' for Nepal) and 4 numbers to identify the patient. This reference should be catalogued with the patient's name and address.

```
 REF:          DATE:       SEX:   AGE:

RACE:      OCC:      LOC:     CLIM:

TYPE:     DUR:     STAT:     TREAT:

VA           R:       L:      BROWS:

LIDS         R:       L:      TRICH:

CLOSURE:     TARS:        LAC:

SCLERA       R:       L:      CONJ:

CORNEA OPAC  R:       L:      PTY:

       SENS  R:       L:      NERVES:

PUPIL REACT  R:       L:

      SIZE   R:       L:

      SHAPE  R:       L:      SYN:

IRIS         R:       L:      PEARLS:

LENS         R:       L:

BLINDNESS    R:       L:

HAND MOB     R:       L:

FINGERS      R:       L:

COMMENTS:
```

Figure 1. Proforma for recording data.

In the subsequent sections an attempt has been made to provide data on environmental and occupational factors that may be relevant in the pathogenesis of ocular complications. Details of the type of leprosy, its duration, status and therapy should also be recorded, if possible from case notes or by direct questioning. When data are not available an 'X' should be entered onto the proforma.

OCULAR EXAMINATION

It would be difficult to produce a comprehensive form that covered every possible ocular complication of leprosy, but conditions frequently associated with the disease have been listed and, with the exception of visual acuity measurements, all

All unknowns write 'X'

REFerence: NE 0687 DATE: 1153 (=Nov 1953) SEX: male 1 female 2 AGE: in years

RACE:

African	1
Arab	2
Asian	3
Chinese	4
European	5
Indian	6
S America	7
Mixed	8
Others	9

OCCupation:

Agriculture	1
Labourer	2
Fisherman	3
Factory	4
Professional	5
Clerical	6
School	7
Home	8
Others	9

LOCality:

Coastal village	1
Coastal town	2
Inland village	3
Inland town	4
Mountain	5
Desert	6
Forest	7
Lake	8
Others	9

CLIMate:

Wet tropical	1
Dry tropical	2
Warm temperate	3
Cool temperate	4
Cold	5
Others	6

TYPE of leprosy:

Lepromatous	1
Tuberculoid	2
Borderline	3
Indeterminate	4

DURation:

in years

STATus:

Positive +++	1
Positive ++	2
Positive +	3
Negative < 5yrs	4
Negative > 5yrs	5

TREATment:

Sulphones	1
Chaulmoogra	2
Rifampicin	3
Lamprene	4
Steroids	5
Combined 1-4	6
Combined 1-5	7
Others	8
None	9

VA:

NPL	1
PL	2
HM	3
CF	4
3/60	5
6/60	6
6/36	7
6/24	8
6/18	9
6/12	10
6/9	11
6/6	12

BROWS:

Normal	1
Reduced	2
Absent	3
Transplant	4

LIDS:

Normal	1
Ectropion	2
Lagophthalmos	3
Entropion	4
Temp transfer	5
Others	6

TRICHiasis:

Absent	1
Right	2
Left	3
Bilateral	4

CLOSure:

Normal	1
Inadequate R	2
Inadequate L	3
Inadequate R+L	4

TARSorrhaphy:

Absent	1
Right	2
Left	3
Bilateral	4

LACrimal:

Normal	1
Blockage R	2
Blockage L	3
Dacryocystitis	4

SCLERA:

Normal	1
Nodules	2
Plaques	3
Episcleritis	4
Scleritis	5
Staphyloma	6
Others	7

CONJunctiva:

Normal	1
Conjunctivitis R	2
Conjunctivitis L	3
Conjunctivitis R+L	4
Others	5

Figure 2, part 1

normal findings are designated by the number '1'. In the event of more than one condition being present within a section, the complication most likely to cause visual loss should be recorded and a comment on the second factor made at the end of the proforma.

CORNEA OPACities:

Absent	1
Pannus	2
Mild	3
Moderate	4
Severe	5
Total	6
Leproma	7

PTY (pterygium):

Absent	1
Right	2
Left	3
Bilateral	4

CORNEA SENSation:

Normal	1
Diminished	2
Absent	3

CORNEA NERVES:

Not seen	1
Thickened R	2
Thickened L	3
Thickened R+L	4

PUPIL REACTions:

Normal	1
Diminished	2
Absent	3

PUPIL SIZE:

2–4 mm	1
<2 mm	2
>4 mm	3
Iridectomy	4
Seclusio	5

PUPIL SHAPE:

Normal	1
Irregular	2
Eccentric	3
Multiple	4
Iridectomy	5

SYNechiae:

Absent	1
Present R	2
Present L	3
Present R+L	4

IRIS:

Normal	1
Atrophy	2
Floccules	3
Floccules + atr.	4
Acute iritis	5
Chronic iritis	6
Leproma	7
Others	8

PEARLS:

Absent	1
Present R	2
Present L	3
Present R+L	4

LENS:

Normal	1
Mild cataract	2
Dense cataract	3
Aphakia	4

BLINDNESS (cause):

6/60 or better	1
Corneal opacity	2
Chronic iritis	3
Seclusio	4
Cataract	5
Staphyloma	6
Phthisis	7
Absent eye	8
Others	9
Non-leprosy cause	10
Cataract + cornea	11
Cataract + iritis	12
Cataract + cornea + iritis	13

HAND MOBility:

Normal	1
mild reduction	2
Moderate reduction	3
No function	4

FINGERS:

Normal	1
1 digit affected	2
2 " "	3
3 " "	4
4 " "	5
5 " "	6
All digits absent	7

COMMENTS:

Anything of particular interest not included in the above.

Figure 2, part 2. Key to data form.

VISUAL ACUITY (VA)

This should be measured at 6 m (20 ft) under conditions of good illumination using standard E test-types. Each eye should be assessed separately and the best visual acuity recorded with glasses if worn. Patients with sub-normal vision should be tested with a pin-hole aperture.

BROWS

Partial or complete loss of both eyebrows together should be recorded and the presence of eyebrow transplants noted.

LIDS

Ectropion refers to eversion of the lower lid margin without impairment of lid

closure or blinking. Lagophthalmos is present when there is inability to close the lids completely leaving some of the cornea exposed; it implies facial nerve palsy and is associated with loss of normal blinking and the protective blink reflex. The combination of lagophthalmos and impairment of corneal sensation is a potent cause of corneal scarring and blindness in leprosy. *Entropion* is present when the lid is inverted and is often associated with troublesome trichiasis. The presence of a *temporalis transfer operation* should be noted.

TRICHIASIS

The presence of lashes in the upper or lower lids which rub against the cornea should be recorded—the condition may give rise to corneal scarring.

CLOSURE

A facial nerve palsy is an important complication of all forms of leprosy requiring surgical attention if the health and transparency of the cornea cannot be maintained by medical means. It should be assessed by asking the patient to close the eyes and observing whether the cornea is adequately covered. Closure against resistance is diminished in early cases.

TARSORRHAPHY

The presence of lateral or medial tarsorrhaphies in either eye should be noted.

LACRIMAL

Disturbances of the lacrimal passages are not a common feature of leprosy but may occur when there is extensive nasal destruction. Watering caused by blockage of the tear ducts should be recorded as it may encourage secondary corneal infection. Occasionally a chronic dacryocystitis is present with swelling at the root of the nose, and a purulent regurgitation into the conjunctival sac may be produced by pressure on the swelling.

SCLERA

Leprosy may cause *nodules* to develop astride the corneo-scleral margin and these should be distinguished from the more common pterygium. Flat *plaques* consisting of discrete areas of tissue swelling often greyish in colour may also occur but are rare. Episcleritis and scleritis are seen in the disease and should be distinguished. In *episcleritis* there is superficial redness with a localized engorgement of blood vessels. The redness in *scleritis* by contrast is deep and more diffuse causing considerable pain and local tenderness. A *staphyloma* (a localized

dehiscence of the sclera) is associated with gross scleral thinning often following scleritis and carries a poor prognosis frequently progressing to phthisis bulbi (shrunken eye).

CONJUNCTIVA

Inflammation of the conjunctiva is rarely caused by leprosy itself but occurs in diseases such as trachoma or may represent secondary infection.

CORNEAL OPACITIES

With the exception of pannus and corneal leproma no attempt need be made to distinguish the many types of corneal opacity that may complicate all forms of leprosy. In most cases corneal scarring is secondary to chronic exposure or trichiasis, although a primary keratitis occurs in lepromatous leprosy. *Pannus* occurs in leprosy in the form of a peripheral opacity, often vascularized, which extends around the whole circumference of the cornea, and its affect on vision is negligible. All other types of corneal scarring should be graded according to their effect on visual acuity: *mild* scarring refers to those cases where the scars do not obstruct the vision at all; in *moderate* scarring vision is affected but it is possible to see details of the underlying structures adequately through the scars; in *severe* scarring there is obviously considerable visual impairment and iris details are almost completely obscured. *Total* corneal opacity should be recorded when no details are visible through the scar and the condition is usually associated with vision reduced to bare perception of light. A corneal *leproma* is a rare tumour-like lesion which may extend across the cornea from the sclera.

PTERYGIUM

Pterygium is common in many parts of the world especially in those who work out of doors in a hot dusty environment. The presence of pterygium, although no more frequent in leprosy, may indicate those patients whose occupations predispose to recurrent minor ocular irritation and trauma which would be harmful for eyes with corneal anaesthesia or facial palsy.

CORNEAL SENSATION

Diminished or absent corneal sensation is an important ocular complication since it renders the cornea more susceptible to damage from external factors. It should be tested by touching the centre of the cornea with a wisp of cotton wool and observing the blink reflex, or in those patients with facial palsy by asking whether the stimulus was felt.

CORNEAL NERVES

Thickened corneal nerves are a pathognomonic sign of lepromatous leprosy. They appear as white lines extending towards the centre of the cornea from the edge and they may show localized swelling resembling a string of beads. They are a transient phenomenon occurring relatively early in the disease.

PUPIL REACTIONS

Chronic iritis is an important cause of blindness in lepromatous leprosy and is often associated with cataract. One of the early signs is a diminution or loss of the pupil reactions to light, particularly the recovery phase of the light reflex. The test should be carried out in a darkened room or in the shade.

PUPIL SIZE

Chronic iritis also causes increasing miosis resulting eventually in a pin-point pupil which does not respond to conventional dilating drops. Vision is considerably impaired through these small pupils especially if there are additional corneal or lens opacities. The pupil size should be measured in subdued light with the patient facing away from any bright illumination. In cases who have had previous ocular surgery a broad *iridectomy* may be present so that no assessment of size can be made and this should be recorded. In advanced cases especially those in whom **acute iritis** has occurred previously, the pupil may be bound down with organized **exudate** which fills the pupil aperture—this condition is known as *seclusio* ***pupillae*** **and is usu**ally associated with profound visual impairment.

PUPIL SHAPE

Chronic iritis may also produce abnormalities of pupil shape and position and in some cases advanced iris atrophy can give rise to multiple apertures. Small peripheral iridectomies may be present in eyes that have had surgery for cataract or glaucoma.

SYNECHIAE

Adhesions between the iris and lens (*synechiae*) usually indicate a past history of acute iritis although they are sometimes seen in chronic iritis. Their presence is often difficult to detect especially when the pupil is small, but they should be suspected if there are irregularities of the pupil margin or if the light reaction is unequal in different quadrants of the iris.

IRIS

The most common feature of the chronic iritis of lepromatous leprosy is a progressive atrophy of iris tissue culminating in a loss of all iris mobility and a miotic pupil. Iris patterns vary according to race and age but *atrophy* is characterized by loss of the normal iris landmarks and degeneration of the stroma exposing the underlying pigment epithelium or even the lens. The pupil margin itself may become ragged. In some races whitish deposits may occur at the pupil margin in patients with chronic iritis and lie on the surface of the lens—these are known as *floccules* and they may occur independently or more commonly in association with iris atrophy.

An *acute iritis* may occur in all types of leprosy, particularly the intermediate forms when a change in polarity is taking place, and it may be provoked by alterations in treatment. The clinical signs are characteristic of all forms of acute iritis with pain, redness and visual loss with the formation of synechiae. Patients with acute iritis require urgent treatment with local mydriatic and steroid drops.

Chronic iritis is difficult to detect without a slit-lamp and its presence must often be inferred. Turbidity of the aqueous in the anterior chamber may be visible and the presence of keratic precipitates (kp) on the posterior surface of the cornea is also indicative of the condition. Eyes with chronic iritis usually show very little evidence of inflammation with minimal or no redness and hardly any discomfort. A diagnosis of chronic iritis, therefore, must take a number of different signs into consideration, these include the pupil size, shape and reactions together with its appearance and the presence of iris pearls and synechiae. Single white lesions may occur on the iris in some races but these *lepromas* are rare.

PEARLS

Iris pearls collecting near the pupil margin or in the periphery of the iris are also pathognomonic for lepromatous leprosy and may occur during the early stages of chronic iritis. Like thickened corneal nerves they are a transient phenomenon as they eventually detach from the iris and become absorbed. When present in large numbers they may form a solid white mass in the lower part of the anterior chamber resembling a hypopyon but without any obvious inflammatory signs.

LENS

Cataract is a common cause of blindness in all parts of the world and occurs in the older age groups. Many leprosy patients live to a good age and it is therefore very difficult to decide whether a patient with cataract has developed the condition because of the disease or due to normal senile changes. The detection of cataract may be difficult particularly if the pupil is small but it is usually possible to observe superficial lens opacities.

Surveys

It is intended that the proforma should be used in a number of surveys carried out in areas of the world chosen for their environmental and climatic differences. Initial studies are already being carried out in Nepal, Thailand, India and Kenya by medical students from St Thomas' Hospital and St George's Hospital, London. Each survey should aim to examine at least 100 leprosy patients, preferably those who have been registered with local health authorities so that information on the duration, status and therapy of the disease can be obtained. The students are encouraged to examine a broad spectrum of the leprosy community, not just those with eye complaints—indeed the identification of leprosy patients not affected ocularly is equally important for the analysis. The selection of this representative sample of patients to be studied is of fundamental

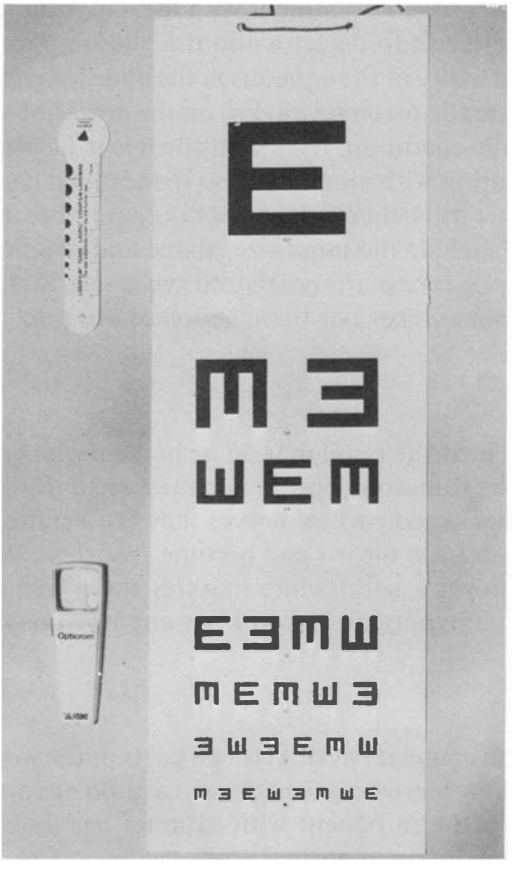

Figure 3. Basic equipment. Folding E test-type (Keeler Instruments Limited). Illuminated loupe (Fisons). Optistick with pin-hole (Allergan).

importance and every attempt should be made to avoid bias towards patients with ocular complications. Studies are therefore preferred in resettlement communities rather than in established leprosaria where the more chronic and disabled cases tend to remain, many of whom have advanced ocular problems. It is of course realized that these criteria for patient selection may not be possible.

The students are provided with basic equipment consisting of a folding E test-type, an illuminated loupe and a pin-hole aperture which also incorporates a scale for measuring pupil size (Figure 3).

The results of the surveys will be entered onto the Iris Fund computer at St Thomas' Hospital, London where an analysis and interpretation can be carried out. From this accumulated evidence it is hoped that information can be derived on the way leprosy affects the eyes in different populations in various parts of the world so that training programmes in eye care for health workers and doctors can be modified to take into account the locality of their work. It is also intended that the information will provide a basis for longitudinal studies on groups of patients in order to gain more data on the natural history of ocular complications.

Acknowledgments

This work was stimulated by visits to the Wilson Leprosy Centre, Suncheon, South Korea and is based on experience gained from the Peace Corps Survey on the ocular complications in leprosy in that country. I am grateful to LEPRA for financial assistance, to the Iris Fund at St Thomas' Hospital for computer facilities and for the help of Dr Andrew Plumb in developing the computer program. Equipment for the student leprosy surveys was donated by Keeler Instruments Limited, Fisons and Allergan. I would like also to thank Mrs Lesley Gibbons for secretarial help.

Reference

[1] Courtright P, Green R, Pilarski R, Smucny J. A survey of the eye complications of leprosy in South Korea. *Lepr Rev*, 1984; **55** (in press).

Lepr Rev (1983) **54,** 283–304

The growth of *Mycobacterium leprae* in nude mice

S CHEHL,* J RUBY,† C K JOB‡ & R C HASTINGS§
** Pharmacology Research Department, National Hansen's Disease Center, Carville, Louisiana 70721 USA; † Louisiana State University Dental School, New Orleans, LA 70119, USA; ‡ Pathology Research Department, National Hansen's Disease Center, Carville, LA 70721, USA; § Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721, USA*

Accepted for publication 25 May 1983

Summary Armadillo-derived *Mycobacterium leprae* were inoculated in graded doses into the left hind foot-pads of 160 nu/nu mice and the same number of BALB/c mice. The course of the infection was followed for 18 months. The nu/nu mice developed massive foot-pad enlargements with eventual dissemination of the bacilli in virtually all organs outside the central nervous system. The acid-fast bacilli were identified as *M. leprae*. BALB/c mice showed a characteristic localized infection. In the nu/nu mice, bacilli were found in large numbers free in the cytoplasm of striated muscle cells and in macrophages, both free in the cytoplasm and within phagosomes. With time, the *M. leprae* in the nu/nu mice showed a tendency to stain more and more non-solidly, suggesting that death of *M. leprae* occurs in these animals despite their lack of the capacity to develop cell-mediated immunity. This model of disseminated leprosy seems useful for a number of studies of the disease.

Athymic, nude mice have been used in leprosy research since 1976. It has been independently demonstrated[1, 2] (also Nakamura & Yogi, private communication) that these animals would support the growth of large numbers of *Mycobacterium leprae*. The present report concerns details of the growth of armadillo-derived *M. leprae* in nude mice at Carville. These findings have been reported in preliminary form in 1980.[3, 4]

0305-7518/83/054283+22 $01.00

Materials and methods

HUSBANDRY

Specific pathogen-free (defined flora) nude mice [(BALB/c An Bom) nu/nu DF], 4–5 weeks' old, were transported by air under sterile conditions from Harlan-Sprague-Dawley, Madison, Wisconsin. Upon arrival these animals were transferred to sterile cages and maintained in a laminar flow animal isolator unit (Model 600, Contamination Control, Inc., Culpsville, PA) situated in a temperature (78–80°F) and humidity (45–55%) controlled room. The laminar flow unit was connected to an emergency generator which was activated within 3 s of an interruption in the electric supply. The animals were provided with sterilized autoclavable rodent food (Service Feed Co., Baton Rouge, LA) and water *ad libitum*. Cages (autoclavable cages, Lab Products, Rochelle Park, NJ), bedding, food and water changes were made twice weekly under aseptic conditions.

Weekly bacteriological monitorings of air flow from the laminar flow isolator, water bottles after use, faeces and urine were made. The cleanliness of the physical facility and laminar flow unit were aggressively maintained.

The counterpart, BALB/c female mice used as controls in this experiment (Tempco Breeding Labs, Houston, TX), were maintained in a conventional environment; food and water were provided as needed; cages, bedding and water bottles were changed twice weekly. Specimens of feed, water, faeces and bedding were monitored monthly for pathogenic bacterial growth on trypticase soy agar.

INOCULUM AND INOCULATION

A suspension of *Mycobacterium leprae* was prepared at 4°C (on an ice bed) using aseptic technique from the spleen tissue of an experimentally infected armadillo as per the methodology of Prabhakaran *et al.*,[5] with slight modifications. The suspension thus obtained (Morphological Index = 10) was tested for sterility in thioglycolate at 37°C for 24 h and was subsequently diluted in Hank's balanced salt solution to contain 1×10^6, 1×10^5, 1×10^4 bacilli per 30 μl. One hundred and sixty nu/nu mice and an equal number of control BALB/c mice were inoculated in the plantar surface of the left hind foot-pad (LHF). Sixty nu/nu and 60 BALB/c mice received 1×10^6 *M. leprae*, 50 from each group received 1×10^5, and 50 received 1×10^4 *M. leprae*. The animals were housed 10 per cage.

HARVESTS

The experimental (nu/nu) and the control (BALB/c) mice were harvested at 5 days (for baseline), and at 90, 180, 272, 365 and 565 days after inoculation. Six to seven mice from the 1×10^6 inoculum groups and 5–6 mice from each of the other groups were sacrificed at each harvest.

HISTOPATHOLOGICAL STUDIES

At each harvest one animal from each of the 1×10^6 inoculum groups of nu/nu and BALB/c mice was autopsied and various organs and tissues were individually weighed and prepared for histopathological studies. The tissues were immediately fixed in 10% buffered formalin and processed for paraffin sections. Five micron sections were cut and stained with haematoxylin and eosin and a modified Fite–Faraco stain for *M. leprae*.

ELECTRON MICROSCOPIC STUDY

A foot-pad of a nude mouse which had been inoculated 217 days before with 1×10^8 *M. leprae* from nude mouse foot-pad passage was cut into approximately 1 mm cubes and fixed in 5% glutaraldehyde solution at 4°C for 3 h. After fixation, the tissue was repeatedly rinsed in several changes of cacodylate buffer containing 0·2 M sucrose for a minimum of 24 h, dehydrated in a graded series of ethanol, immersed in either propylene oxide or dimethylsulphoxide and embedded in pure Spurr's resin. One micron sections were first prepared and stained with 1% toluidine blue for orientation of the tissue. Ultra-thin sections were made from suitable blocks using a Reichert OM-02 ultramicrotome, placed in uncoated grids, stained with uranyl acetate and lead citrate and studied using a Philips EM 300 electron microscope.

BACTERIAL ENUMERATIONS

Foot-pads

In the 5–6 animals from each group remaining after the histopathologic study, a routine harvesting of the inoculated hind foot-pad (LHF) and the contralateral hind foot-pad (RHF) was performed by standard techniques.[6, 7] The technique was slightly modified to process the very large sized foot-pads of the 365- and 565-day harvests. The specimens were individually weighed and stored at −80°C (Kelvinator series 5000, Commercial Products, Inc., Maintowe, WI) until processing.

Liver–spleen

Liver–spleen pools (LS) from each animal were removed, weighed and stored at −80°C until use. They were removed from the freezer, immersed briefly (10–15 s) in liquid nitrogen and homogenized for 2 min in a micro-attachment of a heavy duty Waring blender using normal saline to make a 20% w/v suspension. The LS homogenate was then sonicated for 15–20 s at 50 W (Sonifier Cell Disrupter Model W-185, Heat Systems Ultrasonics, Inc., Plainview, NY). A 1:10 dilution

was made in normal saline and slides were prepared for enumeration by standard acid-fast staining techniques.

Carcass

The carcasses remaining after the removal of the hind foot-pads and liver–spleen, were weighed and stored at −80°C until homogenization. The carcasses were removed from the freezer, immersed in liquid nitrogen for 30–50 s and shattered in a Waring blender. Normal saline was then added to make a 20% w/v suspension and the carcass was homogenized for 3 min. The resulting homogenate was sonicated for 20–25 s at 50 W, and filtered through a single layer of gauze. The filtrate was diluted 1:10 with normal saline and slides were prepared for bacterial enumeration by standard acid-fast staining techniques.

IDENTIFICATION TESTS OF *M. LEPRAE*

Various tests for the identification of *M. leprae* were performed to determine the authenticity of the acid-fast bacilli obtained from the nu/nu, inoculated foot-pads (LHF) at the 365- and 565-day harvests.

Growth pattern in BALB/c mice

An aseptically prepared *M. leprae* suspension from the inoculated nu/nu foot-pad tissue of the 365-day harvest, was diluted in Hank's balanced salt solution to contain 5×10^3 bacilli per 30 μl. Ninety BALB/c mice were inoculated in the plantar surface of the left hind foot with 5×10^3 *M. leprae*. The inoculated foot-pads were individually harvested and enumerated at 90, 120, 150, 180, 272 and 365 days after inoculation. Six animals were sacrificed at each interval.

Dopa oxidase and pyridine extraction

The AFB suspensions from the infected nu/nu footpads at 364 and 565 days after inoculation were tested for DOPA oxidase activity[8] and the pyridine extraction test.[9]

In vitro *cultivability*

The AFB suspensions from the 180-, 272-, 363- and 565-day harvests were inoculated into Lowenstein Jensen slants, Middlebrook 7H9 liquid medium and Dubos liquid medium, incubated at room temperature (23–25°C), 33°C and 37°C, and observed for 6–8 weeks.

Lymphocyte blast transformation (LBT)

Dharmendra antigen was prepared from the acid-fast bacilli (AFB) derived from the experimentally infected nu/nu foot-pad tissue and armadillo lymph nodes using the methodology of Dharmendra with slight modifications.[10] The comparative LBT activity of these antigens was studied in 6-day mononuclear cell cultures prepared from peripheral blood of lepromin-positive normal human subjects and from lepromin-negative leprosy patients. The methods have been previously described[11] with minor modifications. Dharmendra antigen was used to the concentration of 5 μg/well and the medium was supplemented with 10% v/v autologous plasma instead of 20% v/v AB serum.

Results

SURVIVAL

Based on spontaneous deaths, the survival of nude mice maintained under sterile, laminar flow housing conditions was satisfactory and approached that of the conventionally housed, immunologically competent BALB/c mice (Figure 1).

WEIGHTS

The inoculated foot-pads of the nude mice became erythematous and began to

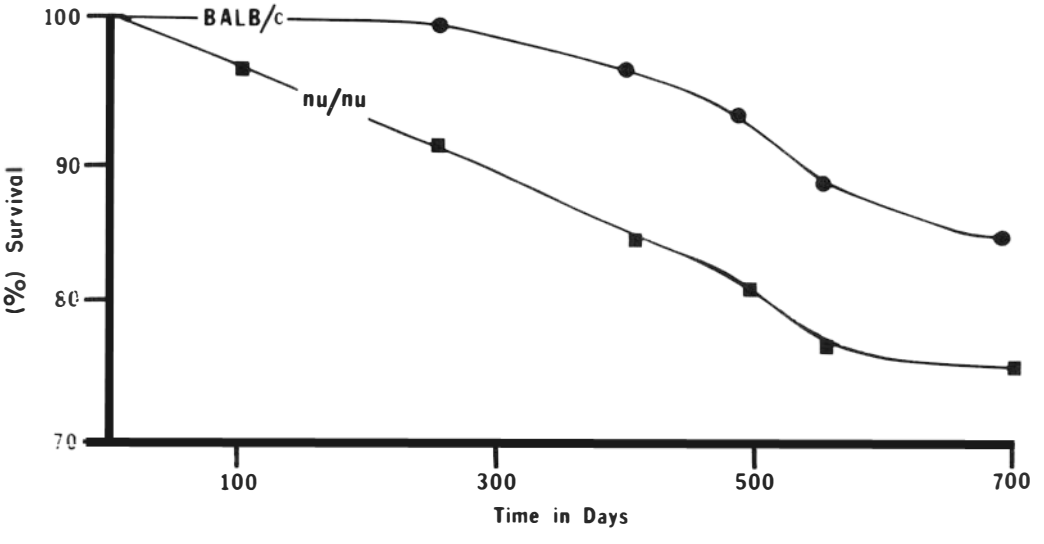

Figure 1. The survival of nu/nu mice housed under sterile laminar flow conditions compared to the survival of conventionally housed BALB/c mice.

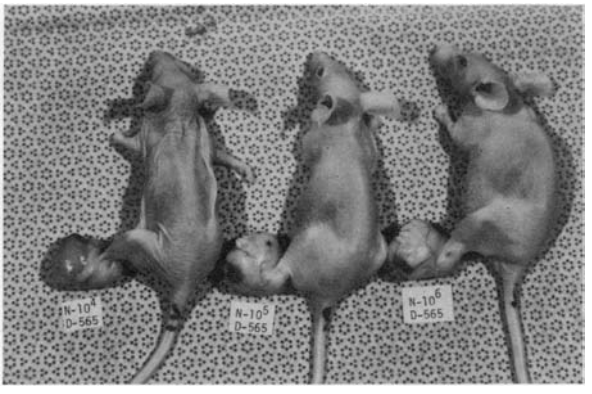

Figure 2. Enlargement of inoculated foot-pads of nu/nu mice 565 days after inoculation with *Mycobacterium leprae*.

enlarge by 272 days after inoculation. There was a progressive enlargement of the inoculated foot-pads and they reached massive proportions by the 565-day harvest (Figure 2). The control BALB/c mice showed no foot-pad enlargement. The weights of the inoculated foot-pads at different time intervals are shown in Figure 3. The weights of the liver–spleen pools are given in Figure 4. There were no noteworthy changes in the body weights of either the nude mice or the BALB/c mice.

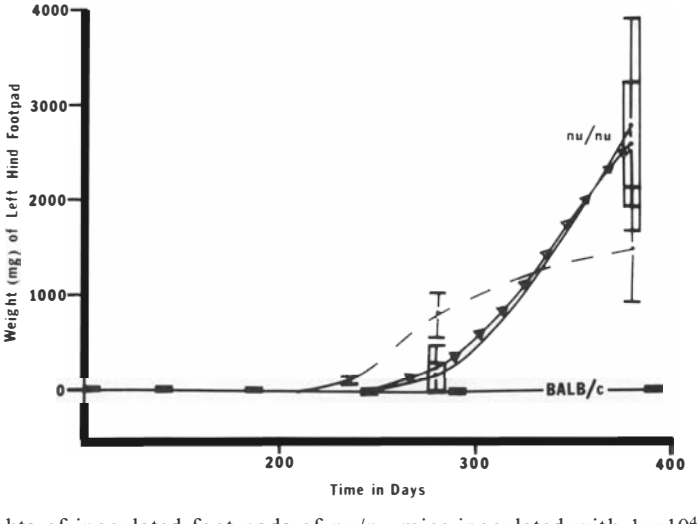

Figure 3. Weights of inoculated foot-pads of nu/nu mice inoculated with 1×10^4 (- - -), 1×10^5 (——), 1×10^6 (▶—▶), and BALB/c mice inoculated with 1×10^4 (■–■), 1×10^5 (■–■) and 1×10^6 (■–■) *Mycobacterium leprae*. Values are depicted as means $\pm$SD, N = 6.

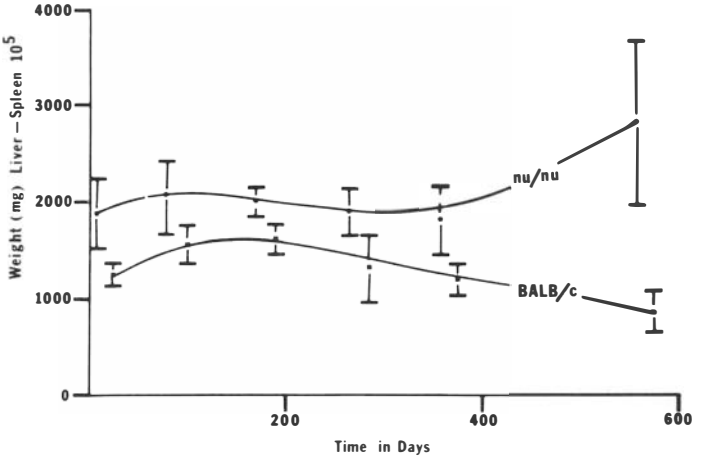

Figure 4. Weights of livers and spleens (combined) in nu/nu and BALB/c mice inoculated with 1×10^5 *Mycobacterium leprae*. Values are depicted as means $\pm$SD, N = 6.

BACTERIAL ENUMERATIONS

The results of bacterial enumerations from the inoculated left hind foot-pads (LHF), showing numbers of bacilli per foot-pad at various intervals, are given in Figure 5. The average level of AFB in the inoculated foot-pads in nude mice reached over 5×10^{10} bacilli per foot-pad, while the BALB/c mice exhibited their characteristic limited growth pattern and plateaus. The dissemination to the contralateral, uninoculated foot-pad (RHF) was seen at a rather surprisingly late stage of the infection in the nude mice (Figure 6).

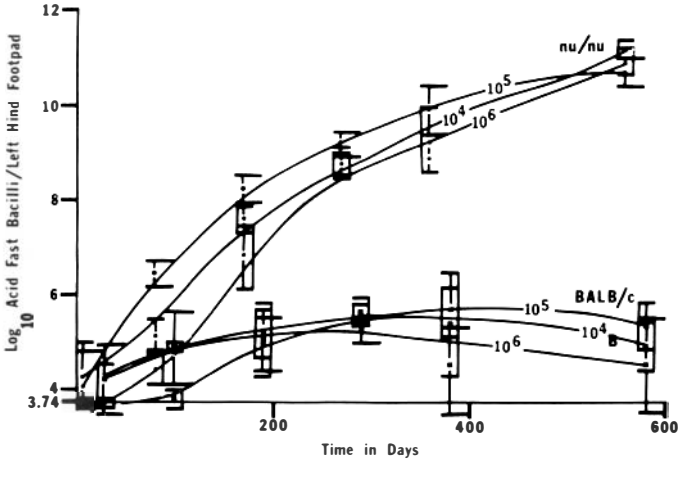

Figure 5. Acid-fast bacilli harvested from the inoculated foot-pads of nu/nu and BALB/c mice after inoculation with 1×10^4, 1×10^5 and 1×10^6 *Mycobacterium leprae*. Values are depicted as means $\pm$SD, N = 6.

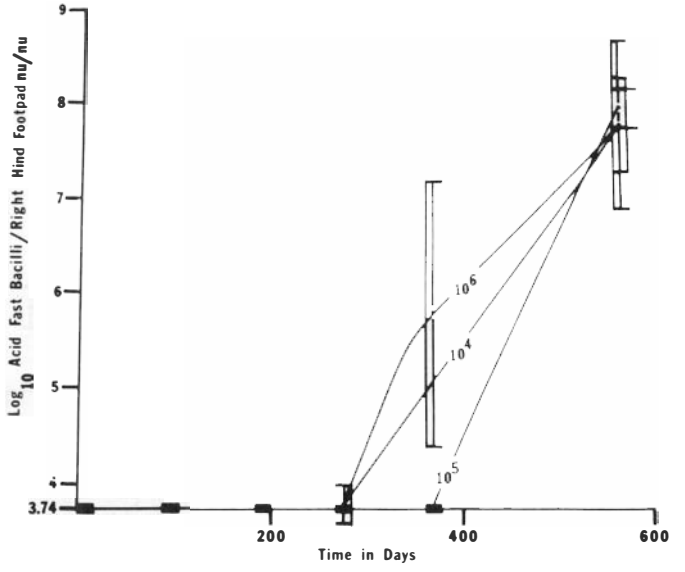

Figure 6. Acid-fast bacilli harvested from the contralateral, uninoculated hind foot-pads of nu/nu mice after inoculation with *Mycobacterium leprae*. No acid-fast bacilli were detected in the contralateral, uninoculated hind foot-pads of BALB/c mice. Values are depicted as means $\pm$SD, N = 6.

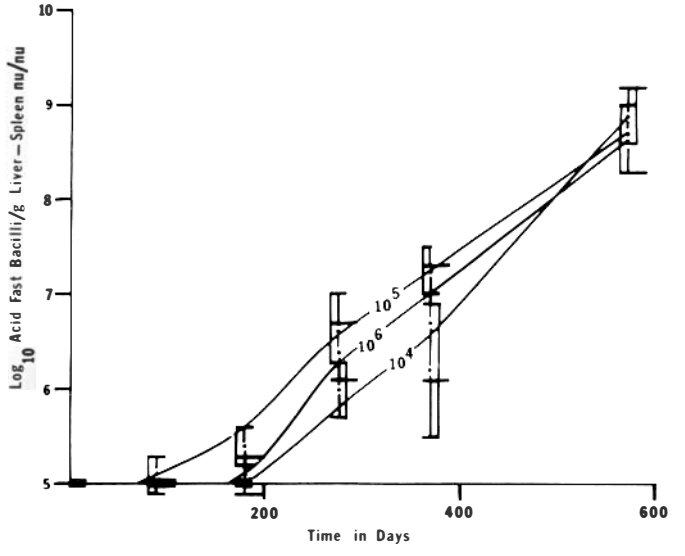

Figure 7. The concentration of acid-fast bacilli per gram of tissue in the liver–spleen pools of nu/nu mice inoculated with *Mycobacterium leprae*. Values are depicted as means $\pm$SD, N = 6.

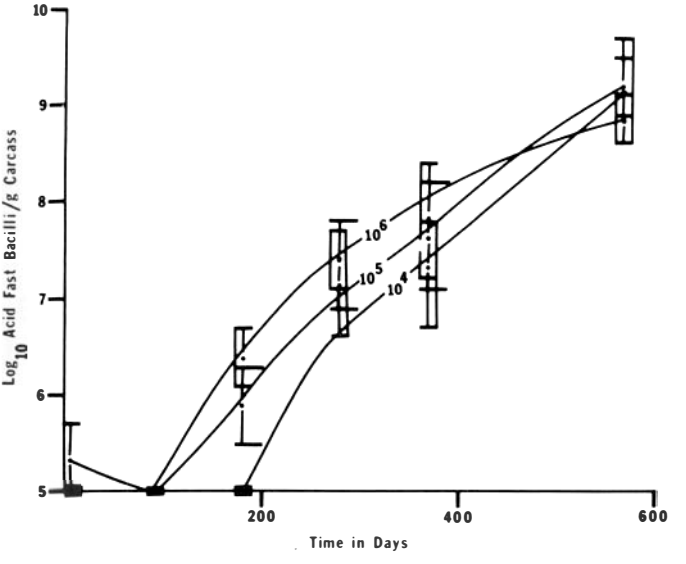

Figure 8. The concentration of acid-fast bacilli per gram of tissue in the carcasses of nu/nu mice inoculated with *Mycobacterium leprae*. Values are depicted as means $\pm$SD, N = 6.

Expressed as concentrations of AFB per gram of tissue in the LHF, the ultimate mycobacteriosis of approximately $1–5 \times 10^{10}$ AFB/g is reached in the nude mice by 272 days after inoculation in all 3 groups. Thereafter, the increase in numbers of AFB is accommodated by enlargement of the foot-pads. The contrasting, characteristic plateaus were reached at approximately 3–4 logs lower concentrations in the BALB/c foot-pads.

Figure 7 shows the AFB per gram of tissue in liver–spleen pools at given stages of infection. A dissemination to the liver–spleen in nude mice occurred by 180 days after inoculation and the AFB concentration approached 1×10^9 bacilli per gram by the 565-day harvest. Figure 8 presents similar dissemination and concentrations of bacilli in nude carcasses remaining after removal of hind foot-pads and liver–spleens. A trend towards a continued increase in AFB per gram is seen in both the liver–spleen pools and carcasses of all groups of nude mice at the time of the termination of the experiment. The final concentration was slightly higher in the carcasses than that in liver–spleen tissues. No dissemination was observed in the BALB/c mice.

MORPHOLOGICAL INDEXES

Figure 9 shows Morphological Indexes which include LHF, liver–spleen pools, and carcasses of nude mice. Interestingly the Morphological Index falls with time in both the inoculated and the contralateral uninoculated foot-pads and in the carcasses of the nude mice. The Morphological Index is highest in AFB from the liver–spleen pool at the time of the final harvest.

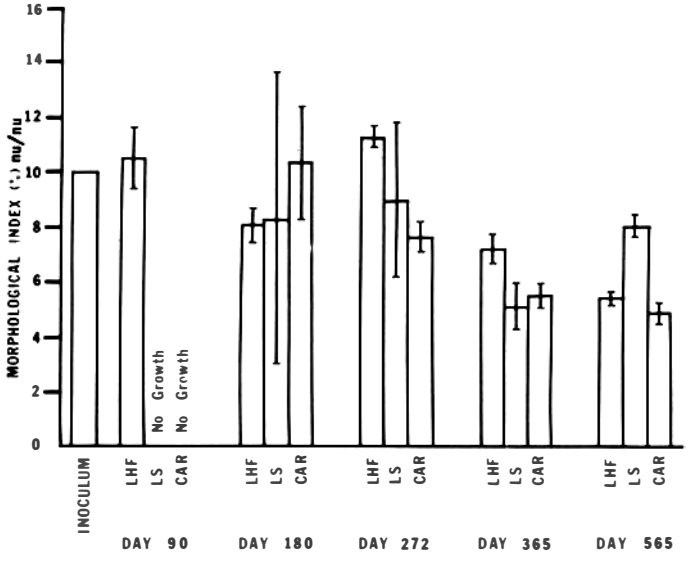

Figure 9. Percentage of solidly staining acid-fast bacilli (Morphological Index) in the various tissues of nu/nu mice after inoculation with *Mycobacterium leprae*. Values are depicted as means ±SEM, N = 6.

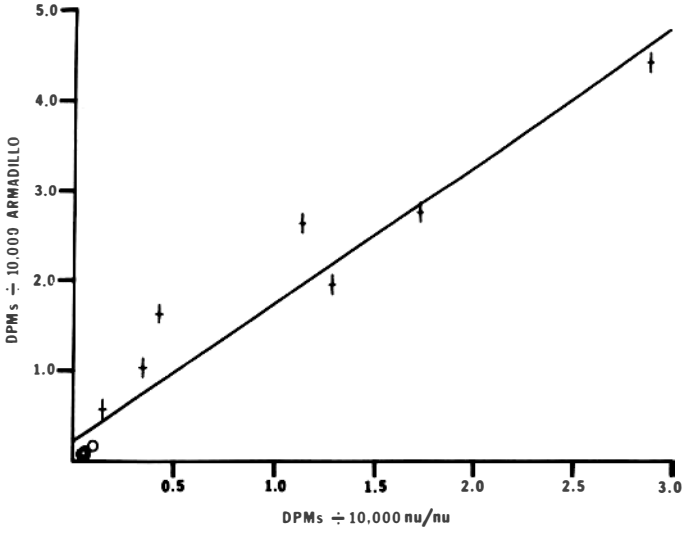

Figure 10. Disintegrations per minute (DPM) ^{3}H-thymidine incorporation in 6-day human lymphocyte cultures in response to Dharmendra antigens prepared from armadillo-derived *Mycobacterium leprae* and acid-fast bacilli harvested from nu/nu mice. 0 = lepromin-negative subjects with lepromatous leprosy. + = lepromin-positive, normal subjects.

IDENTIFICATION TESTS OF *MYCOBACTERIUM LEPRAE*

The acid-fast bacilli recovered from the nude mice behaved like *M. leprae* in lymphocyte blast transformation studies (Figure 10), in their ability to oxidize DOPA, their loss of acid-fastness after pyridine extraction, their inability to grow in 3 mycobacterial culture media at room temperature (23–25°C), 33°C and 37°C, and in their characteristic growth pattern in BALB/c mice.

HISTOPATHOLOGY

In the nude mice, at day 5, an inflammatory reaction was seen in the left hind foot-pad at the site of the inoculation. Small collections of macrophages were seen in the interstitial tissue around blood vessels and around striated muscle cells. Acid-fast bacilli were seen inside macrophages, striated muscle cells and a few mast cells. No lesions were seen in other organs.

At day 90, there was an increase in the number of macrophages in the left hind foot-pad. Acid-fast bacilli were present inside macrophages and striated muscle cells. All the other organs were normal.

At day 180, the left hind foot-pad showed a macrophage granuloma situated beneath the epidermis and incorporating several striated muscle bundles and cutaneous nerves. Acid-fast stain showed bacilli inside macrophages, striated muscle cells, perineurial cells, Schwann cells and fibroblasts. Two Kupffer cells in the liver also showed small clumps of bacilli. The other organs did not show any lesions.

At day 272, the granuloma within the left hind foot-pad had increased in size. The muscle cells incorporated in the granuloma showed loss of striation and vacuolation of their cytoplasm. Acid-fast stain showed large clumps of bacilli inside macrophages, striated muscle cells and nerves. The Kupffer cells in the liver and the reticuloendothelial cells in the spleen, bone marrow (in the tail bone) and the lungs showed collections of bacilli.

At day 365, the granuloma in the left hind foot-pad had become even larger due to an increase in the number of macrophages replacing normal tissues. Both ears were infiltrated by small aggregates of macrophages in the sub-epithelial tissue. The bone marrow of the tail bone showed a macrophage granuloma. Acid-fast stain revealed bacilli inside macrophages, nerves and the reticuloendothelial cells of the liver, spleen and lungs. In addition, the other 3 foot-pads, nose and sub-mucosa of the jejunum showed a few macrophages containing acid-fast bacilli.

At day 565, there were disseminated lepromatous lesions involving almost all organs. The left hind foot-pad had a massive macrophage granuloma replacing almost all the normal tissues (Figure 11). Acid-fast bacilli were present in macrophages, muscle cells (Figure 12) and nerves (Figure 13). The other 3 foot-pads were also infiltrated by macrophages containing acid-fast bacilli.

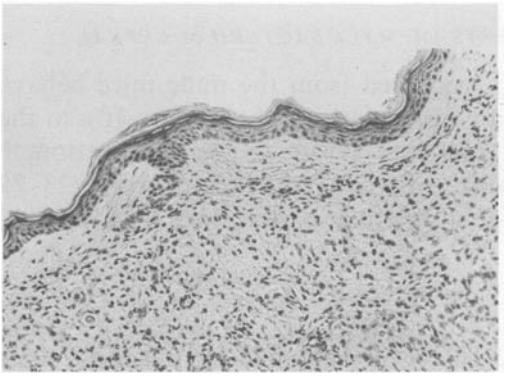

Figure 11. Granuloma in the inoculated hind foot-pad of a nude mouse 565 days after inoculation with *Mycobacterium leprae*. An atrophic epidermis and a clear area separating the granuloma from the epidermis are clearly seen (H&E × 300).

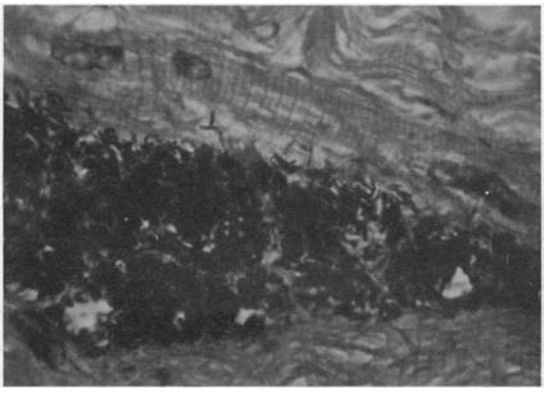

Figure 12. The inoculated hind foot-pad of a nude mouse 565 days after inoculation with *Mycobacterium leprae*. Striated muscle cells contain a large collection of acid-fast bacilli (modified Fite–Faraco stain, counterstained with Harris' haematoxylin × 1100).

Both ears had macrophage granulomas on either side of the cartilage; the outer side seemed to have a larger granuloma than the inner side. Acid-fast stain showed bacilli inside macrophages, perichondrial cells and some cartilage cells.

Skin from the dorsal region, tail and nose had large sub-epithelial granulomas made up of macrophages containing acid-fast bacilli. Besides macrophages, the cells of the hair follicles and the striated muscles in the nose were filled with bacilli.

The organs of the reticuloendothelial system such as the liver, spleen, lymph nodes and bone marrow showed extensive infiltration by macrophages containing acid-fast bacilli (Figure 14). Some liver parenchymal cells also had acid-fast bacilli in their cytoplasm.

The kidneys, adrenals and lungs had collections of macrophages containing

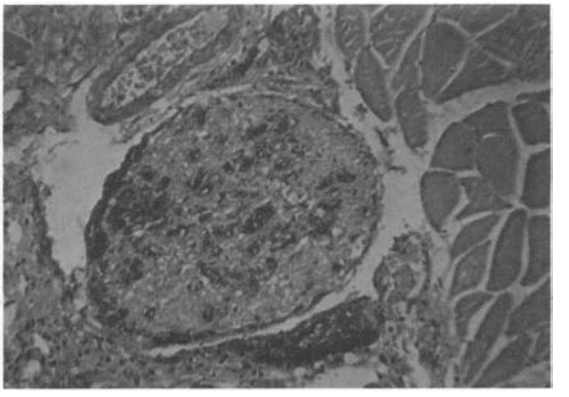

Figure 13. The inoculated hind foot-pad of a nude mouse 565 days after inoculation with *Mycobacterium leprae*. A nerve bundle with clumps of acid-fast bacilli inside Schwann cells, perineurial cells and macrophages surrounding the nerve (modified Fite–Faraco stain, counterstained with Harris' haematoxylin × 300).

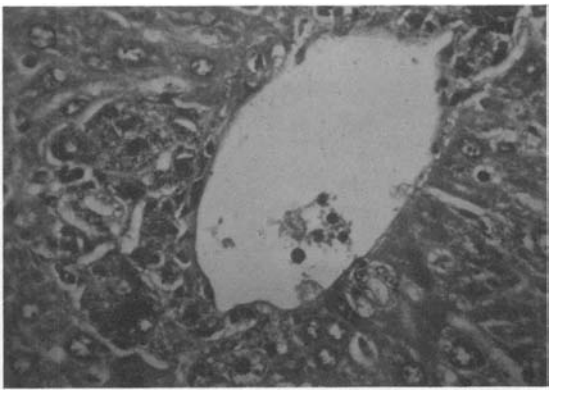

Figure 14. The liver of a nude mouse 565 days after inoculation of the hind foot-pad with *Mycobacterium leprae*. The portal vein is surrounded by a microgranuloma consisting of macrophages containing acid-fast bacilli (modified Fite–Faraco stain, counterstained with Harris' haematoxylin × 900).

acid-fast bacilli in the interstitial tissue (Figures 15 and 16). Some lining cells of the distal convoluted tubules of the kidney, the endothelial cells of the glomeruli and alveolar macrophages contained acid-fast bacilli.

The stomach, duodenum, jejunum (Figure 17), and the ileum showed microgranulomas in the mucosa and sub-mucosa. The colon had only sub-mucosal lesions. Many of the macrophages in the granulomas showed acid-fast bacilli. The heart showed acid-fast bacilli inside cardiac muscle cells (Figure 18). The ovarian tissue was extensively replaced by bacilli-filled macrophages. Graafian follicles were also surrounded by bacilli. The brain showed no lesions at any of the time intervals.

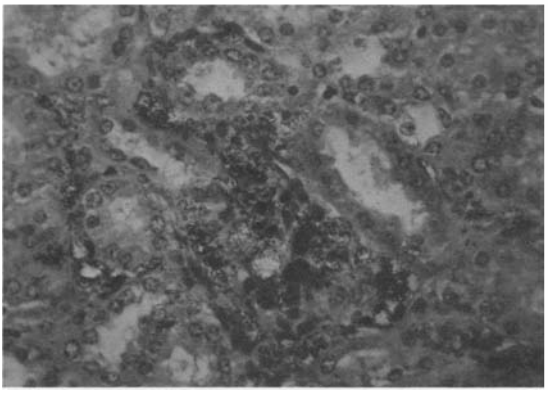

Figure 15. The kidney of a nude mouse 565 days after inoculation in the hind foot-pad with *Mycobacterium leprae*. The interstitial tissue is invaded by macrophages and acid-fast bacilli are present inside the macrophages and in tubular cells (modified Fite–Faraco stain, counterstained with Harris' haematoxylin ×600).

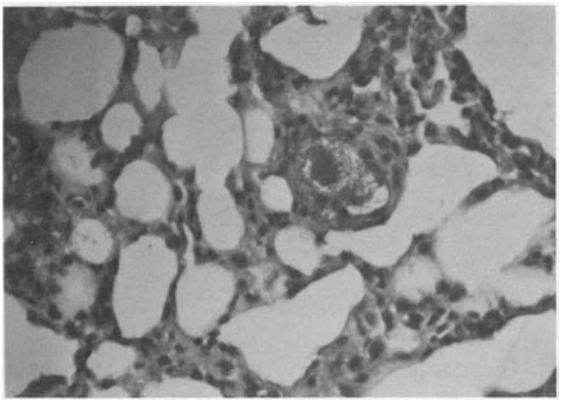

Figure 16. The lung of a nude mouse 565 days after inoculation in the hind foot-pad with *Mycobacterium leprae*. There is a macrophage granuloma containing acid-fast bacilli in the interstitial tissue (modified Fite–Faraco stain, counterstained with Harris' haematoxylin ×600).

ELECTRON MICROSCOPIC STUDY

The majority of the cells of the nude mice which contained bacilli were identified as macrophages. These were large cells with abundant cytoplasm containing numerous phagosomal vacuoles, a moderate number of mitochondria and numerous finger-like villi at the periphery of their cytoplasm. Most of the

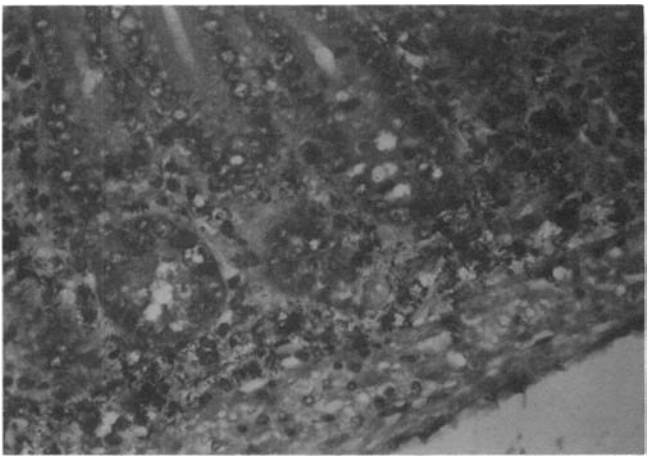

Figure 17. The jejunum of a nude mouse 565 days after inoculation of the hind foot-pad with *Mycobacterium leprae*. The sub-mucosa is infiltrated by macrophages containing acid-fast bacilli (modified Fite–Faraco stain, counterstained with Harris' haematoxylin × 600).

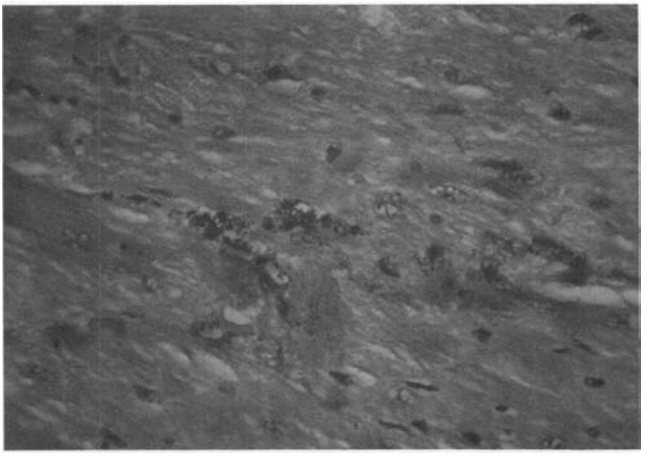

Figure 18. The heart of a nude mouse 565 days after inoculation in the hind foot-pad with *Mycobacterium leprae*. The cardiac muscle shows intracellular acid-fast organisms (modified Fite–Faraco stain, counterstained with Harris' haematoxylin × 600).

phagosomes contained *M. leprae* (Figure 19). Some of the bacilli were seen free in the cytoplasm without a surrounding phagosomal membrane (Figure 20). All of the organisms had a halo of an electron transparent zone (ETZ).

The cutaneous nerve bundles showed bacillary invasion of perineurial cells (Figure 21) and Schwann cells. The cytoplasmic response of these cells to *M. leprae* was the same as that of the macrophages.

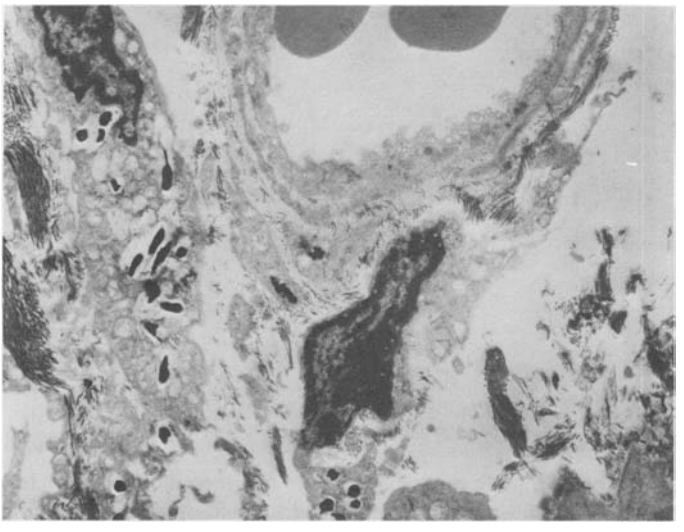

Figure 19. Electron micrograph of the foot-pad of a nude mouse 217 days after inoculation with 1×10^8 *Mycobacterium leprae*. There is a perivascular collection of macrophages containing numerous *M. leprae* within phagosomal vacuoles. Note the electron transparent zone (ETZ) around most of the bacilli. The macrophages have no basement membrane and the periphery of their cytoplasm shows numerous finger-like villi (original magnification $\times 9000$).

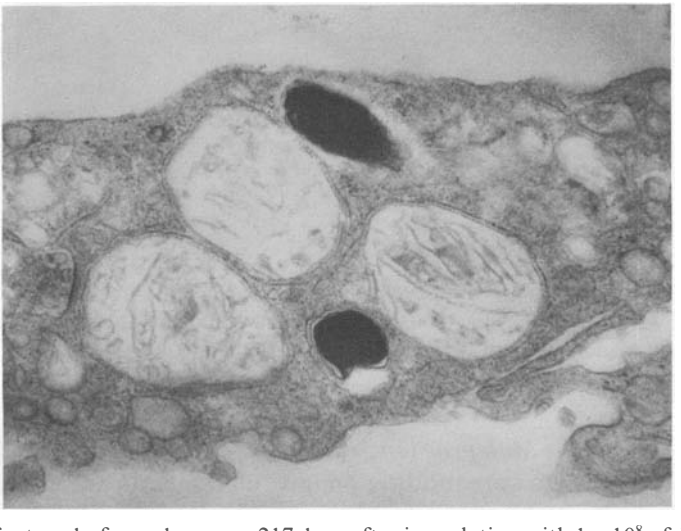

Figure 20. The foot-pad of a nude mouse 217 days after inoculation with 1×10^8 of *Mycobacterium leprae*. A macrophage with its cytoplasm showing several swollen and degenerating Mitachondria and 2 *M. leprae*. One organism (below) is surrounded by phagosomal membrane, and the other (above) is not surrounded by a phagosomal membrane (original magnification $\times 50{,}000$).

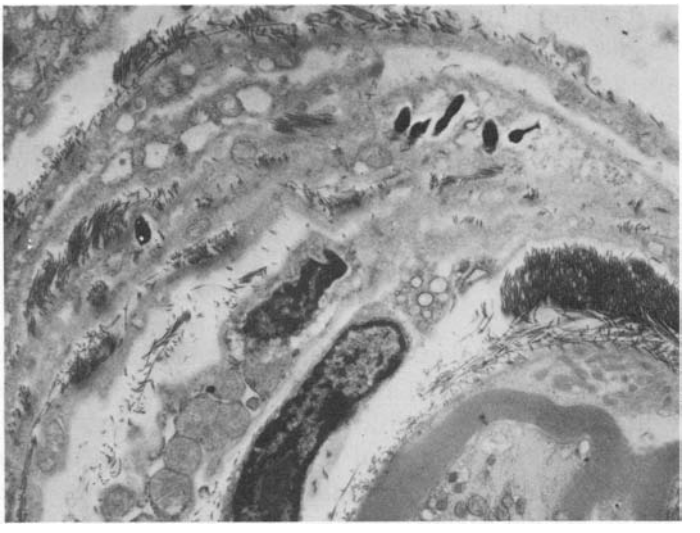

Figure 21. The foot-pad of a nude mouse 217 days after inoculation with 1×10^8 of *Mycobacterium leprae*. A small cutaneous nerve bundle with its perineurial cells containing several *M. leprae* (original magnification × 11,000).

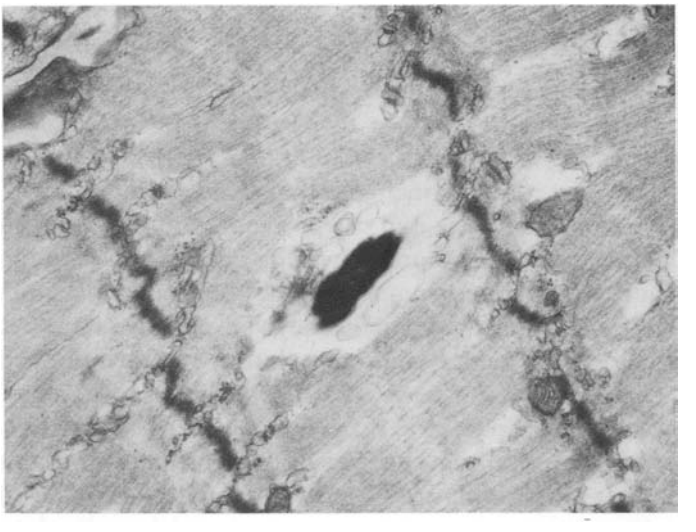

Figure 22. The foot-pad of a nude mouse inoculated 217 days earlier with 1×10^8 *Mycobacterium leprae*. A striated muscle cell contains an organism in its cytoplasm without a surrounding phagosomal membrane. A few small membrane-bound vesicles are seen in the electron transparent zone (ETZ) (original magnification × 41,600).

The most interesting feature of this study was the presence of a large number of *M. leprae* inside many of the striated muscle cells. This confirmed the light microscopic appearance (Figure 22). The organisms were found free within the cytoplasm without any phagosomal membrane. The ETZ was clearly seen around the bacilli and there were also aggregates of membrane-bound vesicles producing a foamy change in the cell cytoplasm.

Discussion

Nude mice were first mentioned in 1962,[12] first described in 1966[13] and were shown to be athymic in 1968.[14] The trait is inherited as an autosomal recessive. The animals have abnormal keratinization of poorly developed hair follicles, a sulphhydryl group deficiency,[13, 15] and under conventional housing conditions have a short lifespan. Under conventional conditions more than half die before weaning and the maximum survival is approximately 25 weeks of age.[13, 16] On the other hand, although susceptible to a variety of infections, nude mice can be kept in good health for the normal lifespan of a mouse if kept under strictly pathogen-free conditions. Such animals can survive for 2 years or longer[15, 17] and in the first year of life can have a mortality of 1–3%.[17]

Nude mice are highly susceptible to many bacterial, fungal and viral infections. Surprisingly, however, particularly when housed under conventional or specific pathogen-free (but not germ-free) conditions, they showed an enhanced resistance to some microbes, including *Candida albicans*, *Brucella abortus*, *Pseudomonas aeruginosa*,[18] *Listeria monocytogenes*,[18, 19] *Aspergillus fumigatus*[20] and *Staphylococcus aureus*.[19] Rao *et al.*[21] showed that peritoneal macrophages from conventionally housed nude mice would not support the growth of vaccinia virus in contrast to peritoneal macrophages of nude mice housed under germ-free conditions and of nude mice that had been reconstituted with thymic transplants. They concluded that there are stimuli in a conventional environment which can activate nude mouse macrophages and this activation is not totally dependent on functioning T lymphocytes. It has been speculated[19] that fixed-tissue macrophages of conventionally housed nude mice are activated as a direct consequence of their thymic deficiency. This macrophage activation could be due to reticuloendothelial system stimulation by normal gut flora in these animals and could be on the basis of (a) a lack of suppressor T cells which normally modulate macrophage activation, (b) macrophage activation by direct acting agents such as lipopolysaccharides of Gram-negative organisms, bacterial phospholipids, or agents similar to *Corynebacterium parvum*; or (c) macrophage activation by lymphokine-like substances produced by stimulated B lymphocytes.

In this regard, it should be noted that the strain of nude mice used in the present study, [(BALB/c An Bom) nu/nu DF] have been described as having a

caecal flora of *Lactobacillus*, α and γ streptococci, *Escherichia coli*, *Proteus mirabilis*, and *Micrococcus*. It should also be noted that non-T lymphocyte-mediated resistance to infectious agents can be markedly influenced by the genetic background of the nude mouse.[22] The present strain of nude mice are from a BALB/c background, a strain already known to be relatively susceptible to *Mycobacterium leprae*.

Nude mice have been utilized in studies of non-leprosy mycobacterial diseases. Nude mice were injected intravenously with the Ravenel strain of *M. bovis*, the BCG strain of *M. bovis* and the Flamingo strain of *M. avium*. In 12–17-day experiments the nude mice had higher numbers of viable organisms in each instance compared to heterozygote controls.[23] In a long-term experiment, $2{\cdot}2 \times 10^6$ viable BCG were injected intravenously into nude mice and the course of the infection was followed for 175 days. The levels of BCG did not decline in any organ in the nude mice but none died of the BCG infection up to day 175. Up to approximately 10^7 viable BCG were found in the lungs of nude mice at the end of 175 days compared to approximately 10^3 viable organisms in the heterozygote controls.[23]

The animal model of disseminated *M. leprae* in mice, other than nude mice, is the thymectomized, irradiated animal.[24] Nineteen months after intravenous injection of 3×10^7 *M. leprae* into these animals, disseminated infections were evident with bacillary yields of 2×10^9 in the nose and ears, 7×10^8 in the foot-pad, 10^8 in the liver, 5×10^7 in the spleen and 10^7 in the lungs, with a total body yield of $1{\cdot}8 \times 10^{10}$ bacilli. Overall, 95% of the bacilli were found in the foot-pads, ears and nose. Foot-pad yields approaching 10^9 organisms could be obtained 10–16 months after foot-pad inoculation of either 10^4 or 10^6 *M. leprae*.[25] In this model, *M. leprae* appeared to preferentially colonize skeletal muscle fibres and later to involve perineural cells and macrophages. Eventually the histopathology of skin lesions was that of advanced human lepromatous leprosy.[26]

It has been demonstrated that these animals support the growth of *M. leprae* and that bacillary yields were considerably higher than those obtained from conventional mice. Colston & Hilson[2] inoculated both hind foot-pads of 30 nude mice with 5×10^5 *M. leprae* from an untreated lepromatous leprosy patient. Two nude mice survived beyond 160 days, one expiring at day 266 and one at day 322. There were approximately 10^8 bacilli per foot-pad at day 266 and approximately 10^9 at day 322. The last surviving animal contained approximately $4{\cdot}0 \times 10^5$ bacilli in its liver, $2{\cdot}0 \times 10^5$ in the spleen, $8{\cdot}0 \times 10^4$ in the nose, $2{\cdot}0 \times 10^4$ in the tail, and 10^4 in each of the forepaws and testes. The acid-fast bacilli were identified as *M. leprae* by the pyridine extraction test and their growth pattern on passage into immunologically intact mice.[2]

Nakamura & Yogi (private communication) reported the growth of *M. leprae* in nude mice. After foot-pad inoculation of $8{\cdot}5 \times 10^3$ bacilli, yields of up to $2{\cdot}5 \times 10^9$ were obtained 17 months later. Similar yields were obtained after inocula of $1{\cdot}5 \times 10^6$ bacilli per foot-pad. In conventionally reared nude mice from

various background strains, inoculated with $4{\cdot}0 \times 10^6$ bacilli, yields of $7{\cdot}7 \times 10^7$ to $1{\cdot}7 \times 10^9$ bacilli were obtained after 12 months. The bacilli were identified as *M. leprae* by lack of foot-pad swelling 6 months after inoculation into normal or nude mice, by pyridine treatment, and by lack of growth on a modified egg yolk medium.

Kohsaka *et al.*[1] initially inoculated 8 nude mice into the hind foot-pad with 10^4 *M. leprae* and reared and maintained the animals in vinyl plastic isolators under specific pathogen-free (SPF) conditions. At 13 months, foot-pad yields of $2{\cdot}6 \times 10^6$ bacilli were found and by 17 months all 3 surviving mice had swollen foot-pads and 2 of the 3 had ulcerations at the base of the tail and swelling of the eyelids. The bacilli were identified as *M. leprae* by lack of *in vitro* growth on Ogawa's egg medium, growth curves in immunologically intact mice, the pyridine extraction test, oxidation of D-dopa and lepromin skin testing. Subsequent work[27, 28] has shown that foot-pad inocula of $1{\cdot}0$–$2{\cdot}2 \times 10^6$ *M. leprae* from nude mice passaged into new nude mice provide yields of $3{\cdot}6 \times 10^8$ bacilli per foot-pad after 10 months and that in one experiment $3{\cdot}6 \times 10^6$ bacilli from human biopsy material yielded $1{\cdot}1 \times 10^{10}$ bacilli in 8 months.[27] Ten months after foot-pad inoculation of $1{\cdot}0$–$2{\cdot}2 \times 10^6$ bacilli from nude mice passage, dissemination was noted with $3{\cdot}6 \times 10^8$ bacilli in the inoculated foot-pad, approximately $4{\cdot}0 \times 10^6$ in the liver, $4{\cdot}0 \times 10^5$ in the earlobes, $2{\cdot}0 \times 10^5$ in the nose, $2{\cdot}0 \times 10^5$ in the lung, $8{\cdot}0 \times 10^4$ in the tail, $6{\cdot}0 \times 10^4$ in the spleen and $5{\cdot}0 \times 10^4$ in the forepaws. In general, bacillary yields were correlated with lower local temperatures in these sites.[27] Bacillary yields in the liver were approximately $2{\cdot}0 \times 10^6$/g and maximum foot-pad swelling was 2–5 mm thickness.[28]

The present results show reasonable survival of *M. leprae*-infected nude mice under sterile, laminar flow conditions. Experiments of 12–18 months' duration are not unreasonable. *M. leprae* multiply to 'saturation' levels in inoculated foot-pads of nude mice and thereafter further bacterial multiplication is accompanied by enlargement of the tissue (leproma formation). Dissemination of bacteria from the inoculated foot-pad is relatively slow despite documented bacillemia occurring relatively early. There is a progressive reduction in the Morphological Index in nude mice with time suggesting that non-immune resistance mechanisms play some role in limiting the proliferation of *M. leprae* in this model. Other than the central nervous system, bacteria were eventually found in virtually all tissues examined. Bacilli were found in macrophages both within phagosomes and free in the cytoplasm. All bacilli showed a halo of an electron transparent zone. There were large numbers of *M. leprae* inside many striated muscle cells, the organisms being found free within the cytoplasm without any phagosomal membrane.

Bacilli from sterile (germ-free rather than SPF) nude mice might be particularly useful in cultivation experiments since one would expect them to be free of contamination by other bacteria due to their life-long sterile environment. This model, similar to other models of multi-bacillary leprosy, may prove useful

in the detection of 'persisters', in the deliberate induction of drug-resistant mutants of *M. leprae*, in chemotherapeutic and immunotherapeutic experiments, and in the production of *M. leprae* in areas without armadillo facilities.

In conjunction with slow-freezing of infected tissues and subsequent storage of the infected tissue at −80°C, interesting strains of *M. leprae* can be grown in nude mice and preserved in a viable state for long periods of time. The model lends itself to adoptive transfer experiments using mononuclear cells from nu/nu–BALB/c heterozygote donors since this combination excludes graft *vs.* host reactions. Because of the immunoincompetence of the homozygous nu/nu animal recipients, host *vs.* graft reactions would also not be expected. Finally, because of the uniform susceptibility of these animals to *M. leprae*, the model has promise as a means of studying the most likely routes of transmission of *M. leprae*.

Acknowledgments

The authors wish to express their deepest appreciation to Geraldine Black, M J Morales, V Richard and Dr E J Shannon, for their technical assistance and Ms Renée Painter for preparation of the transcript. This study was supported in part by Grant CAR-07-78 from the Division of Hospitals and Clinics, Bureau of Medical Services, United States Public Health Service.

References

[1] Kohsaka K, Mori T, Ito T. Lepromatoid lesions developed in nude mouse inoculated with *Mycobacterium leprae*. *La Lepro*, 1976; **45:** 177–87.

[2] Colston MJ, Hilson GR. Growth of *Mycobacterium leprae* and *M. marinum* in congenitally athymic (nude) mice. *Nature*, 1976; **262:** 736–41.

[3] Hastings RC, Chehl SK, Morales MJ, Shannon EJ, Kirchheimer WF. Multiplication of acid-fast bacilli in nude mice inoculated with armadillo derived *M. leprae*. *Int J Lepr*, **48** (4): 490–1.

[4] Hastings RC, Chehl SK, Black G, Morales MJ, Shannon EJ, Kirchheimer WF. Growth of *M. leprae* in nude (athymic) mice. In *Immunological Aspects of Leprosy, Tuberculosis and Leishmaniasis*. Humber David P (ed), Amsterdam–Oxford–Princeton: Excerpta Medica, 1981, pp. 166–76.

[5] Prabhakaran K, Harris EB, Kirchheimer WF. Binding of ^{14}C-labelled dopa by *Mycobacterium leprae in vitro*. *Int J Lepr*, 1976; **44:** 58–64.

[6] Shepard CC. The experimental disease that follows the injection of human leprosy bacilli into footpads of mice. *J. exp Med*, 1960; **112:** 445–54.

[7] Shepard CC, McRae DH. A method for counting acid-fast bacteria. *Int J Lepr*, 1968; **36:** 78–82.

[8] Prabhakaran K, Harris EB, Kirchheimer WF. Confirmation of the spot test for the identification of *Mycobacterium leprae* and occurrence of tissue inhibitors of DOPA oxidation. *Lepr Rev*, 1977; **48:** 49–50.

[9] McCormick GT, Sanchez RM. Pyridine extractability of acid-fastness from *Mycobacterium leprae*. *Int J Lepr*, 1979; **47:** 495–9.

[10] Cochrane RG, Davey TF. Preparation of lepromin. In *Leprosy in Theory and Practice*. Cochrane RG, Davey TF (eds), Baltimore: The Williams and Wilkins Company, 1964, p. 188.
[11] Shannon EJ, Powell MD, Jacobson RR, Hastings RC. Investigations of suppressor mechanisms in clinical leprosy. *Dermatol Rev Mex*, 1981; **25:** 457–68.
[12] Isaacson JH, Lattanack BM. *Mouse News Lett*, 1962; **27:** 31.
[13] Flanagan SP. 'Nude', a new hairless gene with pleiotrophic effects in the mouse. *Genet Res (Camb)*, 1966; **8:** 295–309.
[14] Pantelouris EM. Absence of thymus in a mouse mutant. *Nature*, 1968; **217:** 370.
[15] Helson C. The nude mouse model. In: *Cancer and Chemotherapy*, New York, Academic Press, 1980, Vol. I pp 229–245.
[16] Wortis HH, Nelson S, Owens JJ. Abnormal development of thymus in 'nude' mice. *J Exp Med*, 1971; **134:** 681–92.
[17] Giovanella BC. Experimental chemotherapy of human tumors heterotransplanted in nude mice. *Anti Chemo*, 1980; **28:** 21–7.
[18] Johnson WJ, Balish E. Macrophage function in germ-free, athymic (nu/nu) and conventional flora (nu/+) mice. *J Reticuloendothel Soc*, 1980; **28:** 55–66.
[19] Nickol AD, Bonventre PF. Anomalous high native resistance of athymic mice to bacterial pathogens. *Infect and Immun*, 1977; **18:** 636–45.
[20] Williams DM, Dwight M, Weiner MH, Drutz DJ. Immunologic studies of disseminated infection with *Aspergillus fumigatus* in the nude mouse. *J Infect Disease*, 1981; **143:** 726–33.
[21] Rao GR, Rawls WE, Perez DYE. Macrophage activation in congenitally athymic mice raised under conventional or germ-free conditions. *J Reticuloendothel Soc*, 1977; **21:** 13–20.
[22] Nakamura K, Yogi Y. The nude mouse as an experimental lepromatous model (continued): the NFS/N nude mouse as a new model using the intra-upper tip inoculation method. *Int J Lepr*, 1982; **50** (4): 585–6.
[23] Ueda K, Yamazaki S, Someya S. Experimental mycobacterial infection in congenitally athymic 'nude' mice. *J Reticuloendothel Soc*, 1976; **19:** 77–90.
[24] Rees RJW. Enhanced susceptibility of thymectomized and irradiated mice to infection with *Mycobacterium leprae*. *Nature*, 1966; **21:** 657–8.
[25] Rees RJW, Waters MFR, Weddell AGM, Palmer E. Experimental lepromatous leprosy. *Nature*, 1967; **215:** 599–602.
[26] Rees RJW, Weddell AGM. Experimental models for studying leprosy. *Ann N.Y. Acad Sci*, 1968; **154:** 214–36.
[27] Kohsaka K, Mori T, Ito T. Nude mouse for research in leprosy. *Int J Lepr*, 1978; **46:** 121–2.
[28] Kohsaka K, Yoneda K, Makino M, Mori T, Ito T. Experimental leprosy with nude mice. *Japan J Lepr*, 1979; **48:** 37.

Lepr Rev (1983) **54,** 305–308

Study of the use of nude mice in the cultivation of *Mycobacterium leprae* in a normal, non-specific pathogenic-free room at a temperature of 30–35°C, without air-conditioning

SERI RUNGRUANG, TEERA RAMASOOTA & SURASAK SAMPATTAVANICH
Raj-Pracha-Samasai Institute, Prapadaeng, Samutprakarn Province, 10130, Thailand

Accepted for publication 30 December 1982

Summary From a study of cultivation of *Mycobacterium leprae* in nude mouse foot-pads in a normal, non-specific pathogen-free room at a temperature of 30–35°C, without air-conditioning, it was found that the nude mice in the trial could survive longer than 16 months, which is a sufficient period for laboratory and research activities with this model. Leprosy bacilli cultivated in nude mouse foot-pads could then multiply as well as those cultured in air-conditioned and specific pathogen-free conditions. The authors have some recommendations for the cultivation of *M. leprae* in nude mouse foot-pads and the care of the mice as follows:

1 The room for nude mice on the trial must be kept closed on all sides but an electric fan may occasionally be used to extract foul-smelling air.
2 Prevent ultraviolet rays from sunlight entering the room in order to protect mice from phototoxic dermatitis.
3 The room must be located on the upper floor so that contamination and changes in land temperature are avoided.
4 During breeding and feeding, nude mice must receive special close-care from birth in order to maximize survival.

The cultivation of leprosy bacilli in nude mouse foot-pads using the method described will—until *in vitro* growth is achieved—benefit research in chemotherapy, biochemistry, immunology, immunopathology, epidemiology and other related fields, at relatively low cost.

Introduction

Since 1873 and Hansen's discovery of *Mycobacterium leprae*, there have been

0305-7518/83/054305+04 $01.00

many attempts to cultivate leprosy bacilli on laboratory artificial media and in animals such as normal mice, nude mice and nine-banded armadillos. In 1960 Shepard reported success in the cultivation of leprosy bacilli in normal mouse foot-pads;[1] in 1971 Kirchheimer & Storrs succeeded in cultivating leprosy bacilli in nine-banded armadillos,[2] and in 1976 success in cultivating leprosy bacilli in nude mouse foot-pads was reported.[3–5]

Nude mice naturally have neither hair nor thymus glands, and they lack cell-mediated immunity,[6] being sensitive to infections in normal conditions which may lead to death within 25 weeks.[7] Therefore, nude mice ordinarily have to be cared for in specific pathogen-free (SPF), air-conditioned rooms, involving much work and expenditure. In 1981, however, we started a trial to keep nude mice in a normal clean room, under non-SPF conditions at a temperature of 23–28°C, where nude mice could be kept from birth until the age of 16 months, or longer.[8] The present paper reports our continuing studies, aimed at the discovery of appropriate technology, at reasonable cost, to maintain nude mice in a non-SPF room at a temperature of 30–35°C without air-conditioning, and also to keep them alive long enough to be used for the cultivation of leprosy bacilli.

Materials and methods

1 Leprosy bacilli were prepared from untreated lepromatous leprosy cases in Hank's balanced salt solution and bacillary counts performed.
2 Leprosy bacilli were inoculated into both hind foot-pads, 0·03 ml/foot-pad ($6{\cdot}5 \times 10^5$/foot-pad) of 28 female nude mice, aged 5–6 weeks, weighing about 18–20 g each.
3 After inoculation of leprosy bacilli into foot-pads, 2 mice were sacrificed every month, and bacillary counts were made on the foot-pad tissue.

Results

After inoculation of leprosy bacilli into both foot-pads ($6{\cdot}5 \times 10^5$/foot-pad) of 28 female nude mice, they were kept in a normal room, not equipped with air-conditioning, at a temperature of 30–35°C. During the operation of the work there was no change of clothes and shoes by personnel, and related materials like mouse cages, water, and food were not sterilized in the usual way. From this study it was found that only one nude mouse got thinner, with bending of the backbone and death at 13 months, but the rest remained strong and lived longer than 16 months.

In month 13 after inoculation of $6{\cdot}5 \times 10^5$ leprosy bacilli per foot-pad, the remaining mice got lepromatoid lesions on the feet. Harvest counts showed that during the first 3 months of inoculation the numbers of leprosy bacilli decrease.

Table 1. Leprosy bacilli harvest from nude mouse foot-pads, after inoculation of $6{\cdot}5 \times 10^5$ leprosy bacilli per foot-pad, with solid ratio = 3:500

Month no.	AFB/foot-pad harvest	Solid ratio from foot-pad
1	$2{\cdot}4 \times 10^5$	0
2	$3{\cdot}0 \times 10^5$	0
3	$5{\cdot}3 \times 10^5$	1:500
4	$3{\cdot}8 \times 10^6$	2:500
5	$8{\cdot}4 \times 10^6$	4:500
6	$1{\cdot}8 \times 10^7$	10:500
7	$6{\cdot}6 \times 10^7$	13:500
8	$1{\cdot}2 \times 10^8$	18:500
9	$2{\cdot}9 \times 10^8$	23:500
10	$4{\cdot}2 \times 10^8$	28:500
11	$5{\cdot}6 \times 10^8$	31:500
12	$1{\cdot}3 \times 10^9$	33:500

They began to increase in the fourth month and this continued until the twelfth month (Table 1). The D-dopa oxidase test,[9] was used to confirm that the bacilli harvested from nude mouse foot-pads were *Mycobacterium leprae*.

Discussion

This study on the cultivation of *Mycobacterium leprae* in nude mouse foot-pads has been conducted successfully in a normal room, not equipped with air-conditioning or permanent ventilation fan, at a temperature of 30–35°C, using non-SPF conditions for cages, bottles, water, mouse food and clothes. Our results show that in spite of these conditions bacilli could multiply effectively, as previously reported[3–5] with the loss of only 1 nude mouse at the age of 13 months. This indicated that at temperatures of 30–35°C when the weather was rather hot and the ventilation was not good, nude mice could mostly adapt themselves and live longer than 16 months, thus being of practical value in research work, at relatively low cost. During the course of this work, it has been noted that direct sunlight entering the room produces skin abnormalities and this may lead to weakness and death. Furthermore, we advise that nude mice should be kept in an elevated room away from the influence of the ground temperature and in an atmosphere which may be bacteriologically cleaner than that at ground level. It is important to pay very careful attention to newly-born mice and their feeding in the early stages of life so that they develop and grow up in good condition, capable of survival until 16 months or over.

Acknowledgement

The authors wish to thank the Department of CDC for supporting the trial on cultivation of *Mycobacterium leprae* in nude mouse foot-pads at Raj-Pracha-Samasai Institute Leprosy Division, and the Sasakawa Memorial Health Foundation of Japan for providing scientific materials, fellowships and an adviser from Osaka University, Japan, to help conduct the operation.

References

[1] Shepard CC. The experimental disease that follows the infection of human leprosy bacilli into foot-pads of mice. *J exp Med*, 1960; **112:** 445.

[2] Kirchheimer WF, Storrs EE. Attempt to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. I. Report of lepromatoid leprosy in an experimentally infected armadillo. *Int J Lepr*, 1971; **39:** 693.

[3] Colston MJ, Hilson GRF. Growth of *Mycobacterium leprae* and *M. marnium* in congenitally athymic (nude) mice. *Nature*, 1976; **262:** 370.

[4] Kohsaka K, Mori T, Ito T. Successful transmission of *Mycobacterium leprae* to nude mouse. *Proceedings of the Second International Workshop on Nude Mice.* Tokyo: University of Tokyo Press, 1977/Stuttgart: Gustav Fischer Verlag, 1977; p. 77.

[5] Kohsaka K, *et al.* Experimental leprosy with nude mice. *Jap J Lepr*, 1979; **48:** 37.

[6] Pantelouris EM. Absence of thymus in a mouse mutant. *Nature*, 1968; **262:** 399.

[7] Fogh J, Giovanella BC. *The Nude Mouse in Experimental and Clinical Research.* New York/San Francisco/London: Academic Press, 1978; p. 2.

[8] Rungruang Seri, *et al.* Preliminary report on application of nude mice in cultivation of *Mycobacterium leprae* under non-specific pathogen-free condition. *Commun Dis J*, 1981; **7:** 31.

[9] Prabhakaran K. A rapid identification test for *Mycobacterium leprae*. *Int J Lepr*, (Correspondence) 1973; **41:** 121.

Lepr Rev (1983) **54,** 309–315

The effect of clofazimine on the plaque-forming cell response

ANA VALDES-PORTELA,* A M VAZQUEZ† & C M FINLAY*

* *Department of Microbiology, 'Pedro Kourí' Tropical Medicine Institute, Avenida 15 y calle 200 Siboney, Ciudad Habana, Cuba;*

† *Immunology Laboratory, National Center for Scientific Research*

Accepted for publication 12 May 1983

Summary The plaque-forming cell (PFC) technique was used to assay the effect of clofazimine, an antileprotic drug, on the immune system. Inbred mice were force-fed the drug for different periods of time at concentrations of 1 and 10 mg/kg of body weight per day. After 14 and 21 days of treatment, a significant increase in PFC response was observed. It is concluded that clofazimine exerts an effect on the early events involved in the antibody-forming cell response.

Introduction

The treatment of leprosy has been confronted by serious problems. The early hope that the sulphone drug series would be all-sufficient for leprosy has not been fulfilled.[1] At present, various drugs are used in conjunction with the sulphones in the treatment of the disease. To achieve improved therapeutic methods, the mode of action of the drugs should be understood. For this purpose, our laboratories have been engaged in studying the mechanism of clofazimine, an antileprotic drug, at the cell level. Recent studies reveal that this drug exerts its action on the macrophage lysosomal apparatus by increasing *de novo* synthesis of lysosomal enzymes and the phagocytic capacity.[2, 3]

Macrophage–lymphocyte interaction is a central event in the initiation and regulation of the immune response to both soluble protein and particulate antigen through antigen handling, an important event in the generation of a macrophage-associated antigenicity for T-cell recognition of antigen.[4] It is now clear, for at least some antigens, that interaction of 3 cell types is required in T-dependent antibody formation systems: thymus-derived lymphocytes (T-cells),

Correspondence: Ana Valdes-Portela, 'Pedro Kourí' Tropical Medicine Institute, Avenida 15 y calle 200 Siboney, Ciudad Habana, Cuba.

0305-7518/83/054309+07 $01.00

bone-marrow-derived lymphocytes (B-cells) and the macrophage.[5–7] Since the macrophage participates in the immune response and clofazimine exerts its action on the macrophage, it could be assumed that the drug would have an effect, either directly or indirectly, on the lymphoid system. This study was undertaken to evaluate the effect produced by clofazimine on the functional capacity of the cell population involved in the early events of cellular cooperation.

Materials and methods

MICE

Female mice of the inbred stain, IOR/Hab, 8 weeks of age and weighing between 20 and 25 g, were used. This strain, developed in the animal colony of the Oncology and Radiobiology Hospital in Cuba, is in the process of being recognized internationally (personal communication, *Mouse Newsletter*). All mice were obtained through the courtesy of Licenciado Raul Castillo.

EXPERIMENTAL DESIGN

Two groups of mice were fed by gavage during different time periods with two different concentrations of clofazimine dissolved in 0·3 ml of sunflower oil. The doses used were 1 mg/kg of body weight per day and 10 mg/kg of body weight per day. Two control groups were used, one receiving only 0·3 ml of sunflower oil and the other group receiving no treatment at all. Mice also received mouse pellet food and water *ad libitum*.

PRIMING WITH HETEROLOGOUS ERYTHROCYTES

Sheep red blood cells (SRBC) were obtained from a single animal assigned to this work at the animal colony of the National Center for Scientific Research. One week prior to use, blood was collected and stored in Alsever's solution. When required, the cells were washed 3 times in saline and finally re-suspended to an appropriate volume for injection and for use in the PFC assay. Four days prior to sacrifice, mice were immunized intraperitoneally with 0·5 ml of a 1% solution of SRBC.

PREPARATION OF CELL SUSPENSIONS

Mice were killed by medular elongation and the spleen removed taking care to eliminate all adhering fat. The spleen was disrupted and strained through nylon and steel mesh. The cellular suspension obtained was washed once in Parker-Tris-HCl medium (0·2 M, pH 7·2). The cell counts were done in a 2% solution of crystal violet in acetic acid. Instead of counting the total number of spleen cells,

only small mononuclear cells, easily recognizable in the crystal violet solution, by their round nucleus and scarce cytoplasm, were counted. This modification was used to adjust cell concentrations to 2×10^6 cells/ml.

PLAQUE-FORMING CELL ASSAY

Direct PFC were detected by the method of Cunningham & Szenberg.[8] Briefly, to a 1 ml spleen cell suspension, 0·1 ml of a 26% solution of SRBC, 0·1 ml of cold medium and 0·1 ml of fresh guinea-pig serum as complement source, were added. 0·1 ml of this suspension was seeded into Cunningham incubation chambers, and the residual space filled with a chaser of target cells in medium. The chambers were sealed with paraffin and incubated at 37°C for 90 min. Plaques were counted with a 6·5 magnification.

STATISTICAL ANALYSIS

A comparison of the number of PFC for groups of mice was calculated by Student's *t*-test when the same variances were proven by the Fisher test. In the cases when variances were significantly different, a modification was used to calculate *t* values. Since the number of PFC for groups of mice does not follow a normal distribution, ln values were also calculated and compared to the normal values.

Results

CELL COUNTING MODIFICATION

A modification was introduced in the cell counting method in an attempt to reduce the variances usually observed with this method. In the crystal violet solution, cell morphology is easily recognizable. In this manner, only cells presenting a round nucleus and little cytoplasm were counted assuming that this population would consist, primarily, of responder cells of the lymphocyte series. Consequently, by eliminating cells not directly involved in the cell cooperation response, variability in the drug, if any, should be more evident. Table 1 presents a comparison of the two counting methods, the conventional way and the one proposed by us. As can be observed, with this modification, the number of PFC is greater and more uniform, while the variance is greatly reduced. In view of these results, the modification was used to count cells in the following experiments.

EFFECT OF CLOFAZIMINE ON SPLEEN CELLS

The effect of clofazimine on the primary IgM–PFC response was measured across a spectrum of 21 days. Two clofazimine concentrations were used since it has been

Table 1. Cell-counting modification

	PFC/10^6	ln		PFC/10^6	ln
Conventional	1007	6·915	Mononuclear	1365	7·219
spleen	801	6·686	spleen	1202	7·092
cell count	1027	6·934	cell count	1180	7·073
	1524	7·329		1026	6·933
	611	6·415		1222	7·108
	669	6·506		1033	6·940
	637	6·457		1201	7·090
	926	6·831		1236	7·120
$\bar{X} \pm SD$	900 ± 300	$6{\cdot}759 \pm 0{\cdot}308$		1183 ± 110	$7{\cdot}072 \pm 0{\cdot}094$

Values given are the mean of triplicate plates for each mouse. Variances are significantly different for $P < 0{\cdot}05$.

reported[9] that these concentrations, when given to mice inoculated with *Mycobacterium leprae* in the foot-pad, were effective in preventing bacterial multiplication. Mice were given the drug daily and groups of mice were killed at weekly intervals. Figure 1 gives the difference observed, in percentage, after 7, 14 and 21 days of treatment as compared to the control group receiving only sunflower oil. As can be seen PFC response is greatly increased at 14 days of treatment and begins to decrease after 21 days. The PFC response in clofazimine-

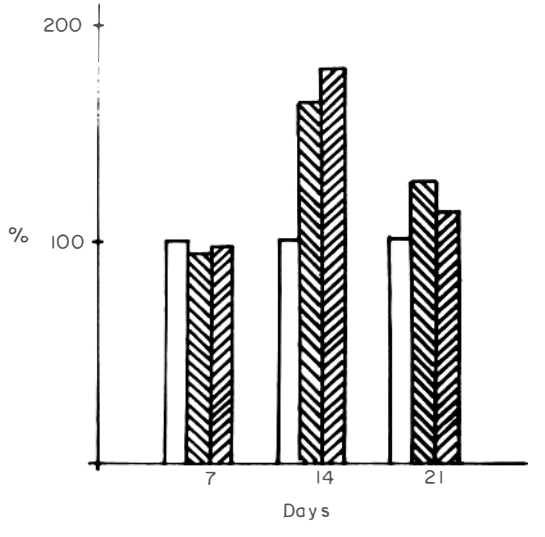

Figure 1. PFC increase in clofazimine-treated mice: □, control group; ▧, 1 mg/kg of body weight per day; ▨, 10 mg/kg of body weight per day.

Table 2. Plaque-forming cell response in clofazimine-treated mice

	Day 7		Day 14		Day 21	
Treatment	*n*	$\bar{X} \pm SD$	*n*	$\bar{X} \pm SD$	*n*	$\bar{X} \pm SD$
Untreated*	8	1183 ± 110 (7·072 ± 0·102)	8	1200 ± 270 (7·069 ± 0·215)	7	811 ± 109 (6·688 ± 0·154)
Sunflower oil	6	1183 ± 199 (7·063 ± 0·179)	8	1170 ± 330 (7·029 ± 0·290)	7	796 ± 67 (6·677 ± 0·084)
1 mg/kg of body weight per day	6	1106 ± 127 (7·004 ± 0·115)	8	1917 ± 187† (7·554 ± 0·101)‡	7	1009 ± 66‡ (6·916 ± 0·064)‡
10 mg/kg of body weight per day	6	1136 ± 191 (7·022 ± 0·178)	8	2080 ± 173† (7·070 ± 0·215)‡	7	897 ± 52‡ (6·798 ± 0·058)‡

* The values of the untreated mice were considered as those referring to the control group as well as the day 0 treatment. Numbers in parentheses are the ln transformed data.

† $P < 0{\cdot}0005$ as compared to the sunflower oil control group.

‡ $P < 0{\cdot}005$ as compared to the sunflower oil control group.

treated mice throughout 21 days of treatment can be observed in Table 2. Both concentrations of the drug increase after 14 days of drug treatment, although the 10 mg concentration response is greater. By 21 days, the response is decreased, but both are higher than the control group and the 1 mg/kg of body weight per day has a higher response than the other drug concentration.

Discussion

The PFC response of mice receiving clofazimine at concentrations reported to prevent bacterial multiplication[9] was significantly increased after 14 and 21 days of treatment with the drug. The response observed could be a collateral effect of macrophage function since studies carried out in our laboratories have shown that clofazimine exerts an action on macrophages not only in *in vitro* methods but also in *in vivo* systems within the same range of drug concentration. Several studies support the idea that macrophage presentation of immunogens to T-cells requires intracellular handling of the antigen.[5] Then, after lysosomal digestion, antigen fragments would be transferred to accessible sites at the cell surface or released to the extracellular space where they may interact with I-region products and with specific T-cells. Since SRBC are a T-dependent antigen and are the antigen used in this model, the response would be due to an interaction between macrophages and T-lymphocytes. Also, there is ample evidence that monocytes can play a regulatory function in the proliferative response of lymphocytes to

antigens[10, 11] and in the induction of antibody synthesis.[11, 12] Previous experiments with the trypanocidal drug, suramin, have shown that drug treatment of BCG-primed spleen cell cultures prevents the enhancement of anti-SRBC response to bacteria added to the cultures[14]. Suramin is known to accumulate in macrophages and interfere with the activity of lysosomal enzymes.[13, 14]

If the reverse can be assumed to be true, then clofazimine which exerts its effect on macrophages, precisely in the lysosomal system, should enhance the anti-SRBC response in spleen cells. Nevertheless further research should be carried out to decipher the precise action of the drug on each immunocompetent cell population, i.e. the B-lymphocyte and the T-lymphocyte. Studies are being carried out in this laboratory to clarify the events which occur in the cellular cooperation mechanism.

Acknowledgments

The authors wish to thank Isabel Cristina Pérez for her excellent technical assistance, Dr Carlos García Santana for his helpful advice and Eslivia Préstamo for typing the manuscript. This investigation received support from the UNDP–World Bank WHO Special Programme for Research Training in Tropical Medicine.

References

[1] Research in Leprosy. A report of a committee set up by the medical council to study future prospects. *Clin exp Imm*, 1979; **36:** 1.

[2] Sarracent J, Finlay CM. The action of clofazimine on the level of lysosomal enzymes of cultured macrophages. *Clin exp Imm*, 1982; **48:** 261.

[3] Sarracent J, Finlay CM. Phagocytosis and intracellular degradation of I^{125}-labelled immune complexes by clofazimine treatment of macrophage cultures. *Clin exp Imm*, 1982; **48:** 268.

[4] Zeigler HK, Unanue ER. Decrease in macrophage antigen catabolism caused by ammonia and chloroquine is associated with inhibition of antigen presentation to T cells. *Proc Natl Acad Sci USA*, 1982; **79:** 175.

[5] Miller JFAP, Mitchell GR. Cell to cell interaction in the immune response. I. Hemolysin-forming cells in neonatally thymectomized mice reconstituted with thymus or thoracic duct lymphocytes. *J exp Med*, 1968; **128:** 801.

[6] Mitchell GF, Miller JFAP. Cell to cell interaction in the immune response. II. The source of hemolysin-forming cells in irradiated mice given bone-marrow and thymus or thoracic duct lymphocytes. *J exp Med*, 1968; **128:** 821.

[7] Maillard J, Bloom BR. Immunological adjuvants and the mechanism of cell cooperation. *J exp Med*, 1972; **186:** 285.

[8] Cunningham AJ, Szenberg A. Futher improvements in the plaque technique for detecting single antibody forming cells. *Immunology*, 1968; **14:** 599.

[9] Sheppard CC, Chang YT. Activity of antituberculosis drugs against *Mycobacterium leprae*. Studies with experimental infection of mouse foot pads. *Int J Lepr*, 1964; **32:** 196.

[10] McCombs C, Michalski JP, Talal N. Cellular interactions in lymphocyte proliferation: effect of syngeneic and xenogeneic macrophages. *Cellular Immunol*, 1976; **23:** 283.

[11] Johansson A, Hefors E, Biberfeld R. An electron microscopic study of the contacts between human peripheral T-lymphocytes and autologous monocytes in culture. *J Submicr Cytol*, 1978; **10:** 281.

[12] Rosenthal AS, Shevach EM. The function of macrophages in T-lymphocyte antigen recognition. *Contemp Top Immunol*, 1976; **5:** 47.

[13] Buys Ch CM, Elferink Marieka GL, Bouma JMW, Gruber M, Nieuwenhuis P. Proteolysis of formaldehyde-treated albumin in Kupffer cells and its inhibition by suramin. *J Reticuloendothel Soc*, 1973; **14:** 209.

[14] Pesanti GL. Suramin effects on macrophage phagolysosome formation and antimicrobial activity. *Infect Immun*, 1978; **20:** 503.

Lepr Rev (1983) **54,** 317–325

An investigation of dapsone compliance using an isoniazid-marked formulation

J N A STANLEY,* J M H PEARSON* & G A ELLARD†
* *Dhoolpet Leprosy Research Centre, Hyderabad 500 006, Andra Pradesh, India;* † *National Institute for Medical Research, London NW7 1AA, England*

Accepted for publication 12 May 1983

Summary In a study conducted among out-patients in Hyderabad, it was shown that the precision of the dapsone/creatinine method for monitoring dapsone compliance could be improved if patients were prescribed specially formulated dapsone capsules containing 6 mg isoniazid as an innocuous marker. Urine samples were obtained by means of surprise home visits; the ingestion of the isoniazid marker was revealed by a simple colorimetric procedure which gives reliably positive results for about 18 h. The study showed that such capsules were acceptable to the patients and, in the short run, were taken more regularly than the standard tablets. However, a small proportion of patients took both capsules and tablets very irregularly, indicating that poor compliance was not overcome by simply changing the dapsone formulation.

Introduction

The dapsone/creatinine ratio (D/C ratio) method has been widely used for monitoring the regularity with which leprosy patients ingest their prescribed daily dapsone treatment.[1] Estimates of the overall level of dapsone compliance have been obtained by comparing the mean D/C ratios of urine samples collected from out-patients with those from suitable groups of controls. Although such an approach has demonstrated the ubiquity of poor dapsone compliance, it suffers from 3 important limitations:

1 Because of the lack of specificity of simple colorimetric procedures for determining dapsone and the presence of interfering compounds in normal urine samples, such samples appear to have significant dapsone concentrations. Thus in order to assess the compliance of out-patients, the apparent D/C ratios of untreated controls must also be determined.

2 Because of the relatively slow elimination of dapsone[2] and its metabolites

0305-7518/83/054317+09 $01.00

from the body (half-life about 27 h), D/C ratios typical of those of fully compliant patients are only reliably achieved after giving some 4 consecutive supervised daily dapsone doses; this normally requires access to hospitalized patients.

3 Although determining D/C ratios virtually eliminates the effect of diuresis on interpreting dapsone urine tests,[3] there are considerable individual differences in creatinine excretion rates, and for this reason the D/C ratios of urine samples collected from supervised controls typically vary over a 3-fold range.[4–7] It is therefore not possible to detect the omission of single dapsone doses or to assess how many doses have been missed, unless the D/C ratios of individual patients are first established during fully supervised treatment.

Recent investigations have demonstrated the feasibility of using minute doses of the antituberculosis drug isoniazid (INH) as an innocuous marker for studying the compliance of drugs prescribed for daily self-administration.[8, 9] Since INH is white, tasteless and odourless, preparations tagged with milligram amounts of the drug should be indistinguishable from standard formulations, while world-wide experience in the treatment of tuberculosis testifies to the safety of INH at daily doses of 300 mg when given for periods of at least a year.[10, 11] The ingestion of such INH doses can be demonstrated using a simple colorimetric urine-test procedure for its metabolites isonicotinic acid and isonicotinylglycine. Studies conducted among volunteers showed that 6 mg doses of INH gave reliably positive urine-test results up to about 18 h and uniformly negative results from 48 h onwards.[9]

It was therefore anticipated that a much more precise evaluation of dapsone compliance might be obtained by employing capsules tagged with 6 mg INH, since if the daily doses were recommended to be taken first thing each morning, the finding of a negative urine-test result would unequivocally indicate failure to have taken a dose that day. This paper describes a study of dapsone compliance among out-patients in Hyderabad, India to investigate the potentialities of such an approach and to compare the acceptability of INH-marked dapsone capsules with standard dapsone tablets.

Methods

PATIENTS AND SAMPLES

The investigation was conducted among a group of patients living within 6 km of Dhoolpet Leprosy Research Centre and typical of those registered for treatment there. Patients were only excluded from the study on 4 grounds:

1 If they had been treated for less than a year, since the compliance of such patients might be anticipated to be atypically good.[7]

2 If they had a record of habitual irregular attendance, since it seemed unreasonable to include those who might be extremely difficult to contact

regularly or who might take too few of their prescribed capsules or tablets for comparison of their acceptability to be possible.

3 If they were being treated with other drugs.

4 If there was any suggestion that they might be suffering from undiagnosed tuberculosis.

During the course of a routine clinic visit patients were interviewed, the study explained and they were asked if they would be willing to have clinic staff visit their homes approximately once a week to collect a urine sample. The study was presented as an investigation of treatment with a new capsule with urine tests being undertaken to evaluate its effectiveness.

When a patient agreed to participate in the study, his/her home address was obtained and shortly thereafter an initial home visit was undertaken. After locating the patient's dwelling, the patient and family were interviewed and their willingness to participate in the study verified. A urine sample was obtained at this visit. Inclusion in the study was finally confirmed by finding the patient at home and collecting a further urine sample on the next visit, and the investigation was initiated by giving the patient the first month's supply of tablets or capsules for daily self-administration immediately on rising each morning.

Twenty male and 14 female patients were included in the study. They were aged from 15 to 73 years (mean 37 years), weighed from 31 to 68 kg (mean 46 kg) and had been previously treated for up to 16 years (mean 5 years). They were randomly allocated to receive daily treatment with standard dapsone tablets (100 mg) for 8 weeks, followed by INH-labelled dapsone capsules (100 mg dapsone plus 6 mg isoniazid) for 8 weeks, or vice versa. The stocks of dapsone tablets or capsules were delivered every 4 weeks. Urine samples were collected by means of weekly surprise home visits between 7 a.m. and 12 noon which followed a randomized schedule so that patients did not know when they would next be visited. If they were out at the time, their homes were revisited later that day and, if necessary on the following day also. Urine samples were stored at 0–4°C in plastic containers to which a crystal of thymol had been added until shipment by air (without refrigeration) to London for subsequent analysis.

ANALYTICAL METHODS

D/C ratios were estimated by determining the urinary concentrations of dapsone plus its diazotisable metabolites (as dapsone equivalents) and creatinine by modifications of the Bratton and Marshall and alkaline picrate procedures, respectively.[12] Urine samples were tested for the presence of the INH metabolites isonicotinic acid and isonicotinylglycine by pipetting aliquots (0·5 ml) into small test-tubes together with 0·2 ml 4 M pH 5·0 acetate buffer and reacting by the sequential addition at 15-s intervals of 0·1 ml 10% aqueous potassium cyanide, 0·1 ml 10% aqueous chloramine-T and 0·5 ml 1% barbituric acid in acetone/water

(1:1 v/v). A positive result was indicated by the appearance within 30 min of a blue, green or grey colour depending on the natural background colour of the urine sample. Samples from patients who regularly smoked or chewed tobacco often gave characteristic orange colours after reaction presumably due to the excretion of metabolites of nicotine. Among such patients ingestion of isoniazid-marked dapsone capsules was indicated by urine samples which on reaction gave grey or brown rather than orange colours.

Results

COMPARISON OF OVERALL D/C RATIOS DURING TREATMENT WITH TABLETS OR CAPSULES

Analyses could only be carried out on a total of 320 urine samples, 187 collected while patients were prescribed standard dapsone tablets and 133 during treatment with the INH-marked capsules, because about a third of those originally collected were lost on account of faulty packing during shipment to England. The mean D/C ratios (μg apparent dapsone/mg creatinine) of these two sets of samples averaged 83 ± 37 and 92 ± 37, respectively ($0{\cdot}025 < P < 0{\cdot}05$).

INGESTION OF INH-MARKED DAPSONE CAPSULES, ESTABLISHMENT OF 'COMPLIANT' D/C RATIOS AND INTERPRETATION OF OTHER D/C RATIOS

The D/C ratios of the 320 urine samples are set out in Table 1. Four-fifths (108/133) of the urine samples collected during the time that the patients were issued with INH-marked dapsone capsules gave positive tests for isonicotinic acid indicating that at least one such capsule had been swallowed during the previous 48 h. The arithmetic mean D/C ratios of such samples are hereafter referred to as 'mean compliant' D/C ratios. Positive isonicotinic acid tests were not obtained from 3 patients during the period that dapsone capsules were prescribed (numbers 3, 21 and 28). Approximate estimates of their mean compliant D/C ratios were therefore obtained by averaging their obviously clustered higher D/C ratios when dapsone tablets were given (see Table 1). For clarity the data are presented in order of increasing mean compliant ratios. These ranged from 56 to 152 and averaged 91 and 117 for the 20 male and 14 female patients, respectively ($P < 0{\cdot}001$). With the exception of the results from patient 6, which will be considered below, there was a marked tendency for the compliant D/C ratios of individuals to cluster together, their variation about the mean being equivalent on average to only $\pm 12\%$. This clustering of individual compliant D/C ratios is similar to that encountered previously when successive urine samples were collected immediately before a series of daily supervised dapsone doses.[4]

The great majority of urine samples were collected between about 8 and 11 in

Table 1. Dapsone/creatinine ratios (μg apparent dapsone/mg creatinine)

Patient	Compliant ratios Marked capsule ingested*		Tablet ingested†	Number of missed doses†			
	Individual	Mean ± SD		1	2	3	⩾4
1	58, 57, 54	56 ± 2	86, 64, 62, 52	32, **30**‡			8, 7, **7**
2	86, 83, 80, 76, 65, 62	75 ± 10	80	**48**, 42	**22**		
3		(77)§	94, 79, 76, 71, 64	39, **34**			
4	84, 77, 71	77 ± 9	82, 76, 66	50	29		
5	83, 77, 76, 74	78 ± 4	62	**52**, **49**	**32**, **32**, 25	17	11, 11, 8
6	86, 86, 84, 78, 73, 61, (253, 222)¶	78 ± 10	93, 71, 69, 67 (158, 147)**				
7	81, 79, 75	78 ± 3	128, 109, 90, 89, 87, 78, 70	**38**			
8	97, 86, 79, 79, 75, 74, 72	80 ± 9	108, 95, 90, 82, 72, 71				11
9	88, 80	84 ± 6	75	**53**, 53			
10	94, 91, 68	84 ± 14		53, 38		18	
11	94, 77	86 ± 12	82, 71	57, **45**			
12	108, 93, 84, 63	87 ± 19	99, 92, 78, 72, 70, 69	61, **42**			
13	106, 71	89 ± 25	100, 87, 85, 71, 70, 70, 68	54, **50**			
14	100, 93, 92, 84	92 ± 7	102, 89, 84, 83, 78, 71	68, 49, 43			
15	124, 104, 85, 83, 76	94 ± 20	97, 93, 85				
16	101, 100, 100, 100, 100	100 ± 0		64		25	14
17	111, 103, 102	105 ± 5		**67**, 46	41, 39, 32		9, 5, 3
18	108	108	179, 145, 133, 123, 111, 103				
19	123, 113, 106, 101, 98	108 ± 10		66, **63**, 62, 55			
20	114, 111, 101	109 ± 7	103, 102, 102, 92	**79**, **77**			
21		(109)§	154, 122, 117, 110, 108, 101, 80, 79				
22	112	112	162, 156, 153, 122, 122, 117	83			
23	124, 102	113 ± 16	128, 128, 119, 87	71			
24	127, 102	115 ± 18	123, 104, 104, 102, 99, 98	54			
25	126, 123, 117, 113, 96	115 ± 12	129, 125, 124, 116, 116, 110, 108, 104				
26	127, 106	117 ± 15	92	81, 80, 59	49, 48		
27	132, 120, 119, 117, 113, 100	117 ± 10		81, 71, 70, 65, 57, 55	39, 35		
28		(119)§	133, 105	57	49	26, 25	
29	134, 107	121 ± 19		75, 67, 55			
30	150, 147, 132, 127, 122, 97	129 ± 19	111,110	84			
31	173, 121, 101	132 ± 37	116, 103	**89**, 82, 73, **71**	**47**, 34	**31**	8
32	137	137	154, 144, 132, 132. 120, 109	101		32	
33	173, 148, 107	143 ± 33	161, 139, 136, 134, 125, 115	84			
34	156, 147	152 ± 6		87, 81, **78**, 68			

* Obtained when urine samples gave positive isonicotinic acid tests. † For basis of interpretation see text. ‡ Bold type when capsules were prescribed, normal type when tablets were given. § Approximate estimate (see text). ¶ Triple doses assumed to be taken. ** Double doses assumed to be taken.

the morning. It therefore follows that a positive isonicotinic acid urine test would normally be due to a marked capsule having been swallowed either less than 5 h previously (earlier that morning) or more than 10 h before (the previous day). The small variability in the compliant D/C ratios consequently indicates that most positive isonicotinic acid urine-test results were due to a single marked capsule having been swallowed earlier in the morning that the urine sample was collected.

The one exception to these findings (the results for patient 6) were especially interesting. During treatment with the INH marked capsules all 8 of his urine samples gave positive isonicotinic acid tests results, indicating excellent compliance. However, while the D/C ratios of 6 of the samples were closely clustered (range 61 to 86, mean 78), those of the remaining two were 3 times higher (222 and 253), indicating that on those occasions he had ingested 300 mg of dapsone. In addition 2 tests while on tablets showed levels twice as high as the compliant ratios (147 and 158), indicating that he had taken 200 mg of dapsone on each of those 2 days.

It was possible to obtain an approximate estimate of the numbers of dapsone doses that had been missed prior to the home visits by comparing the D/C ratios of each urine sample with the mean compliant D/C ratio for the patient. Thus it was assumed that in the average patient, after correcting for the contribution of natural diazotisable compounds in the urine (equivalent to a blank D/C ratio of about 6), D/C ratios would fall by about 50% every 27 h.[3] D/C ratios would therefore be expected to decline to about 73% after 12 h, 40% after 36 h, 21% after 60 h and 12% after 84 h. Hence among the isonicotinic acid negative samples corrected D/C ratios of more than 73% of the mean corrected compliant ratios were taken as indicating that the daily dose of standard dapsone tablets had been taken as prescribed, whereas values of 40–73%, 21–40%, 12–21% and less than 12% were interpreted as implying that either 1, 2, 3 or 4 or more doses, respectively, of capsules or tablets had been missed.

RELATIVE ACCEPTABILITY OF CAPSULES AND TABLETS

Table 2 shows the estimated number of doses of dapsone capsules or tablets that were missed by the 34 patients. It was apparent that the capsules were more regularly self-administered (133 tests, 39 doses missed) than the normal tablets (187 tests, 119 doses missed). Each patient failed on average to take about 3–4 dapsone tablets but only missed 1 capsule. Moreover when individual patients were reviewed, only 2 appeared to take their tablets more regularly.

VARIABILITY OF DAPSONE COMPLIANCE

Patients differed greatly in the regularity with which they ingested their dapsone capsules and tablets (Table 3). Thus the compliance of 10 of the 34 patients was excellent, the urine test results indicating that they had missed at the most only a

Table 2. Relative acceptability of dapsone capsules and tablets

Interpretation of urine tests, no. of missed doses	No. of samples tested		Total no. of missed doses	
	Capsules	Tablets	Capsules	Tablets
None	108	119	0	0
One	17	42	17	42
Two	4	11	8	22
Three	2	5	6	15
Four (or more)	2	10	8	40
Total	133	187	39	119

Table 3. Variability of dapsone compliance

Compliance	No. of patients	No. of tests	No. of missed doses		
			Capsules	Tablets	Total
Excellent	10	97	1	4	5
Average	19	167	15	57	72
Poor	5	56	23	58	81
Total	34	320	39	119	158

single dose of dapsone. By contrast the compliance of patients 1, 5, 17, 28 and 31 was very poor, whether they were prescribed capsules or tablets, with individual totals of missed dapsone doses ranging from 9 to 23. These 5 patients accounted for about a half of the missed capsule and tablet doses in the whole study. The remaining 19 patients missed on average a single dose of the marked capsules and 3 doses of dapsone tablets. Among this relatively small group of patients dapsone compliance appeared not to be influenced by either age, sex or duration of treatment.

Discussion

Previous studies using the D/C ratio method to assess the compliance of patients with their prescribed treatment have usually been cross-sectional in design with the ratios of a group of out-patients being compared with those of groups of fully

supervised hospitalized patients and untreated controls. The present study, however, differed from such studies in 3 important respects:

1 Urine samples were obtained from out-patients by unannounced home visits rather than on the occasion of their attendance at a routine clinic. Surprise home visits should yield results more typical of the patients' general compliance than findings based on samples collected at clinic visits. In the latter situation compliance estimates could be positively biased if the forthcoming visit reminded the patient to take his treatment. Alternatively negative bias could arise if the clinic visit were prompted by the patient having finished his stock of dapsone tablets.

2 The study tried to assess whether dapsone-containing capsules would be as acceptable to patients as their normal dapsone tablets and whether, if such a formulation were regarded as 'new treatment', it might even improve their compliance. It showed conclusively that such capsules were acceptable to the patients and demonstrated that, at least in the short run, they were actually taken more regularly than the standard tablets. However, the extremely irregular self-administration of both capsules and tablets by 5 of the 34 patients showed that the problems of poorly compliant patients cannot be overcome by simply changing the dapsone formulation.

3 By utilizing dapsone capsules containing a 6 mg marker dose of INH, testing for their ingestion by the simple colorimetric isonicotinic acid procedure and comparing the D/C ratios of samples giving either positive or negative isonicotinic acid results, it was possible to assess the regularity with which patients ingest their prescribed dapsone with much greater precision than was formerly attainable. Such an approach clearly showed that the capsules were being taken more regularly than the tablets, a conclusion which could only be hinted at on the basis of a simple comparison of the mean D/C ratios during the two treatments, and demonstrated in a very compelling way the great variability in individual dapsone compliance.

As a result of this investigation one can envisage a much improved standard approach to monitoring the dapsone compliance of out-patients. Patients would be given an initial course of daily treatment with INH-marked dapsone capsules or tablets, to be taken at home at the usual time. During this period several urine samples would be collected by means of clinic or home visits. After testing for the ingestion of the marker by the isonicotinic acid procedure, the D/C ratios of those giving positive results would be determined. Once a reliable estimate of the individual's mean compliant D/C ratios had been obtained, treatment would be continued with ordinary dapsone tablets. The patients' compliance would then be followed by collecting further urine samples and comparing their D/C ratios with the mean compliant value. Such an approach would minimize the slight risk that repeated ingestion of marker doses of INH by an individual with unsuspected tuberculosis might lead to the selection of INH-resistant tubercle bacilli and

thereby prejudice subsequent treatment with INH-containing regimens.[8] It would also be an economical way of using limited stocks of specially formulated INH-marked dapsone capsules or tablets.

Acknowledgments

We wish to thank the International Dispensary Association, Amsterdam, The Netherlands, for the gift of the INH-marked dapsone capsules which were specially prepared for this study. The home visits were undertaken by Mr Paul Sukumar, Mrs Deveena Prabhaker and Mr Syed Yousuff Jani. J M H Pearson is a member of the staff of Victoria Hospital, Dichpalli, on secondment from the National Institute for Medical Research, London. Dhoolpet Leprosy Research Centre is managed by Victoria Hospital, Dichpalli, in collaboration with the Medical Research Council (of Great Britain), and also receives support from the British Leprosy Relief Association.

References

[1] Ellard GA. Drug compliance in the treatment of leprosy. *Lepr Rev,* 1981; **52:** 201–13.

[2] Colston MJ, Ellard GA, Gammon PT. Drugs for combined therapy: experimental studies on the antileprosy activity of ethionamide and prothionamide, and a general review. *Lepr Rev*, 1978; **49:** 115–26.

[3] Ellard GA. Profile of urinary dapsone/creatinine ratios after oral dosage with dapsone. *Lepr Rev*, 1980; **51:** 229–36.

[4] Low SJM, Pearson JMH. Do leprosy patients take dapsone regularly? *Lepr Rev*, 1974; **45:** 218–23.

[5] Jesudasan K, George B, Chacko CJG, Taylor PM, Kurian PV, Job CK. An evaluation of the self-administration of DDS in Gudiyatham Taluk. *Lepr India*, 1976 (Suppl); **48:** 668–76.

[6] Cates CJ. An assessment of dapsone self-administration in Gudiyatham Taluk. *Lepr Rev*, 1981; **52:** 55–64.

[7] Barton RPC, Rees RJW, McDougall AC, Ellard GA. The nose in lepromatous leprosy: Bacteriological and histological studies of patients treated with dapsone monotherapy for varying periods of time. *Int J Lepr*, 1982; **50:** 58–67.

[8] Ellard GA, Jenner PJ, Downs PA. An evaluation of the potential use of isoniazid, acetylisonizid and isonicotinic acid for monitoring the self-administration of drugs. *Br J clin Pharmac*, 1980; **10:** 369–81.

[9] Ellard GA, Jenner PJ, Allen BW. Who is taking their tablets? *Br Med J*, 1982; **ii:** 1660–1.

[10] Fox W. General considerations in the choice and management of regimens of chemotherapy for pulmonary tuberculosis. *Bull Int Un Tuberc*, 1972; **47:** 49–67.

[11] Girling DJ. The hepatic toxicity of antituberculosis regimens containing isoniazid, rifampicin and pyrazinamide. *Tubercle*, 1978; **59:** 13–32.

[12] Ellard GA, Gammon PT, Helmy HS, Rees RJW. Urine tests to monitor the self-administration of dapsone by leprosy patients. *Amer J Trop Med Hyg*, 1974; **23:** 464–70.

Lepr Rev (1983) **54,** 327–335

A review of side-effects experienced by patients taking clofazimine

VICTORIA J MOORE*
23 Colebourne Road, King's Heath, Birmingham B13 0EZ

Accepted for publication 5 May 1983

Summary The incidence of specific side-effects experienced by 65 patients taking clofazimine was assessed. All but one had suffered side-effects; skin and conjunctival pigmentation being the commonest. Abdominal pain was a complaint in one-third of the patients.

The 57 patients who were still taking clofazimine were divided into 2 groups according to dosage. The incidence of side-effects was similar in both groups. The average times of onset of the side-effects since starting clofazimine, in all instances, were greater in the lower dosage group. The possible significance of this is discussed.

Eight patients who had discontinued clofazimine therapy were seen. The disappearance of the side-effects was, in the majority of cases, 6 months or more after stopping therapy.

The difficulties associated with assessing dimness of vision and time intervals retrospectively were discussed.

No patients had stopped clofazimine due to side-effects. Five patients expressed dislike of the side-effects, 3 of which were in the lower dosage group. One patient *asked* for an increase in dosage to control an ENL reaction. The tolerance of side-effects, in general, was very good.

No patients suffered serious adverse reactions.

Introduction

Clofazimine is a substituted phenazine[1] which is active against *Mycobacteria*. It has been used in the treatment of leprosy for more than 10 years, both for its anti-mycobacterial effect and at relatively high doses to suppress moderate to severe erythema nodosum leprosum (ENL).

Clofazimine is highly lipophilic and tends to be deposited in fatty tissue and cells of the reticulo-endothelial system.[2] It has a tendency to remain in human tissue a long time, its half-life being at least 70 days.[3]

* Elective Student, Schieffelin Leprosy Training and Research Centre, Karigiri, Tamil Nadu, Southern India.

0305-7518/83/054327+09 $01.00

A very large number of papers have been published on the side-effects of clofazimine and have been reviewed.[4] Several workers have found potentially serious side-effects relating to the GI tract,[5–8] histopathological evidence of widespread clofazimine crystal deposition being found in some studies.[9]

The aim of this study is to obtain firm figures of the incidence of side-effects related to dose and duration of treatment, their subsidence after withdrawal and to assess the patients' tolerance of these.

Material and methods

All patients currently taking clofazimine and a few who had previously taken it, who attended Schieffelin Leprosy Training and Research Centre, out-patients or village clinics were interviewed with specific questions about anticipated side-effects.

The side-effects were considered in 3 sections: skin, eye and gastrointestinal tract. An estimate of the time relation of taking clofazimine and the onset of side-effects was obtained.

The patients were divided into 3 groups: those taking up to 700 mg/week as anti-mycobacterial therapy (group A), those taking higher doses for anti-inflammatory effect (group B) and those who had discontinued clofazimine treatment (group C). Capsules (100 mg) were taken after meals and in groups B and C, in divided doses.

Thirty-four patients taking clofazimine at a dose of 700 mg or less per week were seen (group A), the duration of treatment ranging from 1 to 83 months.

Twenty-three patients on higher doses averaged 10·5 months of treatment (group B); the maximum dose of 300 mg daily being given 3·1 months on average (with a maximum of 9 months). Four of the 23 patients had never received a dose of more than 200 mg daily.

The 8 patients seen who had had clofazimine previously for its anti-inflammatory effect, averaged 16·8 months of treatment, with a maximum number of 11 months at 300 mg daily (averaging 4·4 months at this dose).

Presentation of results

Tables 1–3 show the results in each group. They show the numbers and percentages of patients suffering the named side-effect, the times of onset since starting clofazimine and in the case of group C, the times of disappearance of the side-effect after stopping clofazimine.

Only one patient did not complain of any side-effects, having had 100 mg daily for 1 month. The average number of complaints per patient was 2·7 in the low dosage group, 2·8 in the higher dosage group and 3·0 in group C.

Table 1. 'Skin' side-effects of clofazimine in patients from groups A–C

	Discoloration	Pigmentation	Dryness	Other
Group A (34)				
Number (and percentage)	8 (23·5%)	18 (52·9%)	12 (35·3%)	1 (2·9%) (a complaint of generalized pruritus)
Average times (and ranges)	3·5 months (immediately–8 months)	2·5 months (immediately–6 months)	4 months (immediately–10 months)	—
Group B (23)				
Number (and percentage)	4 (17·4%)	17 (73·9%)	8 (34·8%)	2 (8·7%) (two cases of generalized pruritus)
Average times (and ranges)	1 month (immediately–3 months)	3 weeks (immediately–3 to 4 months)	5 weeks (1 month–3 to 4 months)	(1 month and 5 months)
Group C (8)				
Number (and percentage)	1 (12·5%)	7 (87·5%)	3 (37·5%)	0 —
Average times (and ranges)	(5–6 months)	4·2 months (1 week–2 months)	(1 month and 3 months)	—
Times of disappearance	(3 months)	8 months (6 months–1 year) (One patient is still pigmented 4 months after stopping clofazimine)	8 months (in 2 cases dryness is still present 1 year and 3 years since stopping clofazimine)	—

Table 4 shows the percentage of patients complaining of each side-effect, in total and in each group.

Discussion of results

The most common side-effect was skin pigmentation and conjunctival pigmentation, being complained of by 64·6% and 49·2% of all patients respectively. Abdominal pain was a complaint of just over one-third of all patients.

Table 2. Ocular side-effects of clofazimine in patients from groups A–C

	Conjunctival pigmentation	Dimness of vision	Dryness	Other
Group A (34)				
Number (and percentage)	16 (47·1%)	7 (20·6%)	0 —	11 (32·4%) (various complaints including burning (4) itching (4), irritation (2) and pricking (1) of the eyes)
Average times (and ranges)	3 months (immediately–1 year)	11 months (immediately–30 months)	— —	7·5 months (3 months–22 months)
Group B (23)				
Number (and percentage)	13 (56·5%)	1 (4·3%)	1 (4·3%)	3 (13·0%) (burning, irritation and watering)
Average times (and ranges)	3 weeks	(1 month)	—	—
Group C (8)				
Number (and percentage)	3 (37·5%)	0 —	0 —	1 (12·5%) (prescription of eye drops once only)
Times of appearance of side-effects	(1 month, 1 month and 2 months)	—	—	(7 months)
Times of disappearance of side-effects	3 months (2 patients still have pigmentation 15 and 4 months after stopping)	—	—	(one prescription)

In general both groups A and B have similar incidences of side-effects. In view of the small numbers involved in the study no formal evaluation of significance of differences has been performed. Some side-effects in group C were inferred from the records of drug therapy.

The percentage of patients complaining of skin colour changes (pigmentation and/or discoloration) was very similar in groups A and B. One patient in group A

Table 3. Gastro-intestinal side-effects of clofazimine

	Abdominal pain	Nausea	Diarrhoea	Other
Group A (34)				
Number (and percentage)	10 (29·4%)	3 (8·8%)	1 (2·9%)	6 (17·6%) (weight loss (2), 'no digestive power' (2), blood in stools (1) and vomiting, 4 bouts (1))
Average times (and ranges)	6·5 months (immediately–16 months)	(immediately and 7 months)	—	(3, 3, 5, 10 and 18 months)
Group B (23)				
Number (and percentage)	8 (34·8%)	1 (4·3%)	4 (17·4%)	1 (4·3%) (appetite loss, weight loss and one bout of vomiting—one patient)
Average times (and ranges)	4·5 months (1 month–13 months)	—	(1 month, 2 months and 5 months)	(1 week)
Group C (8)				
Number (and percentage)	4 (50·0%)	2 (25·0%)	1 (12·5%)	2 (25·0%) (vomiting—4 bouts (1), nausea, vomiting and weight loss (1))
Times of appearance of side-effects	(2 months, 2 months and 11 months)	(immediately and 2 months)	(immediately)	(2 weeks)
Times of disappearance of side-effects	2 years (2 patients are still complaining 1 year and $1\frac{1}{2}$ years after stopping clofazimine)	(2 months and 2–3 months after *starting* clofazimine)	(a few days after *starting* clofazimine)	—

Note. Averages were not calculated for four or less values. In such cases the reported times are given in parentheses.

Table 4. Percentage of patients complaining of each side-effect

	Skin				Eye				GIT			
	Disc.	Pigm.	Dry	Other	Conj. Pigm.	Dim. vision	Dry	Other	Abdo pain	Nausea	Diarrh.	Other
Group A	23·5%	52·9%	35·3%	2·9%	47·1%	20·6%	0	32·4%	29·4%	8·8%	2·9%	17·6%
	73·5%						32·4%					
Group B	17·4%	73·9%	34·8%	8·7%	56·5%	4·3%	4·3%	13·0%	34·8%	4·3%	17·4%	4·3%
	78·3%						17·4%					
Group C	(12·5%)	(87·5%)	(37·5%)	0	(37·5%)	0	0	(12·5%)	(50·0%)	(25·0%)	(12·5%)	(25·0%)
	(100%)						(12·5%)					
All patients	20·0%	64·6%	35·4%	4·6%	49·2%	12·3%	1·5%	23·1%	33·8%	9·2%	9·2%	16·9%

and 3 in group B complained of both discoloration and pigmentation, in all cases redness preceded pigmentation. There were two cases of obvious discoloration and one of pigmentation in the low dosage group which were not complained of; they were not included in the tables. Sometimes pigmentation was only in patches (in reaction spots or sites of nodules). The average time between emergence of skin colour changes and taking clofazimine was noticeably less in the higher dosage group.

All patients in group C noted skin colour changes: pigmentation occurring on average 1 month after starting treatment. (This is similar to the average of 3 weeks noted by patients still taking clofazimine for its anti-inflammatory effect.) The pigmentation disappeared 8·5 months (on average) after stopping the drug—the maximum being 1 year.

Dry skin was a complaint of 30% of patients in both groups. Average times before the onset of these symptoms were considerably shorter in the higher dosage group. One case of dryness (in group C) was a severe fissuring dermatitis still present 3 years after stopping therapy.

Conjunctival pigmentation was complained of by 56·6% of group B patients compared with 47% of group A; the average time before onset being 3 weeks and 3 months respectively. One patient in group B and 3 in group A had obvious pigmentation which was not complained of. Pigmentation disappeared on average 3 months after stopping treatment (group C); but one patient still has this feature 15 months after stopping clofazimine.

Complaints of dry eyes have been combined with complaints such as eye burning, itching, etc., in the percentage tables. These latter symptoms may well be a manifestation of dry eyes; they are common complaints in leprosy in general. Such complaints were found to be much more common in low dosage patients.

Dimness of vision since clofazimine treatment was also complained of much more frequently in group A. There are many difficulties associated with assessing this complaint. Several of the patients who complained of dim vision since taking clofazimine (after reviewing their ophthalmology notes) had, in fact, had dim vision before taking the drug and/or had had visual acuity checked since starting clofazimine with no change detected. These were not included in the tables. The ones that are included have had no formal eye examination to confirm their complaint.

The percentage of patients complaining of gastro-intestinal symptoms relating to the onset of clofazimine treatment was similar in both groups.

Abdominal pain was a common complaint but was never severe. One patient only had pain with initial treatment; another 4 said the pain was very mild. Two patients in group C still have pain 1 year and $1\frac{1}{2}$ years since last taking clofazimine.

Nausea was often a vague complaint. One case was only at night, another occurred once per month and often the nausea was only an occasional problem.

Diarrhoea was a relatively rare complaint. Three of the cases had only

occurred with initial therapy (lasting a few days to 2 weeks). A fourth case was a complaint of occasional diarrhoea.

The accuracy of times stated is dubious in some cases. A few patients had difficulty remembering time relations, especially those on long-term therapy or discontinued therapy. There are many instances of patients vaguely saying a side-effect was noted immediately the drug was started; the concept of 'immediately' may vary markedly between patients. Also the concept of time in terms of weeks or months is probably foreign to many villagers. On several occasions a patient would state the same time relation for all symptoms complained of, which may indicate inaccuracy of time associations in general. However, these considerations apply to all groups and the overall differences between groups may still be valid ones.

In general the *ranges* of time intervals are wider in group A. This is marked in the dimness of vision section, in which the stated time of onset after starting clofazimine varies from immediately to 30 months. Since all patients in this group (apart from one) were taking 100 mg daily at the onset of treatment the possibility of this not being a real side-effect but an incidental occurrence is raised. The much larger percentage of patients complaining of this side-effect in the lower dosage group supports this possibility. Other explanations such as variation in absorption, metabolism and distribution of clofazimine could also explain such variations in time relations for the same side-effect in one group.

A few patients related subsidence or disappearance of side-effects with decreasing doses of clofazimine. Skin dryness, eye dryness and conjunctival and skin pigmentation were the symptoms noted to diminish.

Two patients voluntarily stated a dislike of taking clofazimine; one for no specific reason and the other (an Anglo-Indian) because of increasing skin pigmentation—the degree of pigmentation decreasing since a dose decrease. Two female patients were moderately displeased about facial discolouration when directly asked. They were in the low dosage group. Although skin colour changes were a common side-effect these were the only patients who disliked it. This may be due to the fact that Southern Indians are generally more pigmented than the Northern Indians and also lack of knowledge of the association of this side-effect and leprosy amongst the general population.

One patient, when taking 200 mg per day, experienced another ENL reaction and *asked* for an increase in dosage. Several other patients (especially in group B) said they did not mind the side-effects to gain the benefits of clofazimine.

I did not find any patients who had stopped taking clofazimine due to side-effects. In general side-effects were considered easily tolerable.

Acknowledgments

The encouragement and guidance of Dr P M Taylor and the assistance of the staff

and patients at Schieffelin Leprosy Training and Research Centre were invaluable and much appreciated. My thanks must also be extended to Dr R J W Rees and LEPRA for making the research possible. I also wish to thank Dr E P Fritschi (Director of SLT & RC) for his advice with regard to this publication.

References

[1] Barry VC, Belton JG, Connalty ML, Denney JM, Edward DW, O'Sullivan JF, Twomey D, Winder F. A new series of phenazines (Rimino compounds) with high affinity anti-tuberculosis activity. *Nature*, 1957; **179:** 1013.

[2] Mansfield RE. Tissue concentrations of clofazamine (B663) in man. *Amer J Trop Med Hyg*, 1974; **23:** 1116.

[3] Levy L. Pharmacologic studies of clofazamine. *Amer J Trop Med Hyg*, 1974; **23:** 1097.

[4] Jopling WH. Complications of treatment with clofazamine (Lamprene: B663). *Lepr Rev*, 1976; **47:** 1.

[5] Mason GH, Ellis-Pegler RB, Arthur JF. Clofazamine and eosinophilic enteritis. *Lepr Rev*, 1972; **48:** 175.

[6] Ramu G, Iyer CGS. Side-effects of clofazamine therapy. *Lepr India*, 1976; **48:** 722.

[7] Plock H, Leiker DL. A long term trial with clofazamine in reactive lepromatous leprosy. *Lepr Rev*, 1976; **47:** 25.

[8] Sardari Lal, Garg BR, Hameedulla A. Gastro-intestinal side-effects of clofazamine. *Lepr India*, 1981; **53:** 2.

[9] McDougall AC, Horsfall WR, Hede JE, Chaplin AJ. Splenic infarction and tissue accumulation of crystals associated with the use of clofazamine (Lamprene: B663) in the treatment of pyoderma gangrenosum. *Br J Derm*, 1980; **102:** 227.

Lepr Rev (1983) **54,** 337–340

Quantitative estimation of *Mycobacterium leprae* in exhaled nasal breath

CHRISTINA A GREEN,* V M KATOCH† & K V DESIKAN†
**School of Clinical Medicine, Cambridge, England; †Central JALMA Institute for Leprosy, Taj Ganj, Agra 282001, India*

Accepted for publication 6 March 1983

Summary A quantitative estimation of leprosy bacilli in exhaled nasal breath was undertaken in 20 patients of borderline and lepromatous leprosy. Out of the 20 patients, 8 were untreated, 6 had treatment for 1 month and the remaining 6 were on treatment for a period of 3 months. Non-cultivable acid-fast bacilli could be demonstrated in nasal breath in all the cases studied. The average number of bacilli excreted were $3{\cdot}8 \times 10^4$, $2{\cdot}9 \times 10^4$ and $2{\cdot}8 \times 10^4$ per breath in the 3 groups respectively. The quantum of bacilli exhaled increased with duration of the disease.

Introduction

It has been established for over 80 years that large numbers of *Mycobacterium leprae* are excreted from the nose and mouth in the majority of lepromatous cases of leprosy. It has also been shown that leprosy bacilli can be demonstrated in a large majority of such cases in nasal washings,[12,13] nasal smears[2] and in nasal blows,[3] in the deep nasal breath.[1] The positivity in mouth washings as well as in nasal smears has been studied.[6] Together, these papers present a surge of interest directed recently towards evaluating the nose and mouth as exits for transmittable *M. leprae*. The present study was undertaken to find out if bacilli could be extruded through normal or just harsh breathing and if so the possible numbers of bacilli that could be thrown out in the environment.

Material and methods

The patients included in this study were selected from those attending the

* Present address: Renal Transplant Unit, Bristol, England.
† Reprints may be obtained from Dr Desikan.

0305-7518/83/054337+04 $01.00

out-patient clinics at JALMA. They were all very advanced and highly bacillated cases. Of the 20 cases included in the present study, 19 belonged to the lepromatous type whereas one was of the BL type.[11] Of these 8 were untreated, 6 had treatment for 1 month and the remaining 6 were on therapy for a period of 3 months.

Each patient was asked to breathe into a flask containing 10 ml of physiological saline. In order to dislodge the bacilli from the glass surface, a few drops of 1% Tween 80 were added and the sides of the flask were washed down by swirling the fluid. The fluid was then centrifuged at 10,000 rpm for 30 minutes. The supernatant was pipetted off and the remaining pellet was suspended in 1 ml of physiological saline. The bacilli were counted and calculated according to the method of Hart & Rees.[5] The remaining suspension was decontaminated by modified Petroff's method as described by Shepard.[12] It was then inoculated into Lowenstein–Jensen medium to observe growth of any cultivable *Mycobacteria*. Absence of growth on Lowenstein–Jensen medium was taken as an indirect proof of the acid-fast bacilli being *M. leprae*. Slit smears were performed in the usual way from the ear lobes. Bacteriological Index (BI) was assessed and graded according to the method described by Dharmendra.[4]

Results

The number of bacilli per exhaled nasal breath is shown in Table 1. The untreated

Table 1. Bacilli exhaled in nasal breath and bacteriological indices of bacilli in skin smears

	Number of patients	Skin smears	Bacilli exhaled
		Mean BI* ± SD	Mean number per breath
Group I (Untreated)	8	2·1 ± 0·69	$3{\cdot}8 \times 10^4$ ($2{\cdot}25 \times 10^3 - 1{\cdot}1 \times 10^5$) (a) range
Group II (On treatment up to 1 month)	6	2·0 ± 0·4	$2{\cdot}9 \times 10^4$ ($2{\cdot}25 \times 10^3 - 7{\cdot}1 \times 10^4$) (b) range
Group III (On treatment up to 3 months)	6	2·0 ± 0·5	$2{\cdot}8 \times 10^4$ ($4{\cdot}5 \times 10^3 - 1{\cdot}3 \times 10^5$) (c) range

* BI = Bacteriological Index.

P values calculated on the basis of individual values are: between (a) and (b) > 0·05; between (b) and (c) > 0·05; and between (a) and (c) > 0·05.

group consisted of 6 men in the age group of 16–50 years and 2 women both aged 30 years. The mean duration of the disease was 7 years. The BI varied from 1+ to 4+, the mean being 2·1+. All of the patients exhaled bacilli in their nasal breath, the average number of bacilli being $3{\cdot}8 \times 10^4$ per breath.

There were 6 patients who had received treatment for up to 1 month. All of them had received dapsone 50–100 mg for 5 days to 1 month. Four patients were on combination therapy with clofazimine, pyrazinamide, thiacetazone and isoniazid. All were men belonging to the 22–50-year age group. The average duration of illness in this group was 8 years. The mean BI was 2+. Again bacilli could be found in the exhaled nasal breath in all of these patients, the average number being $2{\cdot}9 \times 10^4$ per breath.

The third group comprised 6 cases whose mean period of treatment was $2\frac{1}{2}$ months. As in the second group, all the patients had taken dapsone 50–100 mg. Two received other drugs also. All the patients in the group were men aged between 21 and 45 years and the mean duration of their illness was 8 years. The average number of bacilli extruded numbered $2{\cdot}8 \times 10^4$ per breath.

Taking the duration of disease into consideration, it was seen that the number of bacilli in breath increased with duration of disease ($P < 0{\cdot}05$) (Table 2). There was no growth on the Lowenstein–Jensen medium in all the samples cultured.

Table 2. Relationship between the disease duration and exhalation of bacilli in nasal breath

	Disease duration < 5 years	Disease duration > 5 years
Number of patients	8	12
Mean number of bacilli excreted	$1{\cdot}0 \times 10^4$/breath	405×10^4/breath

Standard error = 2·56 (on the basis of individual excretion rates and $P < 0{\cdot}05$).

Discussion

In the present study, non-cultivable acid-fast bacilli could be demonstrated in nasal breath of all the cases examined. Taking the absence of growth on Lowenstein–Jensen medium as a negative proof, these bacilli were considered in all probability to be *Mycobacterium leprae*. In one earlier study,[3] 54% positivity was found in nasal discharges whereas in another[6] bacilli were found in 50% of the cases in nasal smears. It must be mentioned that in the present study all the cases selected were highly bacillated patients with little or no treatment. Pedley[7] and Pedley & Geater[8] have drawn attention to the importance of nasal secretions as the portal of exit of leprosy bacilli. While the presence of bacilli in the nasal

secretions and nasal blows is well recognized, it was of interest to find bacilli even in slightly harsher breath in the very highly bacillated cases of lepromatous leprosy. The present study has shown that in advanced cases of lepromatous leprosy a sizeable number of bacilli are exhaled with a harsh breath. These observations support the findings of Bedi *et al.*[1] wherein they had described acid-fast bacilli in deep breath of multibacillary cases. There was no significant difference in the number of bacilli in untreated cases as compared with cases treated for 1 month or up to 3 months. On the other hand, there was a correlation with the duration of the disease, cases with longer duration extruding a larger number of bacilli. These observations would be underscored in view of the fact that the values are obtained from different groups of patients and not from the same group followed up with treatment.

It is clear from the studies carried out so far that a large number of leprosy bacilli could be excreted from the upper respiratory passages as assessed by the examination of the nasal smears, nasal blows and nasal breath. These findings signify the importance of nasal infection in the epidemiology of leprosy.[3,9,10] However, none of these new findings need cause alarm to the patients or to the medico-social workers since it seems obvious that leprosy is only mildly contagious as judged by its low attack rate in the community.

References

[1] Bedi BMS, Narayan E, Sreevatsa, Kirchheimer WF, Balasubrahmanyam M. Dispersal of *Mycobacterium leprae* by leprosy patients while breathing. *Ann Indian Acad Sci*, 1976; **12:** 1.

[2] Davey TF, Barton RPE. Multiple nasal smears in leprosy. *Lepr India*, 1973; **45:** 54.

[3] Davey TF, Rees RJW. The nasal discharge in leprosy: clinical and bacteriological aspects. *Lepr Rev*, 1974; **45:** 121.

[4] Dharmendra. *Notes on Leprosy*. Ministry of Health, Government of India, 1967; p. 314.

[5] Hart P D'Arcy, Rees RJW. Effect of macrocyclon on acute and chronic pulmonary tuberculosis in mice as shown by viable and total bacterial counts. *Br J exp Path*, 1960; **41:** 414.

[6] Hubscher S, Girdhar BK, Desikan KV. Discharge of *Mycobacterium leprae* from the mouth in lepromatous leprosy patients. *Lepr Rev*, 1979; **50:** 45.

[7] Pedley JC. Composite skin contact smears: a method of demonstrating the non-emergence of *M. leprae* from intact lepromatous skin. *Lepr Rev*, 1970; **41:** 31.

[8] Pedley JC, Geater JH. Does droplet infection play a role in the transmission of leprosy? *Lepr Rev*, 1976; **47:** 97.

[9] Rees RJW, Meade TW. Comparison of the modes of spread and incidence of tuberculosis and leprosy. *Lancet*, 1974; **i:** 47.

[10] Rees RJW, Ridley DS. *Bacteriology and Pathology of Leprosy*, in 'Recent Advance in Clinical Pathology'; series 6 (S C Dyke edn). Edinburgh and London: Churchill Livingstone, 1973.

[11] Ridley DS, Jopling WH. Classification of leprosy according to immunity. A five-group system. *Int J Lepr*, 1966; **34:** 255.

[12] Shepard CC. Acid-fast bacilli in nasal secretions in leprosy. The results of inoculation in mice. *Amer J Hyg*, 1960; **71:** 147.

[13] Shepard CC. Nasal excretion of *M. leprae* in leprosy. *Int J Lepr*, 1962; **30:** 10.

Lepr Rev (1983) **54,** 341

Obituary

AYELE BELEHU BS, MSc, PhD
1943–1983

Ayele Belehu, Director of the Armauer Hansen Research Institute (AHRI) in Addis Ababa, Ethiopia died unexpectedly in an acute asthmatic attack on 17 May 1983.

Ayele was born in Debre Sina, Shoa Region, Ethiopia on 8 December 1943. After completing traditional education and subsequently elementary and secondary school in Addis Ababa he joined Addis Ababa University and took his BS in biology in 1966. He then worked for 1 year as a graduate assistant in the Chemistry Department. During 1967 to 1970 Ayele studied microbiology at Boston University College and the University of Arizona, Tucson, USA and obtained an MSc degree. After 2 years of lecturing in the Department of Biology, Addis Ababa University, he left for London to study pathology and immunology in the Department of Pathology at the Royal College of Surgeons, University of London from 1972 to 1975. He studied under Professor John Turk and produced a thesis for his PhD entitled 'The immunology and pathology of *Leishmania enriettii* infection in rodents and its modification by cyclophosphamide and malaria'.

Returning to Addis Ababa he joined AHRI as a senior researcher and from 1977 until his untimely death he served as the Director of the Institute. In addition to his directorship he conducted investigations in the immunology of Leishmaniasis and leprosy, resulting in more than 30 scientific publications. Ayele was also an honorary assistant professor in the Faculty of Medicine, Addis Ababa University, gave lectures at the Department of Biology and supervised MSc students. He was a board member of the *Ethiopian Medical Journal*, the Advisory Board of Central Laboratory and Research Institute, the Advisory Board of Sinet, the *Ethiopian Journal of Science*, the Cheshire Home for the disabled in Addis Ababa, the All Africa Leprosy and Rehabilitation Training Centre (ALERT). For many years Dr Ayele was a member of the Steering Committee of IMMLEP, a component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

Ayele was a most competent and dynamic scientist and leader. His friendly and interested attitude facilitated the cooperation, necessary for an Institute like AHRI, with national and international organizations. He was a gentle and kind person whom you could go to with personal matters. His interest in his fellow men was closely associated with his deep religious attachment to the Ethiopian Orthodox Church.

Ayele's research in the immunology of leprosy and Leishmaniasis has been of major importance for our knowledge in these fields and his contributions in the different boards, especially the Steering Committee of IMMLEP, has further augmented these contributions. In 1983 he was awarded the Redd Barna Prize of Honour, the Norwegian Save the Children Organization's highest mark of respect, for his long-standing contributions in the field of leprosy research. However, his untimely death when he had reached a state of scientific maturity that would have allowed him to contribute even more has cut short a brilliant career.

Ayele is greatly and deeply missed by his family, his friends in Ethiopia and abroad, colleagues in AHRI, ALERT, the University of Addis Ababa and by his country who lost its first immunologist.

Armauer Hansen Research Institute
Addis Ababa, Ethiopia

A HAREGEWOIN
P OLCÉN

0305-7518/83/054341 + 01 $01.00

Lepr Rev (1983) **54,** 342–344

SPECIAL ARTICLE

ECHO: The Joint Mission Hospital Equipment Board Limited

J BURTON
ECHO, 4 West Street, Ewell, Surrey KT17 1UL

The Joint Mission Hospital Equipment Board (JMHEB) now more universally known as ECHO was established in 1966. The concept of a coordinated charitable agency to supply the specialized medical needs of mission and charity hospitals overseas was the vision of an ex-missionary doctor with the support of the Conference of British Missionary Societies, under whose charitable umbrella the organization began life. The original aim was to coordinate and supply the medical equipment needs of mission hospitals overseas by utilizing the vast quantities of equipment becoming available at that time through the programme of re-equipping National Health Service hospitals. Offers of equipment poured in, as did requests from overseas. As a non-profit making charity with limited capital support of member agencies (UK missionary societies and charities) ECHO had from the very early years to become self supporting. Equipment was sold to cover the running costs, yet items were on average available to hospitals overseas at about a quarter of the comparable commercial cost. From these early beginnings has grown an organization now capable of supplying all the complex medical needs of a modern hospital. Large stocks of medical equipment have been built up over the years, both new, unused and reconditioned with a continuing dedication towards an improvement in the standards of equipment supplied to meet a changing pattern of need, e.g. sophisticated items such as X-ray units and hydraulic operating tables are factory reconditioned by specialist manufacturers to a high quality for a cost often below one-third of the commercial new price.

JMHEB became an independent registered charity in 1972, and in 1973 adopted the trading name of ECHO (Equipment for Charity Hospitals Overseas) to symbolize the broad and evolving role of the organization.

Total annual turnover of £6000 in 1967 had grown to 3·5 million in 1982 and by the end of that year ECHO was in contact with over 1600 hospitals and medical units in 119 different countries. The annual number of consignments sent overseas was nearly 2000 totalling approximately 1500 tons of vital supplies. The full and part time staff now number 55 and these include skills in equipment and pharmaceutical purchasing, order interpretation and selection, packing, export documentation, book-keeping and financial control. ECHO is directed by two permanent officers in the medical and administration fields and broad policy is governed by an honorary Council of Management comprising representatives elected annually from member organizations, together with *ex officio* councillors selected from commerce and government.

0305-7518/83/054342+03 $01.00

Pharmaceuticals

In response to demand, pharmaceuticals were added to the product list in 1975. Having established by market research a basic list of more commonly used generic (non-branded) drugs, dramatic savings were achieved by the bulk buying and stocking of tablets by the million. An increased range of pharmaceutical products covering the majority of the endemic diseases of developing countries were later offered as the demand grew. Today ECHO's pharmaceutical supplies are purchased from many sources in the UK and Europe. All are manufactured to the high British Pharmaceutical standard. A permanent stock of over 250 million tablets, capsules, ointments, etc., and several million injections is held—sufficient to meet a regular demand from overseas and for national disasters as they occur. It is interesting to note that the biggest growth in the ECHO Pharmaceutical Programme has been in the supply of drugs needed for the treatment of leprosy, and by 1980 some 260 million dapsone tablets were being manufactured annually for ECHO for world leprosy needs. The newer antileprosy drugs such as clofazimine and rifampicin have been made available by the manufacturers for distribution through ECHO at considerably reduced prices.

The rural health centre and village clinic

Most concerned authorities agree that health care in developing countries should be tailored to suit the particular needs of the community. Many developing countries have a common characteristic of rural communications. It is therefore likely that the medical needs of such rural areas can best be met by providing small compact health centres instead of expensive traditional hospitals, only economical in areas of dense population. With these considerations in mind, ECHO has evolved full basic equipment kits for a five-department Rural Health Centre comprising: examination room, treatment room, minor operations theatre, gynaecological/family planning clinic and a laboratory. In 1983, this kit can be provided for approximately £5000, with the equipment and medical software for the more basic village clinic for a little over £800.

Packing and freight

In 1974, faced with escalating shipping costs, ECHO set up its own packing and shipping department. It now handles not only ECHO supplies but also the freight needs of many member charities at cost. Wooden crates and boxes are made on the premises at 50% of commercial cost.

Appropriate Technology for Health

This somewhat grandiose title given to a recently created department of the World Health Organization describes something that missionaries have through necessity been doing for years, i.e. adapting available resources.

In the context of health care the latest expensive technological aids to diagnosis may be totally irrelevant in an underdeveloped country. ECHO is helping to make available basic items of equipment; for example, a company long-established in equipment manufacture now makes for ECHO a simple operating table in lightweight alloy, incorporating many of the essential features of the more expensive hydraulic table but at about a tenth of the cost. A whole range of descriptive leaflets are available of Appropriate Technology Medical Equipment specially manufactured for ECHO, including several which use car batteries for electric supply.

The future

The concept of a professional agency supplying (without profit motive) the medical needs of those responsible for developing health care in the Third World is now well established. For the future our plans are as follows.

EQUIPMENT

Continuing the development of the service to supply the equipment needs of charity hospitals and the initiatives of developing and supplying equipment kits for health centres and village clinics. Recently a new technical department has been opened led by a highly trained medical physics technician to ensure that there will always be a technical back-up and advisory service available to the overseas charity hospital.

PHARMACEUTICALS

ECHO has achieved a major breakthrough in the planned goal of a worldwide bulk supply of high quality generic medicines available cheaply on demand. The service continues to expand, whilst every encouragement is being given to the setting up of local production and distribution services overseas.

FREIGHT

A compact organization exists now to service the foreseen expansion. Cooperation between hospitals overseas to achieve larger group orders is now being actively encouraged by ECHO and will bring further savings with the benefits of containerization.

THE HEADQUARTERS

With the move in January 1979 to integrated and enlarged premises in Ewell, Surrey, enough working space was available to cope with developments foreseen in the immediate future. The larger premises have enabled better mechanical handling techniques to be introduced thus speeding up the packing and despatch of increasing numbers of overseas consignments. Now, however, a more permanent headquarters must be found and due allowance made for this in the capital funding programme.

ECHO DEVELOPMENT TRUST

ECHO's future role in supplying the needs of the world's medically under-privileged is now clear—the pace of development must be determined by the availability of capital resources, as expansion dictated by need must be allied with commercial prudence. The expansion programme has been costed as accurately as prevailing economic conditions will allow and a separate charity 'The ECHO Development Trust' set up to administer the capital funding needs. The programme to reduce human suffering is ambitious, the capital underwriting cost is not. Further details of this or any of the ECHO services are available on request.

Lepr Rev (1983) **54,** 345–348

Domiciliary and Field Work

OCEAC and OCCGE

We are most grateful to Mr André Recipon, President of the Association Française des Fondations Raoul Follereau, 33 rue de Danzig, 75015, Paris and to Mr Pierre Van den Wijngaert, General Secretary of ILEP in London for the following information on two organizations with well-established interests in leprosy in some of the French-speaking countries of Africa.

OCEAC (Organisation pour la Lutte contre les Endémies en Afrique Centrale)

This is an international organization which was founded in 1963 and currently includes Cameroun, Gabon and Congo-Brazzaville. An administrative council meets every year and it is composed of ministers of health of the above countries. The yearly budget originates approximately two-thirds from the member countries and one-third from France, but over and above this, France finances the services of a general secretary and a number of local doctors. For some years, the Fondations Follereau have supported training in leprosy, especially for the grade of worker called 'infirmier lèpre'. During 1980, however, training was modified and now consists of a course of 24 months for 20 state qualified nurses, selected from the member countries, with a polyvalent curriculum which includes leprosy, trypanosomiasis, malaria and schistosomiasis. It is held in Yaounde, Cameroon.

Every 2 years, OCEAC organizes a 4/5 day technical conference during which specialists in major endemic diseases present papers with emphasis on the current problems in each country. The main point of these conferences is to provide an opportunity for the chief medical officers of each sector to exchange views and take part in discussions. In April 1982, 44 doctors from the member countries attended a conference in which a whole day was given to the new multiple drug regimens for the treatment of leprosy, under the chairmanship of Dr H Sansarricq from WHO in Geneva. Finally OCEAC publishes a regular review, mainly for the medical officers of its member countries, which keeps them up to date with current events and progress on the main diseases they have to combat.

OCCGE (Organisation de Coordination et de Cooperation pour la Lutte contre les Grandes Endémies)

This is an international organization, founded in 1960 and composed of the following members—Benin, Ivory Coast, Upper Volta, Mali, Mauritania, Niger, Senegal and Togo. Fondations Follereau have supported the training of various categories of leprosy workers from the Marchoux Institute in Bamako, Mali and of nurses and laboratory workers from the Tropical Ophthalmology Institute (IOTA), also in Bamako. Financial support has also been given for doctors of the 8 member countries to attend conferences and for various expenses in the running of the secretariat.

Mr Recipon draws attention to the fact that OCCGE and OCEAC actually own the following important centres in the countries concerned; (1) the Marchoux Institute at Bamako, Mali, which

0305-7518/83/054345+04 $01.00

specializes in leprosy; (2) the Tropical Ophthalmology Institute (IOTA) in Bamako; (3) an organization for research on feeding and nutrition in Africa (ORANA) in Dakar, Senegal; (4) the Institute for Research on Onchocerciasis (IRO) at Bouake in Ivory Coast; (5) a centre for research on cerebro-spinal meningitis in Niamey in Niger; (6) the Centre Muraz which specializes in campaigns against trypanosomiasis, in Bobo-Dioulasso in Upper Volta; (7) a pilot scheme on leprosy–tuberculosis in Nouakchott, Mauritania; and (8) a pilot scheme on nutrition in Lome, Togo.

'Getting Down to Basics'

This is in fact the title of an article by Dr W A M Cutting of the Department of Child Life and Health in the University of Edinburgh, in which he draws attention to the value of 'packaging' essential facts about health care in a manner which will make them acceptable to a wide range of people (*World Health*, April–May 1983, pp. 18–20). He gives examples of brief explanatory texts (on blindness, dental care, diarrhoea and breast feeding), suggesting that they may be modified and illustrated with the help of a local artist, to suit varying conditions in different parts of the world. Particularly for the general public, there is almost certainly a case for including leprosy in this basic approach suggested by Dr Cutting, with appropriate translation into various languages. Although covering a wide range of readership (from lay, non-medical to experienced observers), the following articles and texts have also been brought to our attention as valuable sources of 'basic' information on leprosy:

1 Waters MFR. Leprosy. *Br Med J*, 1981; **283:** 1320–2.
2 Fine PM. Leprosy: the epidemiology of a slow bacterium. *Epidemiologic Rev*, 1982; **4:** 161–88.
3 Binford CH, Meyers WM, Walsh GP. Leprosy. *J Amer Med Assoc*, 1982; **247:** 2283–92.
4 Programme For Appropriate Technology in Health (PATH). Drug resistance; an urgent problem: leprosy. *Health Technology Directions*, 1983; **3:** 2.

Technical Guide for Smear Examination for Leprosy by Direct Microscopy

Published by the Leprosy Documentation Service (INFOLEP) at the Royal Tropical Institute, Mauritskade 61a, 1092 AD Amsterdam, the Netherlands, this 34-page paperback booklet covers all main aspects of smear examination. It was produced with the support of the Netherlands Leprosy Relief Association and the Ordre Militaire et Hospitalier de Saint Lazare de Jerusalem in the Netherlands.

The main headings include—introduction; technique of smear-taking; technique of staining; examination by microscopy. Five thousand copies have been printed in English and arrangements are being made for its translation and printing in French, Spanish and Portuguese.

Leprosy in Infants: Enquiry and Study

Dr Wayne Meyers, Chief, Division of Microbiology, Armed Forces Institute of Pathology has written to a number of colleagues in various parts of the world asking for information about leprosy in infants under 1 year of age. His letter reads as follows:

> For some time now the leprosy registry has been interested in leprosy in infants. In collaboration with Merlin L Brubaker, MD, former Regional Advisor for Leprosy for The World Health Organization, Region of the Americas (PAHO), we are undertaking an in-depth survey of the

problem. We are aware, from personal experience and from a survey of the files of the Leprosy Registry at the Armed Forces Institute of Pathology, of several infants under one year of age who had leprosy, proven by clinical and histopathologic findings. We believe that a detailed study of leprosy in this age group will provide valuable information on at least two controversial issues: (1) The minimal incubation period of leprosy. (2) Intrauterine infection of the fetus by *Mycobacterium leprae*.

As one who is knowledgeable in leprosy we are inviting your cooperation to provide us with any information you may have on leprosy in infants under one year of age. These may be either infants you have personally diagnosed or treated, or any others you know of in the geographic areas where you have worked.

We need as much information for each patient as can be reasonably supplied on the following: (1) Basis for the diagnosis (e.g. clinical manifestations, bacteriologic findings or histopathologic changes). (2) Classification of leprosy, and any information available on the course of the disease. (3) Suspected source(s) of infection (e.g. parents, siblings, other contacts, or unknown). If the contact is a parent, what was the classification and status of disease at the time of birth of the patient? (4) Have the patients been the subjects of any published report? This will help to reduce duplication in reporting.

Would you also please recommend to us any clinicians, pathologists, epidemiologists, public health officers or others in your geographic area that may be able to supply information of interest to this project.

This study is being conducted under the auspices of the Leprosy Registry at the Armed Forces Institute of Pathology. The Leprosy Registry is sponsored by The American Leprosy Missions, Bloomfield, New Jersey and is further supported by The Leprosy Mission (London), the Damien–Dutton Society (New York), and The Sasakawa Memorial Health Foundation (Tokyo). Currently biopsy speciments from approximately 1500 leprosy patients from many parts of the world are evaluated annually by members of the Leprosy Registry.

Write to Dr Wayne Meyers, Armed Forces Institute of Pathology, Washington DC 20306, USA.

Histopathology Services for Developing Countries

Professor Michael Hutt will be retiring from St Thomas' Hospital in London in September 1983 and has recently issued the following letter concerning histopathology services, which include the examination of biopsies for leprosy:

For the last 15 years the Department of Histopathology at St Thomas' Hospital has provided a free, postal, diagnostic service for a number of hospitals, both government and mission, in developing countries. It was originally envisaged that the need for such services would decrease as they were built up locally. For a variety of reasons, differing from country to country, this has not happened and the need is still there and likely to continue. To meet these problems and to provide histopathological expertise in parasitic, communicable and other tropical diseases in the UK a new consultative histopathologist post has been created jointly with the London School of Hygiene and Tropical Medicine and University College Hospital Medical School. This post has just been filled by the appointment of Dr S B Lucas who has spent 2 of the last 4 years in this unit and 2 in the Pathology Department at Nairobi. My own full-time post will terminate in September when I retire, though I will continue my involvement with developing countries on a part-time basis.

Dr Lucas is keen to maintain or increase the diagnostic services for tropical countries and we hope to raise funds to cover the expenses of such work.

As from 6 April 1983, I would be grateful if you could re-route your postal specimens to him:

Dr S B Lucas, Department of Morbid Anatomy,
School of Medicine, University College London,
University Street, London WC1. Telephone 01-387 9300.

I hope to remain in contact with you through my association with Dr Lucas and I am sure that he will provide you with an excellent service.

M S R HUTT, *Professor of Geographical Pathology*

Health Workers for the Third World

The Bureau for Overseas Medical Service (BOMS) is a charity for health workers who are interested in working in the Third World countries of Asia, Africa, the Caribbean and South America. BOMS offers career advice and information about jobs in hospitals, clinics, missions, primary health care units and teaching establishments. Enquiries from doctors with provisional or full registration in the UK are welcomed. The BOMS register has been enlarged to include all health workers, including paramedical workers with state registration and two years' working experience. Nurses must be SRN with a higher teaching qualification.

Anyone interested in joining the register or knowing of a vacancy for a health worker in the Third World is invited to contact Colin Jacobs, Secretary, Bureau for Overseas Medical Service, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, telephone 01-636 8636 ext. 232.

***Atlas of leprosy* (revised): Sasakawa Memorial Health Foundation, 1983**

One volume of the *Atlas of Leprosy* (revised) will be given *gratis* to all the participants coming from the 'developing' countries to the XIIth International Leprosy Congress in Delhi. Those participants from the 'industrialized' countries may obtain a copy, if there are any spare copies available, with a payment of a small handling charge.

The Paul Laviron Prize for Leprosy

The most recent issue of *Médicine Tropicale* (a French language review of pathology and tropical public health, published from Marseille, France), draws attention to this prize, which is offered annually. Applications for 1984 must be made before 1st March of that year. A prize of 8000 French francs is offered for the submission of a written account, in French of substantial and original work, which has not previously been published. The presentation should not fall below the standard of a university thesis. Preference will be given to work on leprosy accomplished abroad, or suitable for application abroad. The prize money may be allocated to one, or divided between several applicants. Apply to: Monsieur le Médicin général inspecteur, Directeur de l'I.M.T.S.S.A., Parc du Pharo, 13998, Marseille Armées, France.

Lepr Rev (1983) **54,** 349–352

Reports, News and Notes

ILEP: International Federation of Anti-Leprosy Associations; 29th Working Session, Berne, Switzerland, 6–9 June 1983

The Medical Commission and the Standing Committee and *ad hoc* working groups met to consider the following subjects: Leprosy and Primary Health Care; Combined TB/Leprosy programmes; Leprosy in Europe; the ILA Congress in New Delhi, February 1984; Public Relations; Health Education and Information; Training; Social Aspects in the Treatment of Leprosy Sufferers. Some of the topics covered by the Medical Commission included the following: research projects; a form for the yearly reporting of patients on multiple drug regimens; the final draft of a document on the introduction of multiple drug regimens; the ILA Delhi Congress; the recent workshop on 'Leprosy in Countries with Developed Health Services' (see below). The ILEP booklet *Guidelines for the Campaign against Leprosy* in now available in a French translation. A similar booklet on *Leprosy Control and Primary Health Care* is also available in English and French. The Commission also had the opportunity to examine and discuss the very first copies of the INFOLEP booklet on smear examination in leprosy. CIBA-GEIGY of Basle very kindly set up a demonstration which included a wide range of publications and reprints on chemotherapy. (Apart from the production of rifampicin (as Rimactane®) and clofazimine (as Lamprene®), readers may be interested to know that this company also markets dapsone (as Servidapsone®) in 50 and 100 mg tablets.)

Workshop in Italy: 'Management of Leprosy in Countries with Developed Health Services'; Santa Margherita, April–May 1983. 'Amici di Raoul Follereau'

This Workshop was the third in a series organized by the 'Amici di Raoul Follereau' (formerly called the 'Amici dei Lebbrosi'), the proceedings of which have been published in *Quaderni di Cooperazione Sanitaria* (Health Cooperation papers). The objective on this occasion was to consider aspects of the leprosy problems in countries with developed health services (essentially Europe, America and some countries in the Far East and South America) and to make recommendations for improvement, bearing in mind the recent WHO advice on multiple drug therapy. The Chairman of the Organizing Committee was Dr D L Leiker of Amsterdam. The programme was as follows:

Session I: Pharmacodynamics of sulphones, J K Seydel (W Germany); The pharmacodynamics of clofazimine, S G Browne (UK); The pharmacodynamics of rifampicin, S R Pattyn (Belgium); Pharmacological and experimental evidence for the selection of drugs in the treatment of leprosy, G A Ellard (UK).

Session II: Drug-sensitive persisters and drug resistance in leprosy, S R Pattyn (Belgium); Microbial persistence in mycobacterial infections, J Grosset (France); Principles of chemotherapy in tuberculosis, J Grosset (France); Principles of chemotherapy in leprosy in relation to disease

0305-7518/83/054349+04 $01.00

control, S K Noordeen (WHO); Compliance and compliance testing, H Huikeshoven (The Netherlands); Drug-sensitivity testing in leprosy, S R Pattyn (Belgium).

Session III: Leprosy reactions and their management, differential diagnosis with relapse, B Naafs (The Netherlands); Release from treatment and follow up, R C Hastings (USA); Present day organization of the leprosy control programme in France, F Cottenot (France); Current management of leprosy in The Netherlands, D L Leiker (The Netherlands); Eradication of tuberculosis and leprosy using chemotherapy, E Freerksen (W Germany); Current management of leprosy in Yugoslavia, Amina Korie-Gackie (Yugoslavia); Current management of leprosy in Spain, J Terencio de Las Aguas (Spain); Drug development, needs and prospectives, J K Seydel (W Germany); Discussion.

Session IV: Rehabilitation, S G Browne (UK); Surgical rehabilitation and surgical prevention of leprosy deformities (excluding the face), P Bourrel (France); Teaching, A C McDougall (UK); Introduction to immunology in leprosy, A Bryceson (UK); Immunodiagnosis of leprosy, J L Stanford (UK).

Session V: Immunotherapy, Marian Ulrich (Venezuela); Vaccination in Tuberculosis, IUAT; Vaccination in leprosy, Marian Ulrich (Venezuela); Discussion; Sub-Group meetings on: Therapy, Immunology and Vaccination, Rehabilitation, Training.

An important development during the course of this most enjoyable workshop was the grant for leprosy research described below.

Raoul Follereau Grant for Leprosy Research

('Amici di Raoul Follereau', via Borselli, 4-40135 Bologna, Italy.)

The Italian Leprosy Relief Association 'Amici di Raoul Follereau', will offer a sum of US $20,000 for a 2-year period of leprosy research, named after Raoul Follereau, to a young research worker in a university or other scientific centre in Europe.

The objective is to stimulate the undertaking of an original research project in the field of leprosy in Europe.

Further details and the necessary application forms may be obtained from 'Amici di Raoul Follereau' at the above address.

WHO Press Release: (WHA/7 07 16.5.83) tuberculosis

To those who sometimes become despondent about the lack of progress in leprosy control and the difficulty of obtaining and distributing the necessary drugs, the following statement on tuberculosis may offer some consolation:

> Noting that little improvement has been achieved in controlling tuberculosis in the developing countries over the past 20 years, the Assembly requested the Director-General to make all possible efforts through collaboration between the WHO Action Programme on Essential Drugs and the pharmaceutical industry to ensure that the most effective medicaments become more widely accessible to developing countries.

WHO: Tuberculosis and Leprosy Control

The *Weekly Epidemiological Record* (1983; **58:** 109–16) devotes considerable space to describing the outcome from a meeting of epidemiologists in Geneva in November 1982 to '. . . identify the main problems which are of immediate importance for tuberculosis and leprosy control, and to indicate areas for research'. As usual, the account is in English and French. The final paragraph is of particular interest:

> *Tuberculosis, Leprosy and other Mycobacterial Infections*
>
> There are similarities, differences and interactions between these two mycobacterial diseases, which are imperfectly understood. Two main areas should be explored: (1) The epidemiological interactions between tuberculosis and leprosy infection and disease, and the immunological role of other mycobacterial infections, particularly in relation to the information being collected in the Chingleput trial. (2) Health services research on the possibility of combining control programmes for tuberculosis and leprosy, and incorporating the control of these two diseases into the primary health care system.

Tuberculosis Course at ALERT, Addis Ababa, Ethiopia, May 1983

The first tuberculosis course was held in ALERT in Addis Ababa in May 1983 and another is planned for November 1984. Dr Styblo of the International Union Against Tuberculosis (IUAT) coordinated a small group of internationally eminent teachers in this subject. Further details may be obtained from the Training Director, ALERT, PO Box 165, Addis Ababa, Ethiopia.

Heiser Program for Research in Leprosy, 1984

Postdoctoral research fellowships, research grants and visiting research awards are being offered by this organization for 1984, as in previous years. Full details of these extremely valuable awards are obtainable from Mrs Barbara M Hugonnet, Director, Heiser Program for Research in Leprosy, 450 East 63rd Street, New York, New York 10021, USA.

Documents Received in Editorial Office

We gratefully acknowledge receipt of the following documents from colleagues overseas:

1 XIIIth Biennial Conference of the Indian Association of Leprologists, Bombay, 18–21 November 1983.
2 Summary of the Proceedings of the Eastern Region Leprosy Workers' Conference, Jamshedpur, India, March 1983.
3 XIth Workshop on Leprosy at the Acworth Leprosy Hospital, Bombay, India, March 1983.
4 Protocol for multidrug therapy for active leprosy cases in the control area of Kasturba Kushta Nivaran Nilayam, Malavanthangal (Professor T N Jagadisan, Madras).
5 News from the German Leprosy Relief Association Secretariat in Madras on Health Education Activities in 1983.
6 Bombay Leprosy Project; Indian Railways Launch Antileprosy Campaign in Collaboration with Bombay Leprosy Project (Dr Revankar in Bombay).

[Space does not allow us to give detailed information on all these interesting activities, but we can supply further details from this office if needed. *Editor*]

Zambia: Leprosy Control and Health Education

The Leprosy Advisory Committee met in August 1982 and gave considerable time to discussing health education and training in leprosy; during 1983 Dr Richard de Soldenhoff (Leprosy specialist) has been able to further define the needs at various levels of the community and to make good some of the defects in the availability of suitably written and audio-visual material. A seminar on 'Disability Prevention' was held in the Mwachisompola Health Demonstration Zone in January 1983 and attended by leprosy control officers, physiotherapists and officers in charge of leprosy referral hospitals (leprosaria). The Permanent Secretary and Director of Medical Services, Dr J M Kasonde published (with J D Martin, Adviser on Primary Health Care in the Ministry of Health in Lusaka) 'Moving towards primary health care: the Zambian experience' (*World Health Forum*, 1983; **4:** 25–30).

HYGIE: *International Journal of Health Education*

There is a change of appearance and format; having previously appeared with a blue cover, about 15 by 20 cm, this is now about double the size and also of greater length; the latest we have recived has 96 pages and includes interesting articles on health education presented at the XIth International Conference on Health Education held in Hobart, Tasmania in August 1982. A particularly important one is that by Dr Halfdan Mahler, Director General of WHO, 'The New Look in Health Education', on pages 74–7. He refers to the importance of making '. . . fuller use of the new media resources at our disposal and to harness the latest technological advances in communications'. Following the report of this paper there is a note from the editors inviting all film and video tape producers to send a written description of their productions for publication, in view of the fact that '. . . audiovisuals are more and more used by health educators as learning aids. Many readers have asked us to introduce a section which informs them of existing audiovisuals and how to obtain them.' The Journal address is HYGIE, *IJHE*, 9 Rue Newton F-75116, Paris, France.

Schisto Update

Already peripheral to the interests of many workers in leprosy, this excellent publication may well be of value to those in parasite immunology and it is mailed free of charge to all who request it; from The Edna McConnell Clark Foundation, 250 Park Avenue, New York, New York 10017, USA. It also has a preliminary section with items of general interest; the latest received here has details of: a tropical disease fellowship funded by the Rockefeller Foundation and the Foundation for Microbiology in the USA; tropical disease applications for WHO; epidemiology fellowships under the US Public Health Service; Wellcome Research Travel Grants; Wellcome Visiting Professorships in Microbiology 1983/84, etc.

Vacancies at Armauer Hansen Research Institute

The Armauer Hansen Research Institute is in the compound of ALERT Leprosy Hospital in Addis Ababa, Ethiopia. Its task is to do basic research in the immunology of leprosy. There will be at least one vacancy from early 1984 on the scientific staff. Applicants with suitable experience in immunology/immunochemistry should apply for further details to the Director of AHRI, PO Box 1005, Addis Ababa, Ethiopia as soon as possible.

Lepr Rev (1983) **54,** 353–356

Letters to the Editor

IS THE LEPROMIN TEST RELIABLE IN CHILDREN?

Sir,

Having recently had occasion to review, in the light of findings in Venezuela and elsewhere, some notes made in Nigeria (1959–65), I must conclude that in young children the lepromin test as ordinarily practised may not be a reliable indication of resistance to leprosy infection or to capacity to mount a degree of cell-mediated immunity parallel with the positivity of the test. I would suggest that workers who are in touch with child contacts of leprosy patients in areas of high leprosy prevalence should institute investigations into this problem.

My own findings suggested that while a positive test—and *a fortiori*, a strongly positive test—was useful in indicating a measure of cell-mediated immunity, a negative test provided no evidence that the individual was incapable of mounting a degree of cell-mediated immunity sufficient to limit the enlargement of a single lesion or a few lesions, and eventually to encompass their spontaneous resolution. Some child patients in whom tuberculoid leprosy was histologically confirmed, with acid–alcohol-fast débris in superficial nerve fibrils, might proceed to resolution while the lepromin test remained negative. On the other hand, some child patients in whom the test was negative gave evidence that their indeterminate macular lesions were in reality prelepromatous by showing large numbers of acid–alcohol-fast rods in the dermis on regular fortnightly slit-smear examinations.

S G BROWNE

16 Bridgefield Road
Sutton, Surrey SM1 2DG

LEPROSY IN SUB-HUMAN PRIMATES: POTENTIAL RISK FOR TRANSFER OF *MYCOBACTERIUM LEPRAE* TO HUMANS

Sir,

In recent years there have been a number of publications[1–7] dealing with the possibility of leprosy, or a leprosy-like disease, in sub-human primates. The risk of transmission of *Mycobacterium leprae* from any source is a function of exposure, and whether this is accomplished by aerosols, direct physical contact, vectors, or fomites the likelihood is that such transmission is dependent upon the duration of exposure. In order to investigate the possible role of sub-human primates in the transmission of leprosy to human beings, we undertook a study in order to (1) compare the prevalence of leprosy in the general population of an endemic area with the prevalence of leprosy in individuals having daily contact with sub-human primates, and (2) investigate the possibility that these sub-human primates may constitute a potential risk for transmission of *M. leprae* to susceptible humans.

Two states in India, Andhra Pradesh and Tamilnadu, contain 15% of India's total population

0305-7518/83/054353+04 $01.00

but approximately 50% of the nation's known cases of leprosy. The prevalence rate in Andhra Pradesh, in 1981, was 14·5/1000 and in Tamilnadu 19/1000.[8] Both states also have a large population of monkeys. The leprosy control programme based in Salur, Vizianagaram District, Andhra Pradesh was selected as the base of operations. Para-medical workers and other staff members were utilized to discover the existence and location of owned monkeys. For the purpose of this study it was decided to use only owned monkeys since feral monkeys have no daily physical contact with humans and have little opportunity for transmission of the agent. India does not permit the capture and export of monkeys for laboratory use, therefore the potential for transmission to humans as a result of this activity is nil. When a monkey was located it was examined and an interview was conducted with the owner, photographic documentation obtained and, if the owner or any other close contacts of the monkey were known patients, or showed evidence of leprosy, a smear was made from the ear lobe of the monkey. (Facilities for performing immunological studies for antibodies against *M. leprae* antigens 5 and 7 were not available.) Information requested during the interview included occupation, residence, description of all individuals having close contact with the monkey, and descriptive information about the monkey (age, sex, species, time of ownership and use).

Twenty-six owned monkeys were found. Ages ranged from 1 week to 12 years. There were 15 males and 11 females. The most common species was *Macaca radiata* ('Bonnet monkey'). Only 3 monkeys were used as pets; the balance were 'working' monkeys that were used by beggars to perform simple tricks to acquire money (Table 1).

The 26 monkeys were in continual daily contact with 71 humans (family members and dependents of owners). Among these, 64 had no visible signs or history of leprosy, 4 had tuberculoid leprosy, 1 had indeterminate leprosy and 2 had lepromatous leprosy; a prevalence rate of 98·6/1000. The 6 monkeys in contact with these leprosy patients had negative ear lobe smears. Twenty-four monkeys were free of any visible signs of disease. One monkey (a pet) had bilateral axillary lymphadenitis due to an unidentified Gram positive, non-acid fast organism and one monkey had a clawed left hand. This monkey belonged to a patient with tuberculoid leprosy. An ulnar nerve biopsy performed on this monkey showed no significant lesions.

On the basis of this limited study it is impossible to establish any cause and effect relationship between daily contact with monkeys and increased incidence of leprosy in humans. In all instances the individuals with leprosy had been diagnosed as such *before* they acquired the monkey examined in this survey.

Two questions remain unanswered, however: (1) the possibility of transmission to these individuals by previously owned monkeys, and (2) the possibility of transmission of *M. leprae* from shedding owners to other humans via these monkeys.

As leprosy control programmes become effective in reducing the prevalence and incidence of leprosy it becomes important to consider non-traditional concepts of the method of transmission of the infectious agent. As anyone who has worked with leprosy patients knows, a large percentage have no idea of their source of infection. This may be explained, in part, by the long incubation period which makes accurate histories extremely difficult to obtain, but may also be explained by the fact that there might never have been a period of 'prolonged and intimate contact' with a person known to be infected. Airborne transmission probably accounts for a number of these cases.[9] The fact that spontaneously occurring leprosy has been identified in sub-human primates adds another possible method of transmission. The degree of risk from infected monkeys depends on the amount of contact between these animals and susceptible humans.

Contrary to what one might assume the monkeys that were kept as pets were not handled by many people. Two of the pet monkeys observed in this study were handled by only one person and the third by only 2 persons. The other members of the family either ignored the monkey or were afraid of it.

Monkeys used for begging, on the other hand, physically contact many people. They are taught

Table 1. Description of owned monkeys examined in Andhra Pradesh, South India during May–July 1982

Species	Age	Sex	Use	Daily human contacts with leprosy*	Daily human contacts without leprosy
Macaca mulatta	4 yr	M	Pet	0	4
M. radiata	3 yr	M	Work	1 (T)	1
M. radiata	1 yr	M	Pet	0	3
M. radiata	12 yr	F	Work	4 (1T, 1I, 2L)	2
M. radiata	3 yr	M	Work		
M. radiata	6 mon.	F	Work	0	4
M. radiata	1 yr	F	Work		
M. radiata	1 week	M	Work	0	12
M. radiata	2 yr	M	Work		
M. radiata	2 yr	F	Work		
M. radiata	4 yr	F	Work		
M. radiata	6 yr	M	Work		
M. radiata	7 yr	F	Work		
M. radiata	4 yr	M	Pet	0	7
M. radiata	6 mon.	M	Work	0	1
M. radiata	3 yr	F	Work		
M. mulatta	2 yr	M	Work	1 (T)	6
M. radiata	5 yr	F	Work	1 (T)	5
M. mulatta	1 yr	F	Work		
M. mulatta	1 yr	F	Work	0	8
M. mulatta	10 yr	M	Work		
M. radiata	6 yr	F	Work	0	1
M. radiata	1 yr	M	Work	0	7
M. radiata	2 yr	M	Work	0	1
M. radiata	2 yr	M	Work	0	1
M. radiata	2 yr	M	Work	0	1

* T, tuberculoid; I, indeterminate; L, lepromatous.

to do simple tricks which usually involve no contact with spectators, but several of the monkeys were observed to go around and kiss as many of the children as possible and all of them were taught to manually accept the coins offered to the beggar.

The *modus operandi* of the beggars adds a bit more to the potential risk. Some beggars remain in one village on a semi-permanent basis but the majority of those that used monkeys travelled from village to village, often over considerable distances, to take advantage of the crowds associated with festivals and pilgrimages.

Acknowledgments

This study was supported by a Fulbright Grant under the Indo-US Subcommission on Education and Culture: Indo-American Fellowship Program, 1981–82.

Acknowledgment is made to Dr C K Job, Chief, Pathology Research Department, National Hansens Disease Center, Carville, Louisiana, USA; Dr C J G Chacko, Head, Radda Barnen Research Laboratories, Schieffelin Leprosy Research and Training Center, Karagiri, South India; and Dr N Victor Babu, Superintendent, Reconstructive Surgery Hospital, Salur, AP, India, for their advice and technical assistance and to Dr R H Thangeraj and Dr E P Fritschi for providing the facilities and personnel for this study.

H V HAGSTAD

Department of Epidemiology and Community Health
School of Veterinary Medicine
Louisana State University
Baton Rouge
Louisana 70803, USA

References

[1] Collier DR. Inoculation of monkeys with leprosy following a diet of puak (Colocasia). *Lepr Rev*, 1940; **11:** 135–40.

[2] Gunders AE. Progressive experimental infection with *Mycobacterium leprae* in a chimpanzee: a preliminary report. *J Trop Med Hyg*, 1958; **61:** 228–30.

[3] Binford CH. The inoculation of human leprosy in the chimpanzee: initiation of a long-term project. *Int J Lepr*, 1965; **33:** 666–8.

[4] Waters MFR, Bakri MD, Isa JH, Rees JW, McDougall AC. Experimental lepromatous leprosy in the white-handed gibbon (*Hylobatus lar*): successful inoculation with leprosy bacilli of human origin. *Br J Exp Path*, 1978; **59:** 551–7.

[5] Donham KJ, Leininger JR. Spontaneous leprosy-like disease in a chimpanzee. *J Infect Dis*, 1977; **136:** 132–6.

[6] Leininger JR, Donham KV, Rubino MJ. Leprosy in a chimpanzee. Morphology of the skin lesions and characterization of the organism. *Vet Path*, 1978; **15:** 339–49.

[7] Meyers WM, Walsh GP, Brown HL, Fukunishi Y, Binford CH, Gerone PJ, Wolf RH. Naturally acquired leprosy in a mangabey monkey (*Cerocebus* sp). *Int J Lepr*, 1980; **48:** 495–6.

[8] Christian M. The epidemiological situation of leprosy in India. *Lepr Rev* (Suppl 1), 1981; **52:** 35–42.

[9] Davey TF, Rees RJW. The nasal discharge in leprosy. *Int J Lepr*, 1973; **41:** 512.

Lepr Rev (1983) **54,** 357–359

Abstracts

MUKHERJEE R, MAHADEVAN P R, ANTIA N H **Organized nerve culture. I. A technique to study the effect of *M. leprae* infection.** *International Journal of Leprosy* 1980; **48** (2): 183–8. **II. DNA synthesis in Schwann cells in the presence of *M. leprae*.** *Ibid.* 189–92

I. Organized cultures of dorsal root ganglia from neonatal mice were infected *in vitro* for 2 weeks with *Mycobacterium leprae*, freshly isolated from lepromatous patients. Bacilli were ingested well by free Schwann cells in the premyelin secretory phase, but less so by those merged with axons. They were also phagocytosed by fibroblasts but not by neuronal cells or axons. There was only a poor uptake of heat-killed *M. leprae*, whereas the uptake of ICRC-C44 strain with a viability of 60–90% was massive.

The Schwann cells containing *M. leprae* were unable to associate with nerve fibres and secrete myelin, which suggested that the presence of bacilli interfered with cell function.

II. This hypothesis was supported by the finding that Schwann cells containing *M. leprae* failed to incorporate a DNA precursor. This indicated a blockage of DNA synthesis which would inhibit proliferation and axon association.

D S Ridley

TOUW J, STONER G L, BELEHU A **Effect of *Mycobacterium leprae* on lymphocyte proliferation: suppression of mitogen and antigen responses of human peripheral blood mononuclear cells.** *Clinical and Experimental Immunology* 1980; **41** (3): 397–405

Evidence is presented that *Mycobacterium leprae* suppresses the *in vitro* proliferative response of human peripheral blood mononuclear cells to antigen and mitogen. Lymphoproliferation induced by PPD or alloantigen stimulation was inhibited by concentrations of *M. leprae* which were not cytotoxic for lymphoblasts, and which were stimulatory for sensitized lymphocytes in the lymphocyte transformation test. In contrast, inhibition of PHA-stimulated peripheral blood mononuclear cells was seen only at 10- to 100-fold higher concentrations of *M. leprae* which proved to be cytotoxic for lymphoblasts. Inhibition of PPD-induced lymphoproliferation occurred both when cultures were initiated with *M. leprae* and PPD together, and when peripheral blood mononuclear cells were incubated with *M. leprae* alone for 2 days, before adding PPD. No inhibition occurred when cells were cultured with PPD alone for 2 days before adding *M. leprae*.

The inhibitory effect of *M. leprae* on the response to PPD of normal peripheral blood mononuclear cells resembled that seen when peripheral blood mononuclear cells both from untreated patients with tuberculoid or borderline tuberculoid leprosy, and from untreated patients with lepromatous or borderline leprosy were cultured with *M. leprae* and PPD. These findings indicate that the inhibitory effect of *M. leprae* on lymphoproliferation in response to PPD antigen does not depend upon a particular cell population present particularly in leprosy patients.

The authors discuss how these results could explain some of the immunological aberrations in lepromatous patients who harbour large numbers of *M. leprae* bacilli in their tissues.

P M Preston

YAMAMOTO Y **[Radiological studies on changes in calcaneus trabecula in leprosy]** *Japanese Journal of Leprosy* (1980; **49** (1): 20–37 [In Japanese] English summary

A useful study was made of the changes observed by X-ray of the trabeculae of the calcaneus in the feet of patients with leprosy. The trabeculae disappear, and in a particular pattern, with alterations of the strains to which the calcaneus and foot bones as a whole are subject in patients with paralysed peroneal nerves (foot drop), with fracture and in deformities. Although the author makes no specific mention of this, presumably X-ray changes would be useful in monitoring increasing deformity and applying corrective treatment. There are 7 excellent plates. All but the tables are in Japanese. The above is based on the English summary.

Ralph Schram

ROBINS K, VIJAYAKUMART, GOPINATH T, VASUDEVAN D M **Liver leprosy. I Functional changes.** *Leprosy in India* 1980; **52** (3): 416–22

DAS R, GOSWAMI A, MITRA A K, ROY I S **Ocular complications in leprosy.** *Journal of the Indian Medical Association* 1980; **74** (1): 5–8

150 cases of leprosy with ocular complications were studied in West Bengal. Despite the advent of sulphone therapy, the ocular damage was extensive and crippling. As many as 38% had uveal lesions. The lids and eyebrows were involved in 56%, cornea in 34%, lens in 46% and the globe in 16% of the cases. Blindness was extensive (65.3%) but those due to cataract were operated on with success. The major factor contributing to the damage was delay in diagnosis and treatment. The obvious remedy is to have regular examination and treatment of the eyes as soon as a case of leprosy is detected.

[The authors' observations are in line with those of other workers in this field, but the list of references in the text is not matched by the bibliography given at the end of the paper.]

D P Choyce

0305-7518/83/054357+03 $01.00

KRISHNA MURTHY K, RAJA BABU K K **Toxic psychosis after accidental ingestion of dapsone—review and case report.** *Leprosy in India* 1980; **52** (3): 443–5

'A case of toxic delirious psychosis in a 5-year-old child after accidental ingestion of dapsone is reported and relevant literature is reviewed. A suggestion is made for a detailed work on the pathological and metabolic effects of dapsone on the central nervous system.'

MILLAN J Le contrôle de la lèpre en Guadeloupe. I Organisation générale—mesures de déclaration et d'enregistrement des malades. **Leprosy control in Guadeloupe (French West Indies). I General organization. Notification and registration of patients** *Médicine Tropicale* 1980; **40** (4): 433–8 II. Règles de traitement et de surveillance dans le secteur de Grande-Terre. **[II. Rules of treatment and monitoring in 'Grade Terre' district]** *Ibid.* 441–5 English summaries

I. The distinctive features of the leprosy control programme in the islands collectively known as Guadeloupe are related to the fact that the programme is organized and supervised by microbiologists with epidemiological training. The two centres from which control is exercised are a general hospital and the Pasteur Institute. The partially integrated leprosy control programme is responsible for the treatment of 44% of the patients who present themselves at the two centres (neither of which is identified by an appellation that includes the word 'leprosy'), and 56% are registered with the mobile teams.

The disease is compulsorily notifiable, but confidentiality is respected when the patient requests it. Usually, particulars of the notified cases are passed by the central medical registering authority to the social and administrative services. An allowance in cash is made for a short period when required (for transport and such matters), or for long periods in the case of permanent disability.

The examination of contacts is held to be very important.

After notification, confirmation of the diagnosis is made by the Institute, with the aid of histopathological examination of biopsy material from every patient and the results of the lepromin reaction.

Due attention is paid to the social aspects of leprosy and the prejudices and cultural background of the people. The doctors practising in the islands are becoming versed in the modern ideas about leprosy, a necessary prelude to enlisting their close cooperation.

II. The treatment protocols favoured in Guadeloupe follow the lines laid down by the WHO Expert Committee: combined therapy initially for all patients suffering from multibacillary forms of leprosy, and continued for 2 years; maximal doses from the beginning of treatment; no interruption of treatment. Tablets are distributed every month to the patients.

For paucibacillary forms of leprosy, the following regimen is followed: monotherapy with dapsone for 5 years; if nerves are already damaged when treatment is begun, corticosteroids are given together with either a long-acting sulphonamide or clofazimine.

For multibacillary forms of leprosy, rifampicin and dapsone are given initially with, perhaps, ethionamide. Thereafter, dapsone is continued 'for life'.

For sulphone-resistant leprosy, clofazimine is the drug of choice. [For some unaccountable reason, dapsone is sometimes reintroduced.]

Case-holding presents problems, as does irregularity of treatment.

Profiting by the example of the treatment of tuberculosis, the aim of the treatment programme of leprosy is to prevent relapse and to postpone indefinitely the emergence of dapsone resistance. To this end, the bacillary status of the patient is established before treatment is begun. Exclusion from school or from work is rarely advised, and only for a maximum period of 2 months. Ambulatory treatment is the rule, and admission to hospital the exception. Sometimes for severe reversal reaction, and in the case of uncooperative patients, admission is advised.

Reconstructive surgery is done at the central hospital.

Follow-up examinations are made every 6 months of those patients who are discharged under observation without treatment.

Chemoprophylaxis is not given.

S G Browne

NILAKANTA RAO M S, SHANKAR S V, NARASIMHA MURTHY D P, VOMSTEIN E, MEERMEIER H **Problem of leprosy in Karnataka.** *Leprosy in India* 1980; **52** (2): 236–44

Karnataka State in south India was created in 1973 on linguistic considerations and is still undergoing development. Contiguous areas of adjacent States on the north, east and south-east have a high prevalence of leprosy. This paper summarizes available data on leprosy in the new State and concludes with an estimated leprosy prevalence of about 10 per 1000 in most urban areas (population 9·42 million) and a total of about 200,000 cases of leprosy for the State as a whole (population 35 million).

T F Davey

VAN EDEN W, DE VRIES R R P, MEHRA N K, VAIDYA M C, D'AMARO J, VAN ROOD J J **HLA segregation of tuberculoid leprosy: confirmation of the DR2 marker.** *Journal of Infectious Diseases* 1980; **141** (6): 693–701

'Families with multiple cases of leprosy were tested for HLA (histocompatibility leukocyte antigen)-linked control of susceptibility to tuberculoid leprosy and association with HLA-DR2. Thirty-one non-HLA genetic markers were also examined for indications of non-HLA-linked genetic factors that might control susceptibility to tuberculoid leprosy. A significant ($P = 0{\cdot}002$) preferential inheritance of HLA-DR2 by siblings affected with tuberculoid leprosy, but not by healthy siblings nor by siblings affected with lepromatous leprosy, was observed. In addition, combined family data showed a significant ($P < 0{\cdot}0025$) excess of identical HLA haplotypes inherited from healthy parents by siblings affected with tuberculoid leprosy. Segregation of non-HLA polymorphisms did not deviate significantly from what would have occurred randomly. These data are compatible with a recessive inheritance of HLA-linked susceptibility to tuberculoid leprosy. The preferential segregation of DR2 observed in children with tuberculoid leprosy ($P < 0{\cdot}001$ for the combined data from India) indicates that the HLA-linked susceptibility gene is either DR2 or in linkage disequilibrium with it.'

REZA K, TALIB S, IMAM S K ***O*-diphenoloxidase concentrations in leprosy.** *British Medical Journal* 1979; **2** (Oct. 13): 900–1

O-diphenol oxidase activity was demonstrated in the skin in all of 15 patients with lepromatous leprosy, and in the serum in long-standing infections, though it could not be demon-

strated in non-lepromatous patients or in normal individuals. The substrate specificity of the enzyme indicated that its origin was bacterial, not mammalian. It is suggested that this enzyme might be used as a diagnostic marker for lepromatous leprosy.

D S Ridley

KUPPUSAMY P, RICHARD J, SELVAPANDIAN, A J **A study of causes of unemployment among agricultural labourers afflicted by leprosy.** *Leprosy in India* 1979; **51** (3): 369–75

The authors followed up after an interval of 4 years the employment data of 116 leprosy patients in their care, all of them agricultural labourers. 17 (average age over 50 years) had changed their employment as a result of deformity and 6 had become unemployed. [No details are given and no comparable data offered regarding the employment risks of people without leprosy in this age group.]

T F Davey

LEW J **The integration of handicapped people due to Hansen's disease into the community.** *Journal of the Formosan Medical Association* 1979; **78** (10): 899–900

This is an interesting account of the way in which Korea coped with its leprosy 'begabonds' after World War II in 1945 when more than 5,000 leprosy patients on the streets added to their 10,000 in-patients in four leprosaria. Most of these begging vagabonds were lepromatous patients. The Korean Leprosy Association gathered most of them and through the Hope-Village movement established 16 villages by the outbreak of the Korean war in 1950. This stopped all efforts until 1955, when dapsone treatment and out-patient care came into being.

Today there are about 100 resettlement villages where some 20,000 leprosy patients are managing their daily lives, mostly in poultry and pig raising.

Ralph Schram

OSKA R **[A survey of the social situation of leprosy patients in JALMA Leprosy Centre, Agra, India. II. Survey on the socio-environmental aspects of inpatients]** *Japanese Journal of Leprosy* **48** (2), 59–66 [In Japanese] English summary

A study of 240 patients. [For part I see *Trop Dis Bull*, 1979, **76,** abstr. (2413).]

SRITHARAN V, VENKATESAN K, BHARADWAJ V P, RAMY G (1979) **Serum lipid profile in leprosy.** *Leprosy in India* **51** (4), 515–520

LATAPÍ F, SAÚL A, RODRÍGUEZ O, MALACARA M & BROWNE SG [Editors] (1980) **Leprosy. Proceedings of the XI International Leprosy Congress, Mexico City, November 13–18, 1978.** pp. xv+403. Excerpta Medica, 305 Keizersgracht, PO Box 1126, 100 BC Amsterdam, The Netherlands (ISBN 0 444 90092 6] [D. fl. 170.00]

The hardback book, which includes an excellent index, represents a *verbatim* account of the latest *International Leprosy Congress* in Mexico: more than 800 specialists came from 83 countries to describe their work. The main headings in this book are those used in the Congress; epidemiology and control; experimental leprosy; clinical aspects; microbiology; immunology; social aspects; experimental chermotherapy; clinico-pathological aspects; nerve damage; therapy, rehabilitation and workshop summaries. The latter are those already published in *Leprosy Review* [see *Trop Dis Bull*, 1979, **76,** abstr. 2411]. Consensus views of world experts in print are obviously of great value in this subject and these proceedings will be appreciated by those working in all branches of leprosy, not only for reference but, more simply, for the purpose of reading in tranquility what was said at the time. The publishers are to be congratulated on the extremely clear and accurate production. A minor point of criticism is that on the spine of the book, there appear the single word 'Leprosy', and those who do not recognize the names of the eminent leprologists alongside may, in furture years, fail to spot this book on the shelves for what it contains—the proceedings of an historic congress. A major criticism is the price, which is so far beyond the private individual's pocket that this book will never reach many who whould profit by the expert views and information it records.

A C McDougall

BROWNE SG (1980) Le contrôle de la lèpre: chimères et possibilitiés. **[Leprosy control: chimeras and possibilities]** Reprinted from *Bulletin et Mémoires de l' Académie Royale de Médicine de Belgique* **135** (3), 208–218

The author reviews the general situation regarding the control of leprosy in the world, particularly in the light of the increasing menace of sulphone resistance and the non-availability of a specific vaccine.

After 30 years of widespread monotherapy with sulphones—touching, however, only a fifth of those needing treatment—leprosy shows little sign of being controlled, except in certain countries where there is a high natural resistance to infection among potential or actual victims. As with other transmissible diseases in countries of the Third World, the situation is bedevilled by the lack of complete and reliable statistics and by irregularity of treatment.

Two serious complicating factors are now upsetting the prospects for effective control and calling into question the epidemiological assumptions of governments and co-operating voluntary agencies. The first is secondary sulphone resistance, with its inevitable consequence of primary sulphone resistance occurring in susceptible contacts; the second is the demonstration of the presence of persister organisims—viable and drug-sensitive—in certain tissues despite the exhibition of effective drugs in adequate dosage for adequate periods.

The possibilities for control of leprosy today depend on such time-honoured principles as the reduction of the reservoir of infection by correct chemotherapy (that is, multidrug regimens for multibacillary disease), and hygienic measures to reduce transmission of viable organisms. Primary prevention by stimulating innate immunological defence mechanisms has not so far proved very encouraging, and the administration of dapsone prophylactically is largely impracticable.

The world still awaits a specific, safe and inexpensive vaccine, and cheap mycobactericidal alternatives to dapsone.

Ralph Schram

CONTENTS

Printed in Great Britain at the Alden Press Oxford